HETEROTROPHIC PRODUCTION OF LIPID BY MICROALGAE AND ITS CHARACTERIZATION AND PERFORMANCE EVALUATION IN CI ENGINE

A Thesis submitted to the Delhi Technological University, Delhi in fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

In

Mechanical Engineering

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DECLARATION

I hereby declare that the thesis entitled "**Heterotrophic Production of Lipid by Microalgae and its Characterization and Performance Evaluation in CI Engine**" is an original work carried out by me under the supervision of **Dr. Sagar Maji and Dr. Amit Pal**, Department of Mechanical Engineering, Delhi Technological University, Delhi. This thesis has been prepared in conformity with the rules and regulations of the Delhi Technological University, Delhi. The research work reported and results presented in the thesis has not been submitted either in part or full to any other university or institute for the award of any other degree or diploma.

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CERTIFICATE

This is to certify that the work embodied in the thesis research proposal entitled **“Heterotrophic Production of Lipid by Microalgae and its Characterization and Performance Evaluation in CI Engine”** by **Amrik Singh, Roll No. 2K13/Ph.D.ME/09** as a Ph.D. scholar in Department of Mechanical Engineering, Delhi Technological University is satisfactory and meets the Ph.D. requirement. This thesis work is useful to utilities based on innovations and research. He has completed his thesis work under our guidance and supervision. The matter embodied in this research work has not been submitted earlier for the award of any degree or diploma to the best of our knowledge and belief.

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Abstract

The problem of fuel depletion and the increase of carbon emissions increased the concern of researchers towards biodiesel. The algae species-rich in oil content were considered to be a potential feedstock for biodiesel which can easily replace diesel. But the biodiesel production from microalgae is still in its development phase and requires cost-effective biodiesel production and extraction techniques in order to commercialize it. Thus, in the present work, advanced biodiesel production techniques were investigated. The transesterification is a long and complicated process, which usually require different stages to complete the reaction. To simplify the above complex phenomena direct transesterification method of wet algae was also investigated. There is a lack of awareness on the techniques for algae oil methyl ester production, and its combustion effect and its performance analysis in CI engine, which are addressed in the present work.

Firstly, the microalgae species *Neochloris oleoabundans* was selected, and its growth conditions were optimized using statistical software Design expert. It is very vital to optimize cultivation conditions in order to minimize the cost. It was observed that microalgae biomass growth depends upon the glucose and sodium nitrate concentration in the media as well as on the temperature of the surrounding. Different experiments were performed according to the number of experiments suggested by design of expert (DOE). DOE has provided two solutions according to the condition applied, in one solution predicted biomass yield was 1.97 g, while in other method biomass yield was 1.59 g. Based on the above solution, validation test was conducted and biomass yield 1.79 g and 1.42 g was obtained. Further, best harvesting technique was analyzed. It was obtained that among all the harvesting techniques chemical flocculation method is the best method of harvesting.

The solvent extraction method was used for lipid extraction, and then the oil was trans-esterified, and biodiesel yield was optimized by varying the molar ratio of oil to alcohol and catalyst concentration. To reduce the production cost of biodiesel, optimization is essential. The optimum yield was obtained at 6:1 molar ratio and 1% catalyst concentration, and maximum yield was obtained within 20 minutes for ultrasonic reactor and 80 minutes for mechanical stirrer method. It was concluded that using advance technique like ultrasonic assisted transesterification; the yield was increased to higher extent.

Fuel properties highly depends upon the fatty acid composition, so its composition must be determined and make sure that it should meet the international standards. Therefore, microalgae biodiesel physiochemical properties were analyzed. It was found that major fatty acids in algal oil were palmitic acid, oleic acid, and linoleic acid. GC-MS data shows the fatty acid methyl ester composition lies in the range of C14-C18, it has 31% saturated fatty acid 67.9% unsaturated fatty acid. Different fuel properties were tested, for example, viscosity, pour point and calorific value. All of these are within the prescribed limit of different standards. The knowledge of microalgae oil methyl ester properties increases our knowledge about algae oil and its potential to fulfill the demand of world.

A significant amount of literature is available on vegetable oil biodiesel but very few on microalgae. Thus, this work increases the knowledge about the microalgae biodiesel production and its performance in the engine. Finally, different blends of microalgae oil were prepared and tested. The examination of microalgae oil methyl ester in CI engine reveals that algae oil is appropriate for the engine. It is the best fuel for the engine because utilizing this fuel all the emissions were reduced to a great extent except NOx emission.

Keywords: Biodiesel, Design of expert, Algal biomass yield, Engine Emissions, NOx, Transesterification, Ultrasonic reactor, Microwave, Direct transesterification.

Dedicated

to

My Parents

and family

List of abbreviations

Symbol	**Full name**
BTE	Brake thermal efficiency
CV	Calorific value
ρ	Density
NOx	Nitrogen oxide
UHC	Unburnt hydrocarbon
BSFC	Brake specific fuel consumption
AOME	Academy of Medical Educators
OECD Nations	Organisation for Economic Co – operation and Development
IEO	International Energy Outlook
GCMS	Gas Chromatography-Mass Spectrometry
FAME	Fatty Acid Methyl Ester
HCl	Hydrochloric acid
CO	Carbon monoxide
HC	Hydrocarbon
IEA	International Energy Agency
B100	Neat Biodiesel
CO_2	Carbon dioxide
ASTM	American Society for Testing and Materials
SO_2	Sulfur dioxide

BSFC	Brake Specific Fuel Consumption
BTE	Brake Thermal Efficiency
TAG	Triacylglyceride
H_2O	Water
N	Nitrogen
P	Phosphorus
Fe	Iron

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CHAPTER 1

INTRODUCTION

1.1 General introduction

In the recent decades, the world has faced a severe energy crisis due to extensive use of irreversible conventional fossil fuels and is continuously increasing. The combustion of fossil fuels results in the emission of CO (Carbon monoxide), HC (Hydrocarbon) and NOx (Nitrogen Oxide), etc. which are highly hazardous to human health. Their use increases greenhouse gases that resulted in global warming. India is the fifth largest consumer of energy, while the country is estimated to be at third position by 2025 after China and USA, which accounts for 4.1% of the entire world energy consumption (India Energy Book 2012). While only 64.5 % of the country has electricity connectivity with an urban electrification rate of 93.1%, however just 52.5 % of the countryside areas are electrified yet which contains largest portion of the population (IEA International Energy Agency, 2009). Despite a lot of efforts by the Indian government, rural areas are not electrified yet due to insufficient power-generation capacity, which hinders the execution and feasibility of government launched programs. In addition, with 1.2 billion of population India is ranked at 2nd position of the crowded nation with massive urbanization that leads to higher oil imports. India has spent Rs 4,559 billion on crude oil import in years 2010 and 2011 (India Energy Book, 2012). India’s oil import charge will surpass the USA by 2035 approximately USD 190 billion (IEA, 2010).

Any fluctuations in costs or issues that influence the constant supply of oil would severely affect any nations. In the perspective of the increased dangers of energy security and environmental hazard, to overcome this situation it is essential to focus on the following angles. Firstly, utilize the current available fuel sources as economically as could be expected under the circumstances. Secondly, settling the issues identified with atmospheric sustainability so that it does not degrade the environment; lastly, investigating broadened or alternative fuel sources. The dependency on petroleum products for our energy production must be reduced significantly.

Looking at the above points, biofuels are perceived as an outstanding player in guaranteeing energy security later in India. Alcohol, biogas, biodiesel are the potential alternative fuels that are being practiced nowadays.

1.2 Biodiesel

Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and alcohol that can be used in diesel engines, alone or blended with diesel oil. Biodiesel is produced from oils (edible and non-edible), and animal fats, transesterification of oils give fatty acid methyl or ethyl ester. The primary feedstock used in India for biodiesel production are non-edible plants, for example, *Jatropha curcas*, *Hevcca brasiliensis*, *Pongamia pinnata*, etc. Biodiesel can be mixed with petroleum diesel to form blends or used in B100 form (Neat biodiesel) (Azam et al., 2005). That’s why vegetable oils are in significant demand for biodiesel, but it's difficult to use edible oil for biodiesel production due to food and land problem. Fortunately, India has a greater portion of waste and unutilized public land that can be utilized for the cultivation of non-edible oilseeds. Moreover, these oilseeds can be grown on land available near the railway tracks, canals and roads (Kumar and Ram Mohan, 2005). It is evaluated that three-million-hectare land is required to deliver oil for 10 % substitution of petrodiesel (The Energy and Resources Institute, 2004). The waste extract of Jatropha after oil extraction can be utilized as natural fertilizers. The government of India signed memoranda of understanding with public and private sector firms to utilise public wasteland for Jatropha cultivation, despite of government incentives and different boosting policies, only few states actively participate (India Biofuels Annual, 2012). There are around 20 biodiesel plants in India having high capacity (10,000 to 200,000 Mt/year million ton per year) that utilize different feedstocks for biodiesel production, a large number of biodiesel plants are not in operating condition because of the unavailability of Jatropha (Biofuels in Asia, 2009).

1.3 Potential Sources of Biodiesel

There are more than 350 biodiesel feed-stocks (Atabani et al., 2012). The wide range of feedstock must fulfill the primary requirement, i.e. large production scale and low production cost. The primary feedstocks of biodiesel are edible oils, nonedible oils, animal fats and other sources as shown in Table 1.1. The oil yield and land occupied are essential parameters to consider any feedstock as a biodiesel source. First generation biofuels are biofuels made from sugar, starch, and vegetable oils. Edible oil resources are considered as first generation of biodiesel. However, edible oil resources such as rapeseed, soybeans, etc use much of arable land for cultivation and give rise to food versus fuel crises and create many environmental problems such as deforestation and destruction of various vital soil resources. So nonedible oil resources are gaining more attention as they are readily available. The main sources of non-edible oil are shown in Table 1.1. The non-edible oils and animal fats are regarded as the second generation of biodiesel feedstock. But second-generation feedstocks are not enough to satisfy the energy demand, and it is reported that the biodiesel produced from vegetable oil has very poor performance in cold weather (Atabani et al., 2012).

Table 1.1: Feedstock of biodiesel (Atabani et al., 2012)

Edible oils	**Non-edible oils**	**Animal fats**	**Other sources**
Soybeans	Jatropha curcas	Pork lard	Bacteria
Rapeseed	Mahua	Beef tallow	Algae (Cyanobacteria)
Safflower	Pongamia	Poultry fat	Microalgae
Rice bran oil	Camelina	Fish oil	Tarpenes
Barley	Cotton seed	Chicken fat	Poplar
Sesame	Karanja		Switchgrass
Groundnut	Cumaru		Fungi
Sorghum	Neem		
Wheat	Tobacco seed		
Corn			
Coconut			
Canola			
Peanut			
Palm			
Sunflower			

Recently, microalgae have received full attention and emerged as a third generation biodiesel feedstock. Due to photosynthesis algae convert sunlight, water and CO_2 (Carbon dioxide) to sugar. It is very popular because of high photosynthetic efficiency and high yield of oil compared to other feedstocks as shown in Table 1.2. The oil produced by microalgae is 25 times higher than the palm oil and 250 times higher than the soybean (Atabani et al., 2012) shown in Table 1.2. As compared to edible resources algae has shorter harvest time, and few algae can double their mass every 24 hours.

Table 1.2: Percentage of yield and oil content in different Feedstocks (Atabani et al., 2012)

Feedstocks	Oil content (%)	Oil yield (L/ha/year)
Microalgae (high oil content)	70	136,900
Palm oil	30–60	5950
Calophyllum inophyllum L.	65	4680
Coconut	63–65	2689
Euphorbia lathyris L.	48	1500–2500[a]

Pongamia pinnata (karanja)	27–39	225–2250[a]
Jatropha	50-60	1982
Jojoba	45–50	1818
Castor	53	1413
Olive oil	45–70	1212
Rapeseed	38–46	1190
Peanut oil	45–55	1059
Sunflower	25–35	952
Tung	16–18	940
Rice bran	15–23	828
Sesame	–	696
Soybean	15–20	446
Cottonseed	18–25	325
Corn (Germ)	48	172

[a] (kg oil/ha).

1.4 Biofuels from microalgae

Microalgae signify to a particularly differing yet profoundly specific gathering of microorganisms adapted to different biological environments. Algae use sunlight that changes CO_2 to useful items, for example, pigments, biofuels and chemicals etc. (Spolaore et al., 2006; Raja et al., 2007). Algae have simple structure due to which it is highly photosynthetic (6-8% efficiency) and grow at faster rates than other terrestrial partners (2.2 %). The concept of utilizing microalgae as a fuel is not new, but rather it is currently being considered important in India on account of the increasing cost of oil each month and more fundamentally, the developing worry about global warming that is nearly connected with the combustion of non-renewable energy sources (Omer, 2012). Algal biomass holds roughly 50 % carbon by dry weight; in this way, it is additionally used to create methane by the anaerobic process. The procedure is, in fact, achievable; however, it cannot compete with numerous other small price organic substrates that are already accessible for anaerobic digestion (Hussain et al., 2010).

Various microalgae species produce numerous types of lipids, hydrocarbons, and other complex oils (Guschina and Harwood, 2006). Microalgal oil methyl esters should follow existing biodiesel standards i.e. ASTM (American Society for Testing and Materials) biodiesel standard D6751 (Knothe et al., 2005). The degree of unsaturation of microalgal oil fatty acids having more than four double bonds can be reduced by catalytic hydrogenation of the oil. To harvest and extract oil from algal biomass is challenging (Jang et al., 2005; Dijkstra, 2006). Biodiesel from microalgae can be produced profitably. Using advanced technologies, biodiesel can meet the needs of environment and energy. Algal biodiesel can be produced without water reduction from the biomass of algae by the energy efficient process known as direct hydrothermal liquefaction (Patil et al., 2008). Microalgae biomass, whether it is aquatic, or terrestrial is thought to be a better option for renewable energy (Chisti, 2008; Raja et al., 2008). The photosynthetic productivity of aquatic microalgal biomass is substantially higher (6–8 %) than that of terrestrial partners (1.8–2.2 %) (Li et al., 2008). Numerous algae are rich in oil, which in turn can produce biodiesel. In some microalgae there is presence of lipids even more than 80 % of the dry weight (Chisti, 2007).

1.5 Transportation and energy consumption

Energy consumption in transportation sector increases at the rate of 1.4% annually; it will grow to 155 quadrillions Btu (british thermal unit) in 2040, which was earlier 104 quadrillions Btu in 2012 (IEO, 2016). Organizations for economic cooperation and development (OECD) have almost stable transportation energy demand, while non-OECD regions require higher transportation energy. In 2012, transportation energy consumption in developed nations was higher than developing parts, however, it is projected that in 2020 transportation energy demand will be equal. Throughout world for transportation energy, liquid fuels and petroleum are used as a major source, their use will decline according to IEO 2016 report from 96% to 88% by 2040, and this is because of increase in natural gas share in transportation sector that will be 11% in 2040 with reference to 3% in 2012. At present, all the transportation energy demand is fulfilled with liquid fuels, only 3% of demand is fulfilled with natural gas its share is very low worldwide, oil consumption increases by 36 quadrillions Btu, among them diesel including biodiesel has the largest percentage as shown in Figure 1.1. Gasoline remains the major source of fuel for transportation sector but by 2040 its use will decline and will be 33% by 2040 as shown in Figure 1.2.

1.6 Energy scenario in world context:

In developing countries, the energy demand is continuously increasing especially in Asia, rather than other markets in OECD. Primary energy consumption growth throughout world stayed low in 2016, and the fuel blending trends shifted towards low carbon fuels. Oil is world’s driving fuel, representing 33% of worldwide energy consumption increased by 1.6 million barrels for each day (Mb/d million barrel per day) China consumes 400,000 b/d (barrel per day) whereas India uses 330, 000 b/d, gave the biggest augmentation whereas worldwide oil production rate growth is 0.4 Mb/d, which is reported as the slowest growth since 2013 whereas the coal share in global market is 28.1% which still holds the second position after oil. Moreover, nowadays natural gas consumption increased rapidly, and the world is consuming 63 billion cubic meters (bcm) of gas. However, the gas production rate

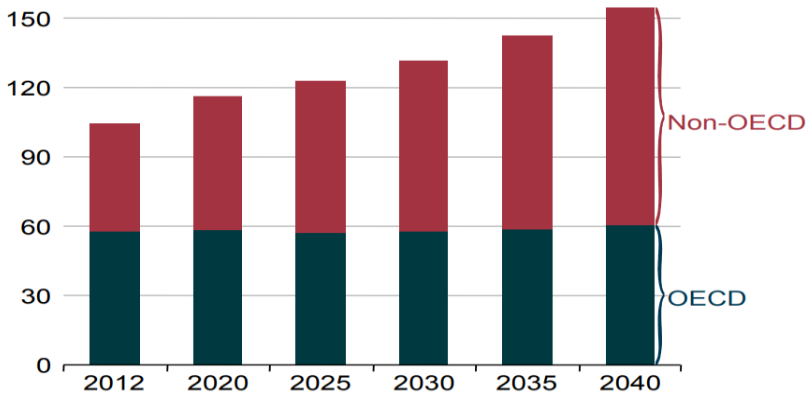


Figure 1.1: Energy consumption share in transportation sector by OECD and Non-OECD nations, 2012–40 (quadrillion Btu)

is very low which is only 21 bcm. In this way, the fossil fuel will soon exhaust. So, it's very important to find alternative sources of fuel.

1.7 Energy scenario in Indian perspective:

Indian economy highly depends upon agriculture, and agriculture further depends upon IC engine for irrigation purpose. Every year India imports approximately 0.3 Mb/d of oil to meet its demand. In 2016 utilization of India's primary energy increased by 5.4% and it is the third most energy consuming country in the world, for coal consumption it holds second position in the world. Moreover, oil consumption in 2016 also increased by 325 Kb/d that makes it third-largest oil-consuming country throughout the world (BP Statistical Review 2017). In few decades, the oil reserves will exhaust, in order to meet the problems of fuel scarcity and air pollution new alternative fuels must be explored. The government of India is focusing on strict national emission norms to move towards cleaner alternative fuels. Recent engines launched must meet the EURO-VI emission norms, the government of India is focusing on this. As India is rich in biodiversity, so we can use biofuels as an alternative to non-renewable fuels. Oil production and consumption in different regions is shown in Figure 1.3, 1.4.

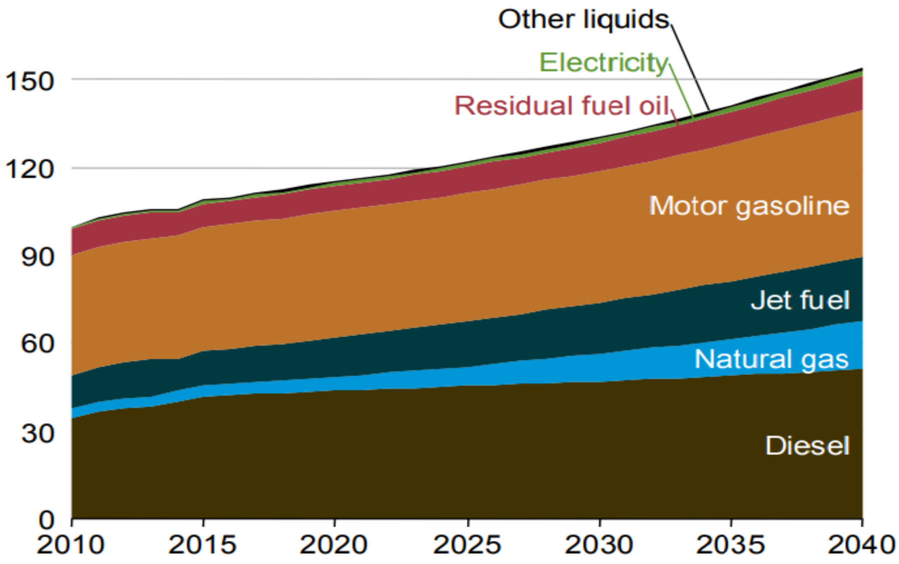


Figure 1.2: Energy consumption in transportation area throughout the globe IEO 2016 (in quadrillion Btu)

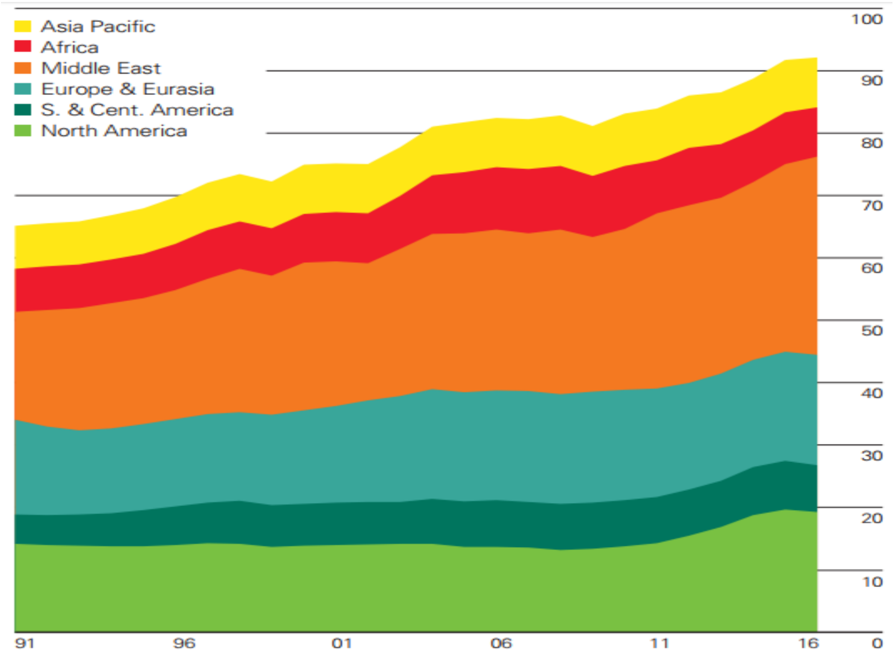


Figure 1.3: Oil Production by continents (Mb/d) (BP Statistical Review 2017)

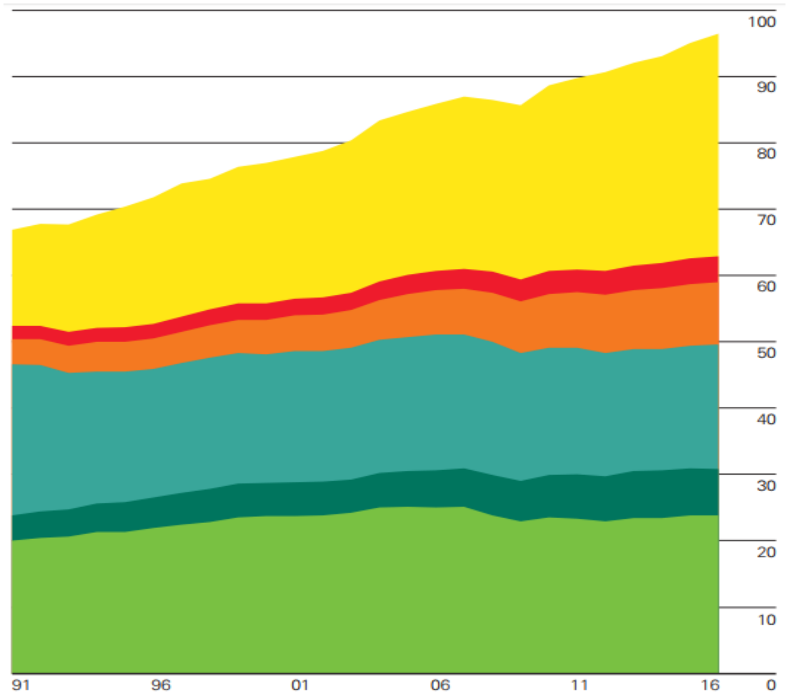


Figure 1.4: Oil Consumption by continents (Mb/d) (BP Statistical Review 2017)

1.8 Why biofuels?

According to BP Statistical Review of World Energy 2017 forecasting, the energy consumption in the world will increase by 32% because the population growth is estimated to 8.7 billion which in turns require 15 Mb/d more oil. These predictions give rise to various essential questions. Are we ready to handle the large demand of energy? How might we go up against environmental change as we solely depend on non-renewable fuels to meet the large demand?

This is the place biofuels can offer assistance. Within two decades, biofuels ought to show 20% growth in transport sector. BP is persuaded that a critical increment in the utilization of biofuels, if carefully chosen biodiesel feedstock having high biodiesel content, will help to decrease the worldwide emissions as well as reduce the greenhouse effect.

A large number of initiatives are being propelled in the entire globe. The EU (European union has focused on decreasing its pollution to at least 20% beneath 1990 levels by 2020. To satisfy the above mentioned goal is by increasing the usage amount of renewable energy sources, including biofuels. The US intend to increment biofuel utilization from 9 billion gallons to 36 billion by 2022. Biofuels as of now represent 2.5% of transport powers. In 2035 this number is relied upon to increment to 4%. At present Biofuels have 2.5% market share in transport sector, by 2035 this amount is estimated to be 4% and in addition expanding security in energy supply and empowering enhancements and advancement inside agriculture field, we are persuaded that this growth in biofuel utilization will promptly decrease CO_2 emissions related with motorway transport. Brazil set an example for whole world at present, 45% of energy utilized is produced from renewable fuels and 90% of its engine working on fuel blends such as gasoline with ethanol, that brings down the CO_2 emissions. Furthermore, biodiesel have low toxicity and biodegradable in nature, can be easily used as fuel for different applications such as boiler and IC engine. Apart from CO_2 emission reduction, sulphate emission and other environmentally dangerous chemical substances' emissions are almost negligible. The current development in biodiesel production depends upon three challenging key factors, energy security, economic development and environmental condition as explained below.

1.8.1 Energy security

The IEA (International Energy Agency) characterizes energy security as "the continuous accessibility of energy sources at a reasonable cost". It is very important to focus on long term energy security as well as short term energy security. In some cases, where price fluctuate according to the supply and demand such as oil market, the danger of physical inaccessibility is constrained to extraordinary occasions. Supply security concerns are principally identified with the financial harm caused by extraordinary value spikes. The worry for physical inaccessibility of supply is more common in markets where transmission frameworks must be kept in consistent balance, for example, power and to some degree, CNG gas. This is especially the case where there are limit requirements or where costs are not ready to fill in as a modification system to adjust supply for the time being. Guaranteeing energy security has been at the focal point of the job of the IEA since its initiation. The IEA incorporated new objective to its mission, to find and develop alternative energy sources so that dependency on oil import will decrease. Moreover, different countries also focusing on producing biodiesel from local feed stock to ensure energy security, such as Jatropha.

1.8.2 Economic development

Biofuel preparation from agricultural items in countryside regions has been broadly sought after as a technique for rural economic improvement. By increasing the production of biofuel in local areas, from different feedstocks can make new employment openings and create monetary spinoffs in countryside zones. This would be highly advantageous for developing nations having large amount of population in agriculture sector. Global economic development raises the living standard of people, which in turn increase the dependency on private or personal vehicle for travel, that consequently increase the energy demand. For all purposes of transportation 94% growth of energy consumption is projected for developing countries by 2040 (U.S. Energy Information Administration and International Energy Outlook 2016).

1.8.3 Exhaust emission pollution

During the combustion of diesel fuel large number of waste products and other emissions are exhausted to the surrounding environment, specifically nitrogen oxides (NOx), particulate matter (PM), sulfur dioxide (SO_2) and hydrocarbons (HC). These emissions badly effect the environment and result in some critical issues such as acid rain and global warming.

Nine nations and some other regions, which together account for 75% of worldwide fuel utilization by light vehicles, have followed compulsory standard for improving fuel combustion efficiency and to decrease GHG (greenhouse gas) emissions. The expectation and structure of the emission policies differ generally around the globe. Since mileage and GHG emission standards has major effect on fuel consumption, vehicle policies are among the most critical segments of future demand for fuel. One region of contrast is the metric quantified in the standard. Few standards concentrate on reducing greenhouse gasses or CO_2 emissions, some give attention on enhancing fuel efficiency (or lessening fuel consumption), and others concentrate on combination of both the aims. The EU and India have principles that particularly plan to decrease CO_2 emissions. Canada's emission standard incorporates limitations on all greenhouse gasses emissions. New substitute fuels for diesel oil have been world widely investigated because of the high oil costs, dangerous engine emissions and interrupted supply of diesel fuels.

Petroleum product usage is unsustainable, and it release greenhouse gasses. Looking for substitute fuel for different engine (e.g. renewable fuel) is a significant issue for better environment and sustainable fuel (Chandrasekaran et al., 2016; Venkanna and Venkataramana Reddy, 2012). In such manner, biodiesel fuels are superior alternative fuels for diesel. They are sustainable and made effortlessly from vegetable oils, animal fats, microalgae oil, and so on. Biodiesel have comparative properties like diesel, and their emissions are superior to diesel. Biodiesel can be produced from various feedstocks such as vegetable oils, algae oil and cooking oils. (Yilmaz et al., 2016; Pandey et al., 2016).

1.9 Indian Scenario in biodiesel

For developing countries such as India, it is very important to have sustainable energy supply for socio-economic development. To attain a sustainable growth, excess amount of energy availability is the major requirement. To meet the demand of oil, India highly depends upon oil imports which badly influence its economy. A coordinated approach will be needed to deal with the energy supply through its proficient use, conservation and uses of renewable fuels (Editorial Energy, 2009). So, finding new environment- friendly substitute fuel is very beneficial which will save our surrounding from poisonous gasses and it’s a great admiration to the environment (World Commission, 1987). The world is facing twin crisis of energy exhaustion and damaging of environment, so utilization of renewable fuels and to save surroundings and ecology are important issues to look after. In India the consumption of petro-products was 193.745 MMT in the year 2016-2017 (BP Statistical Review (2015)). India is at the third position with regards to consumption of oil and petroleum products from 2015 and still holds the same position. Up to 2016, the India had 600 MMT oil reserves. Oil consumption has increased at a Compound Annual Growth Rate of 2.98 % during 2008–17E (E- Estimated) to reach 4.13 mbpd (Million Barrels Per Day) by 2017 as shown in Figure 1.5 (BP Statistical Review (2015).

Demand of diesel will reach 100 MT by 2020, increase with 6% growth annually in its consumption and 90% of its demand totally depends upon import. The above data warns us to pay much attention so as to complete the demand of fuel in sustainable way without any damaging effect on surroundings. Diesel demand in India is very high among all the petroleum products, its shares are 40% which is quite a large figure, therefore some substitute fuel to diesel must be discovered to meet the high demand.

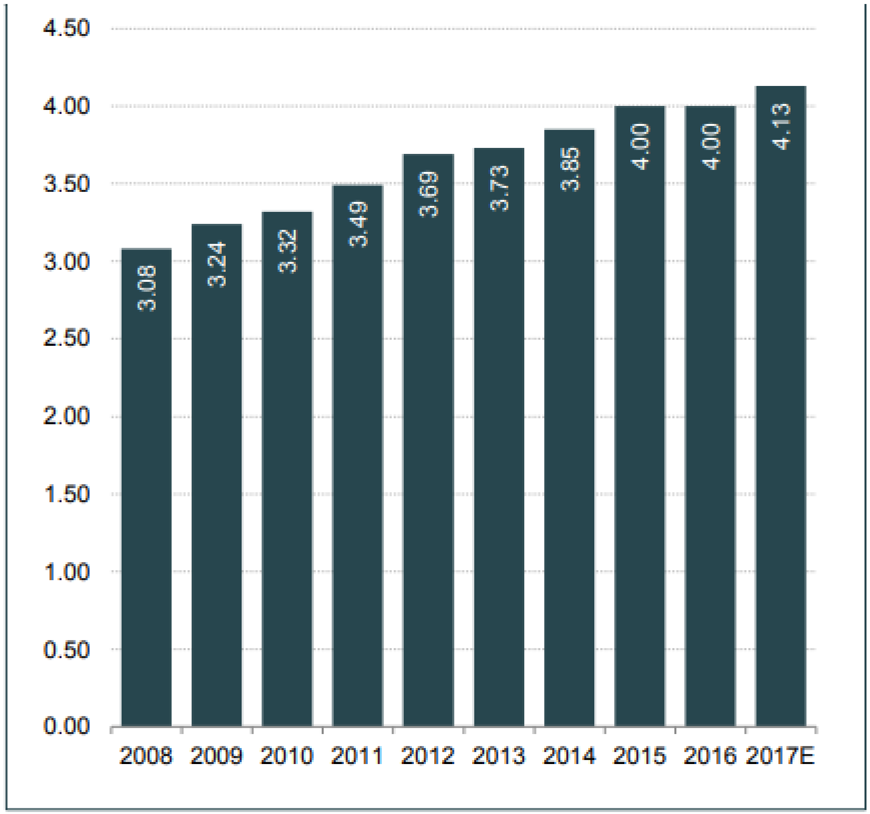


Figure1.5: Oil consumption in India (2008-17) (mbpd) (BP Statistical Review (2015))

It is expected that by 2032, the share of renewable energy in Indian market would be only 5 to 6% which is still very low, thus to meet the oil demand the country has to import fuel which is again a potentially big issue (Planning Commission Report, 2006). According to

India's National Biodiesel Mission 20% diesel demand will be met by biodiesel in the year 2016-2017. To meet the 20% biodiesel blend demand approximately 16.72 metric ton of biodiesel is required while for 10% blending the needed amount of biodiesel is 8.36 metric ton (Noiroj et al., 2009).

India faces a number of challenges for biodiesel production; farmers don't show interest in the cultivation of non-edible oil crops, due to uncertainty in crops yields as well as the reliability of feedstocks. Moreover, for the plants like Jatropha and Pongamia, the gestation period is long, approximately three years for Jatropha and five years for Pongamia crop which is too long, the farmer is not ready to wait so long for one crop. On the other hand, microalgae are the species which can be harvested on a daily basis; it does not require years to be grown and cultivated. That's why these days researchers are focusing on microalgae, but there are some constraints due to which the cost of algae oil is high, soon there will be some efficient methods by which we will accomplish this. To boost up algae oil production government ought to finance the biodiesel projects then ultimately the goal will be achieved.

1.10 Conclusion

Based on this chapter, it is observed that biodiesel from microalgae have a good scope for the India to get rid of energy crisis as well as environmental issues. By keeping this in mind, this work is focused on biodiesel production from algae and its use in CI engine.

Microalgae oil feedstocks are easily available throughout the globe and can be cultivated even in the wasteland, arid and semi-arid land, do not require fertile area, so does not displace food crops. The other primary nonedible oil feedstocks for biodiesel production are Kusum, *Madhuca indica, Jatropha curcas* etc. But these feedstocks have long gestation period as well as low yield. This research work aims to search alternative algae species rich in oil and to maximize the biodiesel yield.

1.11 Organization of the thesis

The thesis is divided into seven chapters. The sequence of the chapters is listed below.

Chapter 1 begins with the significance of energy in the Indian scenario. Gives alternative solution to fulfill the energy demand of transportation system and the potential of biofuels. From energy scenario, it is clear that more dependence of modern society on fossil fuels may result in the depletion of fossil fuels in the next sixty years. It is a well-known fact that fossil fuel constitutes a finite resource so that biofuel can be an alternative source of energy which can replace petroleum based fossil fuels. Biofuels are organic combustibles derived from biomass. These are mainly used for combustion to produce heat for industrial processes, electricity generation and heat engine carburation in internal combustion engines. Microalgae, edible and non-edible sources are used as a feedstock for biofuel. In the present scenario, although biodiesel can not entirely replace petroleum-based fuels, there are various advantages of biodiesel over diesel fuel. It is biodegradable, more than 90% biodiesel can be biodegraded within 21 days. Cetane number and combustion efficiency are higher for biodiesel than in the case of diesel fuel. The Sulfur and aromatic content is lower in case of biodiesel, i.e. use of biodiesel for the combustion process in engines do not produce toxic emission gases. Additionally, it reduces most exhaust emissions such as carbon monoxide, unburned hydrocarbons, and particulate matter except oxides of nitrogen (NO_x). So, biodiesel can provide an alternative solution to avoid the problems of petro-fuel crisis and environmental degradation.

Chapter 2 Literature review based on microalgae cultivation, lipid extraction, biodiesel properties and CI engine performance using biodiesel blend is presented in this chapter. This chapter also discusses the literature gap and limited use of algae biodiesel in diesel engine. Moreover, the literature has been classified into the following categories.

The first part discusses the literature related to culturing and harvesting of microalgae and species selection and its culturing conditions. The second section reviews the literature related to the biodiesel production and transesterification. The third section discusses the literature related to the performance and emission characteristics of diesel engine run on biodiesel.

Chapter 3 The research methodology used in present research work is discussed in this chapter. This chapter explains the methods that are used to gather all the information to complete the research. Algae species-rich in oil was cultivated and harvested, after harvesting oil was extracted using soxhlet apparatus. Further crude oil properties are measured using different instruments. Moreover, transesterification was performed using different instruments that are included in this chapter. The instruments used to determine the biodiesel properties are also tabulated in this chapter.

Chapter 4 Explains the microalgae species selection, growth, and harvesting of algae. The microalgal species *N. oleoabundans* is grown at different conditions using a different amount of substrates. The investigation was performed in a lab at Delhi Technological University, Delhi. Initially, Bristol medium was used to maintain a culture in small test tubes and after that scaled up to 2 L conical flask, and then media was put into larger volume flask.

Chapter 5 Explains the oil extraction and biodiesel production using different technologies from conventional to advanced techniques, such as mechanical stirring, ultra-sonication and soxhlet extraction method. In this work microwave was used to disrupt the cell wall in order to carry out two-step indirect-transesterification (ID-Trans). Lipids were extracted from dry algae using different solvents; Algal cells were disrupted with the help of microwave reactor. Lipids were collected and weighed.

Chapter 6: Laboratory engine setup and test procedure for the engine performance and emissions testing is discussed in this chapter. Also, the results obtained from engine testing of microalgae biodiesel are presented. The performance of algae biodiesel compared with petrodiesel. Experiments were performed on four-stroke single cylinder water cooled engine coupled to a dynamometer. Cylinder pressure and crank angle were measured by various instruments attached to setup. Engine indicator interfaced signals to a computer that plot P-V and P-θ diagram. Air flow, fuel flow, load and temperature were interfaced to computer through different sensors. The performance parameters such as brake specific fuel consumption (BSFC), brake thermal efficiency (BTE) are investigated. To evaluate performance, engine software "Engine soft LV" was utilised, and for testing of smoke opacity, AVL DIX was used.

Chapter 7: This chapter discusses the conclusion of the thesis from the research work. Possible direction for further research are also discussed in this chapter.

Chapter 2. Literature review

2.1 Introduction

A substantial amount of work has been done on various aspects of biodiesel, some part is discussed below. The literature has been classified into the following categories.

The first part discusses the literature related to culturing and harvesting of microalgae and species selection and its culturing conditions. The second section reviews the research related to the biodiesel production and transesterification. The third section discusses the research pertaining to the performance and emission characteristics of diesel engine run on biodiesel.

2.2 Cultivation of Microalgae

Neochloris oleoabundans is fresh water, unicellular microalgal species which was isolated originally from the surface of sand dunes in Saudi Arabia (Chantanachat and Bold, 1962). It is green alga having rounded shape with 3-10 μm diameter which depends on its phase of growth and environmental conditions. The dominant cell type of this microalgae is a vegetative coccoid cell with a single nucleus. The mode of reproduction is asexual, in which it releases zoospores from enlarged mother cells to complete its cell cycle (Da Silva et al., 2009; Loera-Quezada et al., 2011).

Microalgae with high oil content are required for producing biodiesel. Microalgal biodiesel can be produced commercially. It is reported that *N. oleoabundans* accumulate up to 30-50% total lipids of its dry mass, 80% includes neutral TAGs (Triacylglyceride) (Tornabene et al., 1983; Li et al., 2008; Gouveia et al., 2009). Oleic acid (C18:1) predominates among all the long chain fatty acids produced, which is the main fatty acid for biodiesel production. (Tornabene et al., 1983; Gouveia et al., 2009; Santos et al., 2012; Yang et al., 2013, Knothe, 2008). Hence *N. oleoabundans* is a potential oleaginous microalgal species which can be used for the production of biodiesel.

An adequate amount of light intensity, temperature, CO_2, H_2O (water) and inorganic salts are needed to grow microalgae. The metabolism and growth of microalgae depends upon both the light intensity as well as light quality. Light intensity affects both cell size and cell density. The spectral quality determines the physiological as well as biophysical microalgal properties. Generally, microalgae tolerate greater light intensity around their optimum growth temperatures. (Borowitzka, 1998; Carvalho et al., 2011).

Carbon dioxide acts as a source of carbon supply in photosynthesis which is converted to algal biomass. Therefore, light intensity, light quality and concentration of CO2 have a significant impact on photosynthesis. For example, in case of three oleaginous microalgal species, the demand for light raised accordingly, when they were provided 10% (v/v) CO_2. (Yoo et al., 2010). Increasing concentration of CO_2 in the air affects growth as it leads to higher biomass. For instance, when *N. oleoabundans* was provided with 5% CO_2 in air (v/v), the exponential growth phase occurred earlier in comparison to cells that were only provided with ambient air; also, the doubling time decreased from 2.2 days to 1.4 days with increased CO_2 concentration. (Gouveia et al., 2009).

In algae culture, temperature also plays an important role in growth as many physiological activities and the enzymatic reactions depend on temperature. Lipid and biomass highly depend upon the temperature, it varies from one species to another. The temperature below optimal conditions help to sustain the effectiveness of light harvest; thus algae typically boost the photosynthetic enzymes production. On the other hand, when temperature was above optimum condition, carotenoids were produced that further relieves the oxidative stress due to high temperature (Richmond, 2004). Moreover, with temperature variations some other effects were also observed. For example, variation of algal size. At optimum temperature, algae prevent excess utilization of Carbon and Nitrogen by minimizing the cell size. (Goldman and Mann, 1980; Richmond, 2004; Sivakumar et al., 2012).

Essential elements for its growth are N (Nitrogen), P (Phosphorus) and Fe (Iron). Nutritional requirement of microalgae can be estimated using the approximate molecular formula of microalgal biomass; ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) which is based on data presented by (Sudhakar and Premalatha, 2012). Continuous culture during daylight is used for large-scale production of microalgae. In this method of operation, the fresh culture medium is fed at a constant rate, and the same quantity of microalgal broth is withdrawn continuously (Grima Molina et al., 1999). Feeding ceases during the night, but the mixing of broth must continue to prevent settling of biomass (Grima Molina et al., 1999). Figure 2.1 shows the culture harvesting, and biodiesel production process. Practically the methods used for large-scale microalgae production are raceway ponds (Grima Molina et al., 1999; Kenneth and Lawrence, 1985) and tubular photobioreactors (Grima Molina et al., 1999; Tredici, 1999).

2.2.1 Open Pond System

Open pond system is used widely nowadays because it is easier to construct and has low economic as well as operational cost. As algae growth depends upon sunlight that is freely available in open pond system. Open system comprises lakes, ponds and raceway ponds. Open pond system is widely used but still they depend upon prevailing climatic conditions (cloudy days, change of season and rainfall). Furthermore in open pond system predators attack effect the production.

2.2.2 Raceway Ponds

Raceway ponds are made in concrete and have closed loop recirculation channel that is about 0.3m deep. Algae broth and nutrient mixing is produced by paddle wheel. Behind paddlewheel on completion of the loop algae broth is harvested. To prevent sedimentation paddlewheel operates all the time as shown in Figure 2.1.

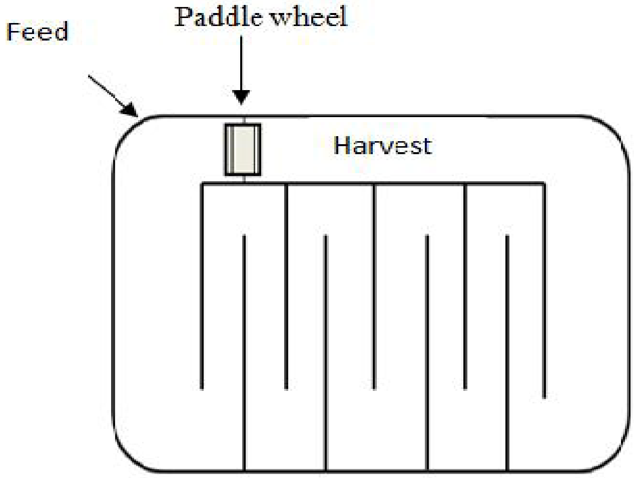


Figure 2.1: View of a raceway pond

2.2.3 Photobioreactor

Some species of algae do not grow in open system, so closed system which is free from contaminants such as heavy metals and microorganisms is used for them. The basic designs are Tubular photobioreactors, Flat bioreactor and Column photobioreactor. Tubular photobioreactor is generally made of transparent plastic or glass. Photobioreactor can be vertical, horizontal, inclined or conical shaped. Pump is used to circulate algae through the tube (Figure 2.2). Solar collector is used to collect the sunlight. The diameter of solar collector tube is less than or equal to 0.1m to ensure light productivity. Diameter is limited because light do not penetrate too deeply in the dense culture. Microalgae is circulated from degassing column to solar collector and back to degassing column. Tubes are arranged horizontally or in the form of helix and are oriented from north to south. The ground is covered with white sheet of plastic (Tredici, 1999) to increase reflectance. To prevent sedimentation in tubes the flow is maintained as highly turbulent. The mixing is done by mechanical pump or airlift pump. Mechanical pumps can damage the biomass (Chisti, 1999; Garcia et al., 2001 and Garcia et al., 2007) but are easy to operate and handle.

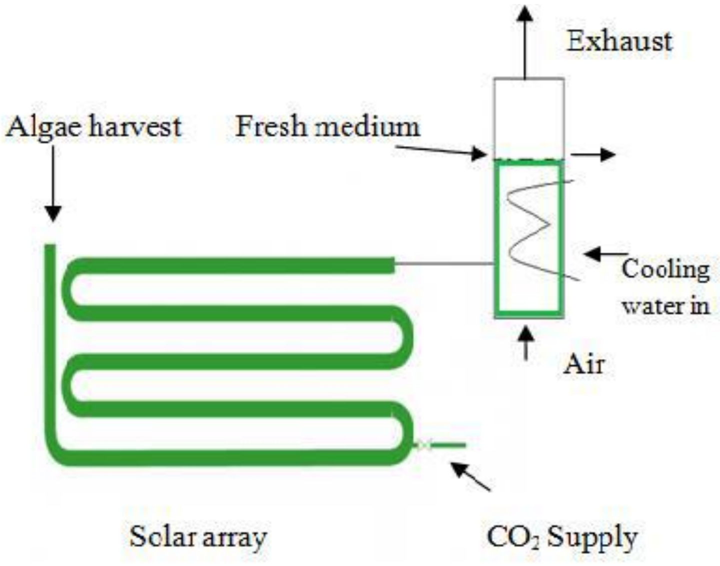


Figure 2.2: Tubular photobioreactor

Liang et al [2009] investigated biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Under autotrophic conditions, higher lipid productivity was obtained which was 38%, whereas it was much lower with heterotrophic mode of growth using acetate, glucose or glycerol. With the use of 1% (w/v) glucose, optimal cell growth i.e. 2 g/l and 54 mg/l/day lipid productivity were obtained. At high concentrations, both glucose and glycerol inhibit growth. Because *C. vulgaris* is mixotrophic, it produces highest lipid productivity while growing on glucose with light.

Devi et al [2012] used domestic wastewater as a source of nutrients and investigated the effect on heterotrophic growth and lipid production of microalgae during sequential growth phase and starvation phase. On the growth of biomass, the effect of various nutrients such as carbon, nitrogen, phosphorus and potassium were studied in which it was shown that availability of nutrients during growth phase increases the biomass but due to nutrient unavailability during starvation phase the formation of triacylglycerides increases, which further increment lipid productivity. It was observed that during sequential growth phase, highest growth in biomass occurred with maximum nitrogen and phosphorus which was 1.69 mg/ml, but during starvation phase, higher lipid produced with maximum 28.2% C concentration. Variable fatty acid profile with abundant saturated fatty acids was found. Good waste water treatment efficiency was elucidated in relation to removal of nutrients and substrate degradation catalyzed by mixed microalgae which shows positive method for treating and cultivating algae in low toxic waste water. Thus, above discussed method is a low cost and highly effective method. Thus dual mode operation ensures increased biomass and lipid productivity which can be applied for up scaling in a cost-effective way. Figure 2.3 shows the complete biodiesel production process.

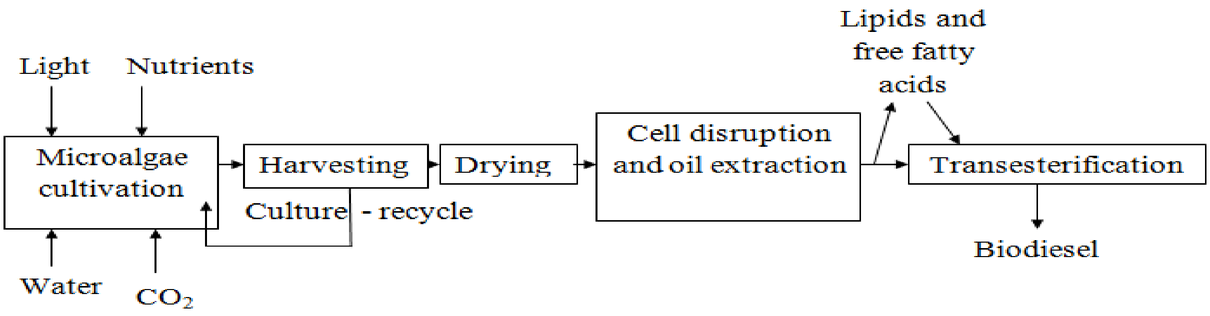


Figure 2.3: The process is showing cultivation, harvesting and biodiesel production from microalgae.

2.3 Harvesting

The cost of biomass recovery from the broth can make upto 20 – 30% of the total cost of producing the biomass. Microalgal biomass can be harvested physically, chemically or biologically. Various combination of these methods is also common (Bernhardt and Clasen, 1991; Danquah et al., 2009 and Kumar et al., 1981). There is no proven single best method of harvesting microalgae (Shelf et al., 1984).

2.3.1 Centrifugation

Separation of algal cells from medium is a difficult task because of smallest size of the algal cell. Mixtures are separated by spinning in a centrifuge. Centrifugation equipment are either fixed wall devices (hydrocyclone) or rotating wall devices (sedimenting centrifuges) (Shelf et al., 1984). The general types of centrifuges used are multichamber centrifuge, Disc – Stack Centrifuge and Decanters. Nozzle type of centrifuges are easily sterilized and cleaned but have high operating cost (Shelf et al., 1984). In multi chamber centrifuge the feed is passed through vertical concentric cylinders rotating at high speed. Larger particles deposit at the inner chamber and finer particles at the outer chamber (Mohn, 1980). Disc stack centrifuge consist of disc stack plate, liquid is collected at the top and solids are removed under gravitation (Shelf et al., 1984; Oswald, 1988). Decanter centrifuge have screw conveyor (rotating at slow speed) fixed with solid cylindrical bowl (rotating at high speed).

2.3.2 Filtration

Filtration is method of harvesting suitable only for large size microalgae. The different type of filtration process available are Tangential Flow filtration, Vacuum filtration, Dead end filtration, Ultrafiltration, Microfiltration and Pressure filtration. Generally broth is passed through the filters. It allows the medium to pass and accumulate the algae. Small size microalgae is also harvested by using diaphragm filter (Borowitzka M.A. and Borowitzka L.J., 1988). Microalgae having coarse particles are harvested by vacuum band filters. Sand filters cannot operate continuously because after some interval of time they have to be rinsed off. Sand filters that are modified can be cleaned continuously by mammoth pump (Borowitzka M.A. and Borowitzka L.J., 1988). Sedimentation is the tendency of the particle to settle down the fluid under the gravity. Benefits of sedimentation are low manpower requirement, low design cost, low power requirement.

2.3.3 Floatation

In floatation method algae is made to float on the surface of the medium and removed as scum. The floatation methods that are generally used are Froth floatation, Dissolved air floatation, Bio floatation. In froth floatation method air is bubbled through column to create froth, so that algae is accumulated above the media. Smaller the air bubble, higher is the floatation efficiency. Because of lower buoyancy in froth floatation bubble size is large, making it less efficient. The stability of air microalgae mixture is governed by the pH of the culture. The pH required depends on different species of algae. This is not suitable because of high cost and lower floatation efficiency. In dissolved air floatation algae is harvested by passing stream of air bubbles. Adhesion of particles to bubbles is required, so chemical flocculants are used. The process is efficient if micro-fine bubbles are introduced but this increases the cost. Use of floatation method is limited due to limited evidence of economic and technical viability (Brennan and Owende, 2010).

2.3.4 Flocculation

Harvesting of the algae depends upon the effective particle size. Chemical flocculants are added to increase the particle size so that another method like floatation and filtration are used to harvest the algae. In the suspension, microalgae have a negative charge that prevents them from self-aggregation. Addition of flocculants reduces or neutralizes the negative surface charge. To flocculate the cells, electrolytes and synthetic polymers are added (Bernhardt and Clasen, 1991).

For charge neutralization ferric cations, aluminium sulfate is often used because they have +3 charges. Natural polymer and cationic starch are also used as flocculants (Pal et al., 2005). Adding cationic polymers and multivalent cations as flocculants, surface charge can be reduced or neutralized (Grima Molina et al., 2003). Flocculants should be non toxic and inexpensive.

2.3.5 Auto Flocculation

The pH of water increases due to photosynthesis in microalgae. Phosphate, calcium and magnesium ions are precipitated with an increase in pH of water (Becker, 1994). These salts have a positive charge so can neutralize the negative charge on the microalgae and flocculate the algae. For auto flocculation pH should be above 10.

2.4 Method for extraction of lipids

Microalgae include polar lipids and neutral lipids. These lipids must be extracted from the cell and collected. The cell disruption can be achieved by chemical and mechanical disruption, sonication, super critical fluid extraction, solvent extraction and combination of these techniques (Lee et al., 2010).

2.4.1 Mechanical cell disruption method

Mechanical disruption is utilized at large processing, homogenization and bead milling (Mercer and Armenta, 2010 and Greenwell et al., 2009). Mechanical disruption maintains the chemical integrity of the substance and contamination from the external sources is minimized. In mechanical pressing high pressure on the microalgae is applied which rupture cell wall and oil is extracted. Mechanical pressing is used to extract oil from plant seeds, but this method can be applied to microalgae (Mercer and Armenta, 2010).

In homogenization, cell wall is ruptured by forcing the biomass to pass through a small orifice at high pressure. At the opening of the orifice large pressure drop and liquid shear forces rupture the cells allowing the lipids to be extracted (Greenwell et al., 2009). Bead milling is packed with very small beads which agitates the algal biomass resulting in cell disruption. This technique is used for size reduction and for the disruption of cells (Doucha and Livansky, 2008). Mechanical force of the beads pulverizes the algal cells and helps in extracting the lipids (DOE, 2010). Degree of disruption depends upon the contact between beads and biomass, size, shape and strength of microalgal cell walls. For the extraction of lipids most effective and simplest method is use of microwave by (Lee et al., 2010) but this technique is very difficult to use for industrial scale (DOE, 2010).

2.4.2 Solvent extraction method

This technique is very effective for lipid extraction from vegetable seed as well as from microalgae (Russin et al.,2010). This is because of high solubility of lipids in organic solvents such as hexane, chloroform, petroleum ether and benzene (Ahmad et al., 2011). To extract lipids from microalgae, five-step mechanism is proposed by (Halim et al.,2011) shown in Figure 2.4. The solvent enters the cytoplasm by penetrating the cell membrane. 2: The non polar lipids interact with the non polar solvent, as both are non polar there exist van der wall forces. 3: Non polar solvent and lipid form a complex. 4: This complex diffuses across the cell membrane. 5: Organic solvent lipid complex diffuses through the static film into the bulk solvent.

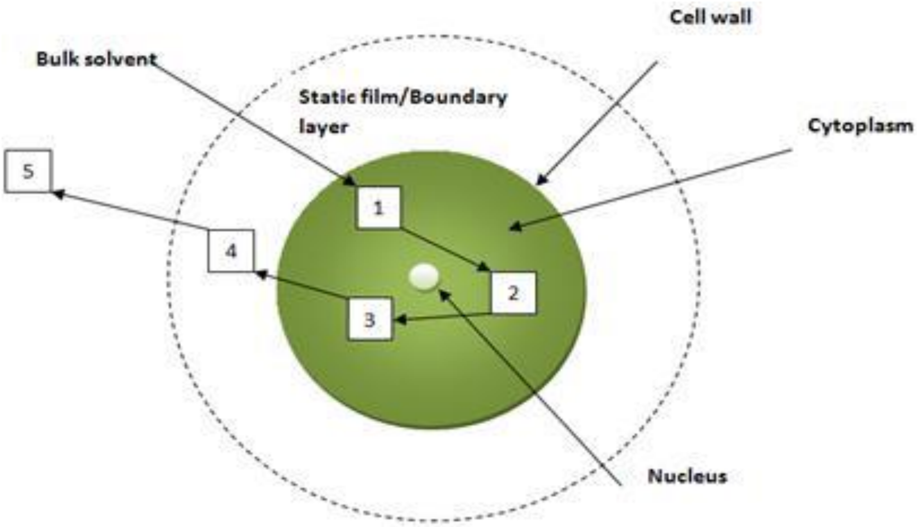


Figure 2.4: Schematic diagram shows mechanism for lipid extraction

Number of techniques exists for extraction such as Bligh and Dyer, Folch extraction and Gold Fisch (Bligh and Dyer, 1959; Folch et al., 1957 and Gloria et al., 1985). The Bligh and Dyer technique use chloroform-methanol solvent to extract lipids from fish tissue. Bligh and Dyer method is modified for the extraction of lipid from the algae and found to recover 70% of total lipids (Enssani, 1987). To obtain optimum result biomass should be dried. If the water is not removed it interfere by shielding lipids from solvent (DOE, 2010; Halim et al., 2011; Converti et al., 2009; and Young et al., 2010). But drying of biomass requires large energy (Lardon et al., 2009). In contrast, ideal extraction method should be safe, non-toxic, inexpensive and have low energy requirement. In industries, alkanes and short chain alcohol are commonly used for lipid extraction. Hexane an ideal solvent to extract edible oil is an alkane hydrocarbon with chemical formula C_6H_{14}. It is nonreactive and nonpolar. Hexane solvent extracts a fraction of lipids available in algae depending on the lipids present in biomass (Halim et al., 2011). Research efforts are being made to determine effective solvent capable of removing maximum lipid from algae (Lewis et al., 2000 and Dufreche et al., 2007).

2.4.3 Supercritical fluid extraction method

Nowadays study is focused on supercritical extraction of lipids because of shortcoming associated with organic solvent-based lipid extraction method (Carrapiso and Garcia, 2000). This method typically uses carbon dioxide. When CO_2 heated beyond critical pressure and temperature, to its supercritical state it exhibits the characteristic of both liquid and gas, making it more diffusive with low viscosity (Herrero et al., 2006 and Gong and Jiang, 2011). Because of this the CO_2 penetrate solids and extract the target molecules. CO_2 is used as supercritical fluid because of relatively low critical temperature (31.10^0C) and pressure (72.9 atm) (Herrero et al., 2006 and Mercer and Armenta, 2010). Extraction efficiency of supercritical CO_2 is affected by four main factors, i.e., temperature, pressure, extraction time and CO_2 flow rate. Use of modifiers (ethanol as a co - solvent) can increase the polarity of the extracting solvent, subsequently viscosity of fluid is altered. Use of CO_2 for the extraction of algae lipid have numerous advantages. Lipid extraction by solvent requires large volume of toxic and hazardous compounds and organic solvents are not required (Carrapiso and Garcia,2000). Supercritical extraction method uses only CO_2 as extracting solvent (Carrapiso and Garcia, 2000 and Halim et al., 2011). CO_2 can be easily removed as it act as a gas at ambient condition leaving behind a pure extract (Mercer and Armenta, 2010).

2.4.4 Ultrasonic extraction

In many species of algae, cells are resistant to solvent extraction, requiring pre treatment to degrade the cells to remove lipids (Grima Molina et al., 2003; Lee et al., 2010; Gouveia and Oliveira, 2008 and Singh and Gu, 2010). After cell degradation lipid yield increases (Lee et al., 2010; Converti et al., 2009 and Widjaja et al., 2009). So ultrasonic cell disruption method is used. Ultrasonic waves are applied with high frequency and energy intensity to a solution containing cell culture. These waves create cavitation bubbles in a solvent material, when these bubbles collapse it creates small regions of extremely high pressure which shear cell walls apart (Chaplin, 2004). It is an effective method of destroying cell walls at laboratory scale (U.S. Department of Energy, 2009). This method requires a large amount of energy per unit volume. So, it is not practically possible for an industrial scale method of cell disruption (Chisti and Moo-Young, 1986).

2.4.5 Soxhlet extraction

Soxhlet extraction is the oldest approach widely used for extraction of solid samples. It consist of a simple distillation process repeated a number of times. In this approach fresh solvent is always in contact with the sample phase which enhances the displacement of target compound from the matrix. The extraction procedure is divided into three stage boiling, refluxing and recovery. The advantage of soxhlet extraction method is that the compounds are not discomposed due to moderate extraction condition (Lee et al., 2010).

2.5 Biodiesel production

Once the lipids are extracted they must be converted to biodiesel. Biodiesel production from any parent oil is performed via a chemical conversion process known as transesterification

(esterification for FFA (Free Fatty Acids)) (Figure 2.5). The oils which are originally in the form of Triglycerides, complex lipids or FFA are converted to alkyl esters, which has physical and chemical resemblance with petroleum base diesel (Meher et al., 2006). Transesterification can be performed by reacting lipids with alcohol with or without the presence of a catalyst (Demirbas, 2006). The various steps followed in transesterification are: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol (Chisti, 2007). R is a mixture of fatty acid chain. Alcohol generally used for transesterification is methanol as it is cheap. Ethanol and Iso-Propanol may also be used, results in biodiesel with better fuel properties.

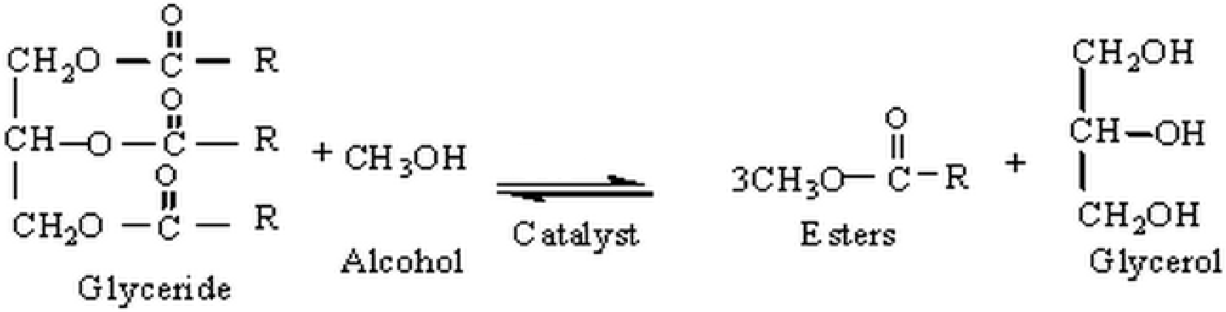


Figure 2.5: Transesterification of oil to Esters.

The catalyst used for transesterification process can be alkali catalysts, acid catalyst, enzyme and heterogeneous catalyst. The basic catalyst commonly used include sodium hydroxide (NaOH), potassium hydroxide (KOH), Potassium methoxide ($KOCH_3$), and sodium methoxide ($NaOCH_3$) (Singh et al., 2006). The acid catalyst commonly used are hydrochloric acid, sulphuric acid and sulphonic acid. The catalyst whether they are alkaline or acidic, they all are homogeneous catalyst which mean the reactants and catalyst are in the same phase. In general, acid catalyzed transesterification is performed at low to moderate temperature and pressure. Acid-catalyzed reaction require high acid catalyst concentration to obtain the best product yields with reasonable reaction time. Acid catalyzed transesterification for sunflower oil is carried at low temperature and pressure, 200:1 molar ratio under these conditions reaction takes 4h to complete (Marinkovic Siler and Tomasevic, 1998). Acid catalyst is generally used because of its insensitiveness to free fatty acid in the feedstock but they are largely ignored because of the relatively slow reaction rate. The concentration of free fatty acid can change according to type and quality of lipid feedstock used (Meher et al., 2006 and Canakc and Van Gerpen, 2003). Fatty acids react differently depending on acid or base is used as catalyst (Marchetti et al., 2007). In alkali-catalyzed transesterification alkalis such as potassium and sodium hydroxide are the two types of most commonly used catalyst at a concentration from0.4 to 2% w/w of oil (Freedman et al., 1984). The main drawback of base-catalyzed transesterification is that the oil containing free fatty acids cannot be converted into biodiesel. The free fatty acid reacts with base catalyst to produce soaps that inhibits the separation and production of biodiesel (Georgogianni et al., 2008). The advantage of alkali-catalyzed transesterification is that it proceeds faster than the acid catalyzed reaction (Fukuda et al., 2001).

There are many feedstocks from which biodiesel is obtained. This oil cannot be directly used to run engine due to high viscosity and low volatility which leads to injector coking and engine deposit (Perkins and Peterson, 1991; Scholl and Sorenson, 1993). However this problem is eliminated by transesterification of oil to alkyl ester (Perkins and Peterson, 1991; Zhang et al., 1988).

Transesterification is also called alcoholysis. Transesterification is reversible reaction in which triglycerides are converted to di-glycerides and to mono-glycerides which finally gives glycerol. Biodiesel floats at the top while glycerol sinks to the bottom which is separated easily (Atabani et al., 2012). In transesterification methanol and ethanol is mainly used as alcohol due to low cost. However, octanol, propanol, butanol and tert butanol can also be used but their cost is higher as compared to methanol and ethanol (Shahid and Jamal, 2011; Balat and Balat, 2010; Demirbas and Demirbas, 2007; Fukuda et al., 2001). If methanol is used in reaction, then process is called methanolysis. General equation for transesterification (Meher et al., 2004). This reaction generally takes place in presence of catalyst which may be acidic or basic in nature as alcohol is scarcely soluble in oil, so catalyst increase the solubility, thus accelerates the reaction (Atabani et al., 2012). The transesterification process removes the glycerin, so viscosity decreases but heating value and cetane number does not change (Awolu and Layokun, 2013).

2.5.1. Kinetics of transesterification reaction

The oil from which biodiesel is produced is known as triacylglyceride (TAG). TAGs are formed by covalent bonding of carboxylic acid with alcohol. In this context, TAG is an ester formed by combining three molecules of fatty acids covalently bonded with glycerol molecule (Gaurav et al., 2013). Fatty acid has carboxylic group while glycerol has three hydroxyl groups, which while combining form ester or TAGs. Transesterification is a chemical process in which carboxylic acid ester is converted into different carboxylic acid esters.

2.5.2. Base-catalysed transesterification

Base catalysts are mostly used for transesterification of vegetable oils (Meher et al., 2006; Kaeida et al., 1999; Antolin et al., 2002; Srivastava and Prasad, 2000; Zhang et al., 2003).

When the triglyceride contains free fatty acids or excess amount of water then acid catalysts are used to reduce the soap formation (Kaeida et al., 1999; Zhang et al., 2003; Freedman et al., 1984). Base catalyzed transesterification reaction is 4000 times faster than acid catalyzed reaction, but it is used only if triglyceride contains less than 2% free fatty acids (Sharma and Singh, 2008). Sodium and potassium hydroxide are mostly used for industrial purpose.

2.5.3. Mechanism of base-catalyzed transesterification

The transesterification using base catalyst involves four steps, pre-step or first step in which base reacts with alcohol and form protonated catalyst and an alkoxide. In the next step carbonyl group of oil is attacked by nucleophile and forms intermediate (Taft et al., 1947; Guthrie, 1991; Meher et al., 2006). In third step alkyl ester and anion of diglyceride are formed. In fourth step the catalyst deprotonates, thus regenerating the base which again reacts with second molecule of alcohol and starts another cycle (Figure 2.6).

Base catalysts are mostly used because reaction takes place at low temperature and pressure that is 60^{o}C and 20 psi and obtain high yield about 98%. However, there are some shortcomings, i.e. it requires high energy, to separate the catalyst from the media after transesterification pro-reaction treatment is required, difficult to recover glycerol after the reaction moreover it forms soap with free fatty acids.

2.5.4. Factors affecting base catalyzed transesterification.

Effect of alcohol to oil molar ratio: the yield of methyl esters generally depends upon methanol to triglyceride molar ratio. Theoretically three moles of methanol are required per mole of oil for transesterification. A vegetable oil (Prafulla and Deng, 2009) studied the amount of alcohol required for transesterification of vegetable oil in terms of alcohol to oil molar ratio.

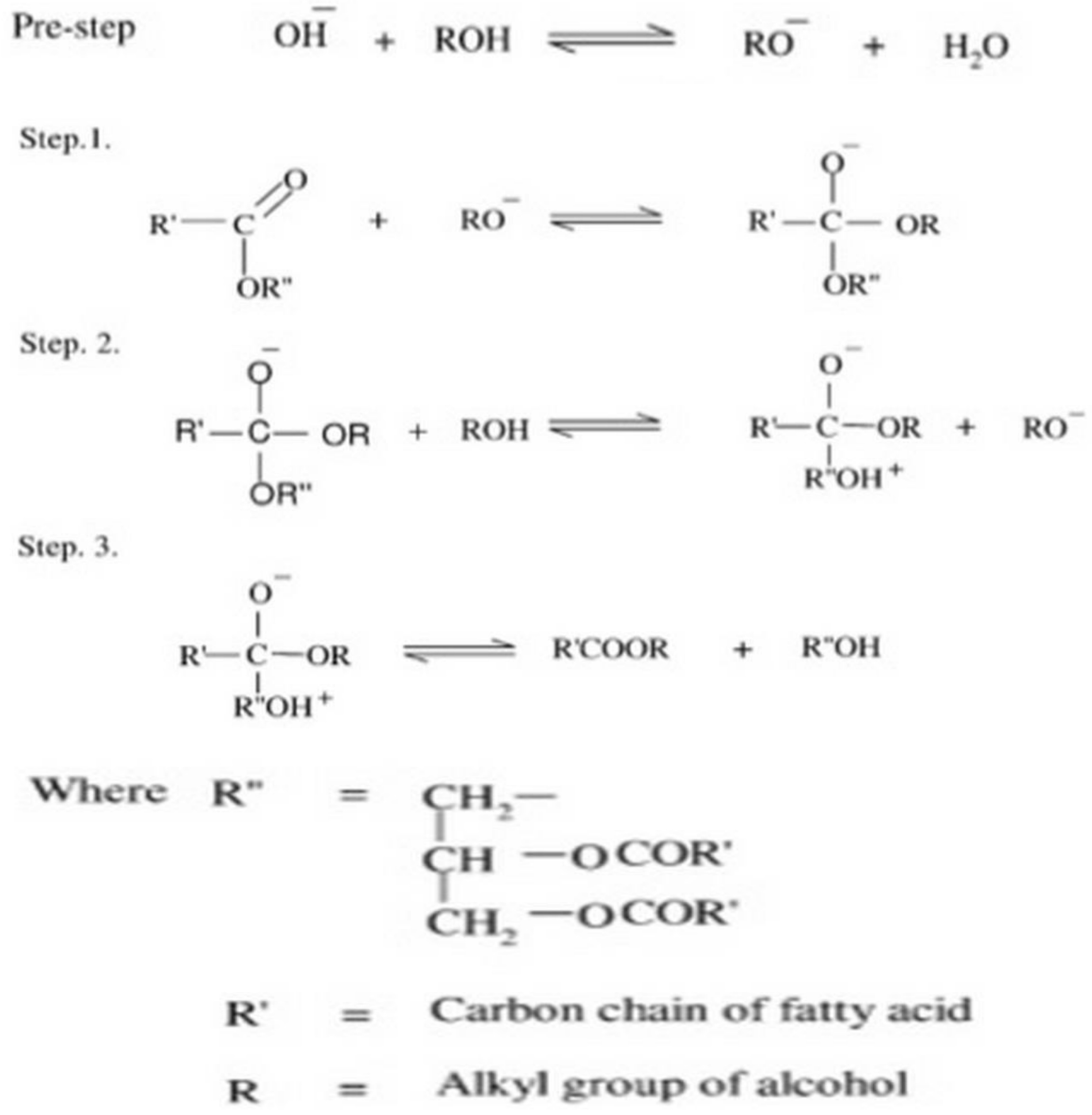


Figure 2.6. Mechanism for base catalyzed transesterification reaction

Shazia sultana et al., (2014) studied transesterification on five different molar ratios in the range 2:1 to 10:1 and obtained maximum yield 92% with 6:1 methanol to oil molar ratio. On further increase in methanol to oil molar ratio the ester yield decreases.

Encinar J. M. et al., (2002) studied different ethanol to oil molar ratio between range 3:1 to 15:1 for the transesterification of cynara oil and reported that reaction is incomplete when molar ratio is less than 6:1. The yield of ester increases as the molar ratio increased upto 12:1 and obtained optimum value at 9:1. However many authors reported that (Lu et al., 2010 and Bousquet et al., 2001) with increase in methanol to oil molar ratio the yield decreases, for instance, Lu et al., (2010) worked on different molar ratio ranges from 1:1 to 1:10 and reported that the maximum yield is obtained at 1:1 and this may be due to inhibitory effect of alcohol on lipase activity.

Similarly Li et al., (2006) has given same trend that with increase in molar ratio, yield decreases, the achieved 95% yield in 12 hour at molar ratio 4:1.

2.6 Effect of catalyst concentration

Mostly alkaline, acid and enzyme catalyst are used. If the oil contains high free fatty acids and large quantity of water, then acid catalyst is used for transesterification. Sultana Shazia et al., 2014 studied the effect of NaOH concentration between the range of 0.1-0.9 wt% and obtained that yield increases with increase in catalyst concentration from 0.1-0.5%. The yield decreases with further increase in NaOH concentration and reduced to 50% with 1.5% NaOH concentration. This is because with increase in the concentration of catalyst, soap formation will take place and reduce the yield with increase in viscosity. Ma F et al., 1998 studied the effect of NaOH and NaOMe (Sodium Methoxide) concentration and found that at 3% and 5% w/w of catalyst to beef tallow oil maximum yield is obtained.

2.7 Acid-Catalyzed transesterification

The acid catalyzed transesterification does not gain much popularity because it is much slower than the alkali catalyzed reactions (Sharma and Singh, 2008). Its performance does not affected by the presence of free fatty acids and can catalyze simultaneously both esterification and trans-esterification. Acid catalyst can produce biodiesel from low cost feed stock having high free fatty acid FFA. The transesterification of triglyceride consists of three reversible reactions. Acid catalyzed transesterification mechanism is shown in Figure 2.7 for monoglyceride. Carbonyl group protonation leads to carbocation which forms tetrahedral intermediate after the nucleophilic attack of alcohol. The glycerol is separated and forms new ester. These reactions should be carried in the absence of water because carbocation reacts with water to form carboxylic acids.

Acid catalyzed transesterification mechanism is shown in fig for monoglyceride. Carbonyl group protonation leads to carbocation which forms tetrahedral intermediate after the nucleophilic attack of alcohol. The glycerol is separated and forms new ester. These reactions should be carried in the absence of water because carbocation reacts with water to form carboxylic acids.

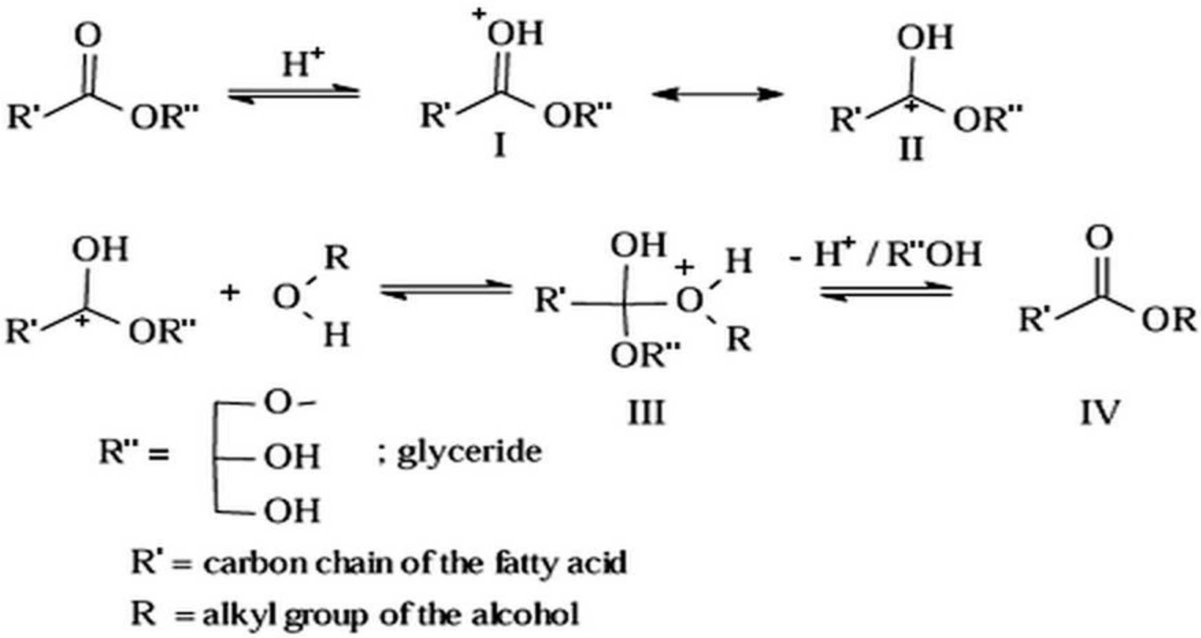


Figure 2.7. Mechanism of acid catalyzed reaction.

2.8. Catalyst for transesterification process

For transesterification following catalyst are investigated, heterogenous, enzymatic and homogenous or alkali catalyst like potassium and sodium hydroxide are mostly used in industrial transesterification because they promote reaction at low temperature also (Sivasamy et al., 2009).

2.8.1. Homogeneous catalyst

Homogeneous catalysts are further divided as homogeneous acids and homogeneous base catalyst. Homogeneous base catalysts are commonly used for transesterification of triglyceride. Homogeneous base catalyst such as carbonates (Arzamendi et al., 2008), alkaline metal hydroxide (Rashid et al., 2008; Gryglewicz, 1999) and alkoxides (Freedman et al., 1984, Freedman et al., 1986) are most commonly used (Sivasamy et al., 2009) the alkoxide are more difficult to handle then hydroxide because alkoxide are hygroscopic in nature. Alkoxide does not form soap from triglyceride saponification due to the presence of hydroxide ion which act as an impurity in alkoxide (Sivasamy et al., 2009). While using alkaline catalyst the free fatty acid content should not increase 0.5% by wt. otherwise soap formation will take place which hampers the production of biodiesel. Various authors reported that 90% yield is obtained by using potassium hydroxide and boiler ashes in the methanolysis and ethanolysis of coconut and palm oil (Graille et al., 1986; Encinar et al., 2002; Ejikeme, 2007; Ejikeme, 2008; Ejikeme et al., 2008). Ma et al., (1998) found that alkaline catalyst NaOH perform better than NaOMe (Sodium Methoxide). However, to obtain higher yield the concentration of NaOMe is slightly higher as compared to NaOH (Ma et al., 1998). Singh et al., (2006) studied about alkaline catalyst (NaOH, KOH, KOMe and

NaOMe) and found that better yield is obtained by potassium based catalyst as compared to sodium based catalyst. Where methoxide based catalyst produces higher yield compared to hydroxide based catalyst.

2.8.2 Homogeneous acid catalyst

For the transesterification of free fatty acid (FFAs) h**o**mogeneous acid catalyst are more effective as compared to base catalyst. Acid catlysed reactions proceed slower than the base catalyzed reaction (Srivastava, and Prasad, 2000). However, acid catalyzed reactions have lower moisture sensitivity as well as non-appearance of soap formation. Acid catalysts are used where oil has higher FFAs (Sivasamy et al., 2009). If base catalyst is used it will form soap. Acid catalyzed reactions are two stage processes, in first stage esterification takes place in the presence of acid catalyst while in the second stage reaction takes place in the presence of base catalyst.

The acid catalyst mostly used are, sulphonic acid, organic sulfonic acid, hydrochloric acid, and phoshphoric acid. Freedman et al., (1986) uses sulphuric acid as catalyst with alcohol oil ratio 30:1 and found that to obtain 90% yield reaction took 50 h to complete at 65^{o} C. Zullaikah et al., (2005) uses sulphuric acid as catalyst for the transesterification of rice bran oil between temperature range of $60\text{-}80^{o}$C.

2.8.3. Heterogeneous catalyst

It is difficult to separate homogeneous catalyst from the reaction mixture so heterogeneous catalysts are developed. Heterogeneous catalysts are advantageous because they do not form soap through saponification of triglyceride and eliminate corrosion problems and reaction requires high temperature and pressure. However, there are some limitations, like they have poor performance compared to homogeneous catalyst, and due to less surface contact catalyst does not participate effectively in reaction so catalyst must be in porous state. The surface of heterogeneous catalyst must be hydrophobic in nature so that it adsorbs triglyceride and to avoid adsorption of polar by products like water and glycerol on surface. Solid catalyst which are mostly used are, alkaline earth oxide, solid organic base, basic oxides supported, basic zeolite, insoluble metal salt and hydroxide, basic metal oxide, hydrotolerite and hetropolyacids (Lee et al., 2014).

2.8.4. Alkaline earth oxide

Ca and Mg are alkaline earth metals which are most widely used as heterogeneous base catalyst. Gryglewicz, (1999) found that alkali earth metal oxides successfully catalyzed the transesterification reaction. Alkaline earth oxides are basic due to M^{2+} and O^{2-} ion pairs (Hattori, 1995). Various authors reported the use of CaO as catalyst for the transesterification of sunflower, and rapeseed oil with methanol (Peterson and Scarrah, 1984, Verziu et al., 2011). Moreover, Strontium Oxide, CaO, MgO also investigated as catalyst for transesterification with high basicity (MacLeod et al., 2008; Montero and Wilson, 2010; Watkins et al., 2004).

Martyanov and Sayari, (2008) used calcium methoxide as catalyst for the transesterification of triglyceride and found that initially reaction is slower as compared to homogeneous sodium methoxide and magnesium methoxide, but at later stage the rate of reaction is higher than magnesium methoxide. Alkaline earth metal oxides assimilate with metal oxide and form composite oxide (Woodford et al., 2014) which can be used as solid base catalyst for transesterification. Composite oxide is more stable and easy to separate from the reaction media.

2.8.5. Acid Zeolite

Zeolites are most widely used as solid acid catalyst for transesterification of oil and made hydrophobic by elimination of water of hydration. Shu et al., (2007) uses La/Zeolite beta catalyst for the batch transesterification of soybean oil and found that La/Zeolite base catalyst have higher conversion rate than zeolite beta heterogeneous acid catalyst used in biodiesel production are mostly mesoporous (Lotero etal., 2005; Dacquin et al., 2010; Kargbo, 2010). Subsume of microporous H-β- Zeolite with secondary meso porosity create a heterogeneous solid catalyst which accelerates microalgae transesterification by reducing the diffusion barriers (Carrero et al, 2011). Koh and Chung, (2008) uses zeolite catalyst for the transesterification of waste cooking oil and found that yield is independent of porosity of zeolite and found that yield increases with increase in strength of the acid.

2.8.6. Hetropolyacids

Hetropolyacids attains much attention due to its superacidic nature (PK H^+> 12) and porous structure. They are highly soluble in polar media in their native form which make their contribution in reaction as homogeneous catalyst (Kozhevnikov, 1998). Chai et al, 2007 uses heterogeneous catalyst ($CS_{2.5}$ $H_{0.5}PW_{12}O_{40}$) for transesterification of eruca sativa gars oil and obtained the same result as by using sodium hydroxide or sulphuric acid with one advantage of easy separation of catalyst from media and its reuse. Cao et al., (2008) used the hetroppolyacids ($H_{35}PW_{12}O_{40}$. $6H_2O$) catalyst for transesterification of waste cooking oil. In

10h 87% yield is obtained using hexhydrate catalyst. The catalyst would be separated easily and was reused many times.

2.9. Microwave irradiation effect on biodiesel production

Traditionally organic reactions are heated by various equipment's such as sand bath, heating jackets and oil baths. These techniques are not effective because they are slower and temperature gradient took place. But now a day's microwave dielectric heating is preferred. In microwave heating radiation passes the wall and only heat the solvent and reactants without heating the vessel (Lidstrom et al., 2001).

Patil et al., (2011) used micro-algal oil to produce biodiesel by transesterification by heating with microwave radiation and observed that microwave irradiation affect the reaction in two way 1. reaction is boosted by thermal effect. 2. Vaporization of methanol due to strong microwave radiation. Ma et al., (2007) observed that microwave heating reduces energy and reaction time due to volumetric heating. N. Azcan and O. Yilmaz produced biodiesel by transesterification of micro-algal oil in the presence of KOH by conventional heating and microwave heating method and find that with conventional heating system reaction completes in 210 minutes while with microwave heating reaction completes in 5 min, obtained 96.54% conversion using KOH 1% wt, 1:8 oil to methanol at 65^{o}C.

2.10. Nanoparticle catalyst in transesterification

For the conversion of triglyceride to methyl esters transesterification takes place in the presence of catalyst. Catalysts used are either base catalyst or acid catalyst. Base catalyzed reactions are much faster than that by acid catalyzed reaction. However basic catalysts have some drawbacks such as loss of catalyst, some catalyst remain in the biodiesel does not separated. To overcome this drawback heterogeneous catalyst are used but require long reaction time and large volume. Therefore, to improve the conversion of free fatty acid, lots of efforts are done to produce catalyst with high surface area. Highest methyl esters can be produced by catalyst with high surface area (Yan et al, 2009). Many authors investigated that nano sized catalyst have large contact area. For instance, Wang et al., (2009) produced biodiesel from waste cooking oil in the presence of Nano-sized catalyst and observed that ≈ 96% conversion was achieved at 55^o C due to large surface area of nano paticle.

2.10.1. CaO/ MgO catalyst

Calcium oxide is heterogeneous base catalyst mostly used for transesterification reaction. It has many advantages, such as easy availability, higher activity, reusability, low cost and mild reaction condition. Pretreatment temperature range between 700-1000 K is used to remove water and CO_2 which is adsorbed on the surface of CaO (Tanabe et al., 1971). Most of the catalyst has adverse effect on yield of methyl ester in the presence of water. However, CaO catalyst performs well in the presence of water, it forms methoxide ion in the presence of methanol which is highly active. Mechanism of transesterification with CaO as catalyst is given in Figure 2.8 (Liu et al., 2008).

As shown in equation 1, Ca^{2+} extracts OH^- and O_2^- extracts H^+ from water so they are easily extracted by reactants during chemical reaction. As shown in equation 2, methoxide anion and H_2O forms when OH^- extracts H^+ methanol. In equation 3 again O^{-2} extract H^+ and form surface methoxide anion. If water exceed by 2.8 wt% of oil it hydrolyzes the methyl esters and forms fatty acid and methanol. Liu et al., (2008) obtained 95% yield at temperature 65^o C by using CaO catalyst. Hsiao et al., (2011) used nano powder CaO as catalyst and obtained

96.6% yield at 1:6 oil to methanol ratio, reaction time 1 hour, 338 K temperature and 3 wt% catalyst.

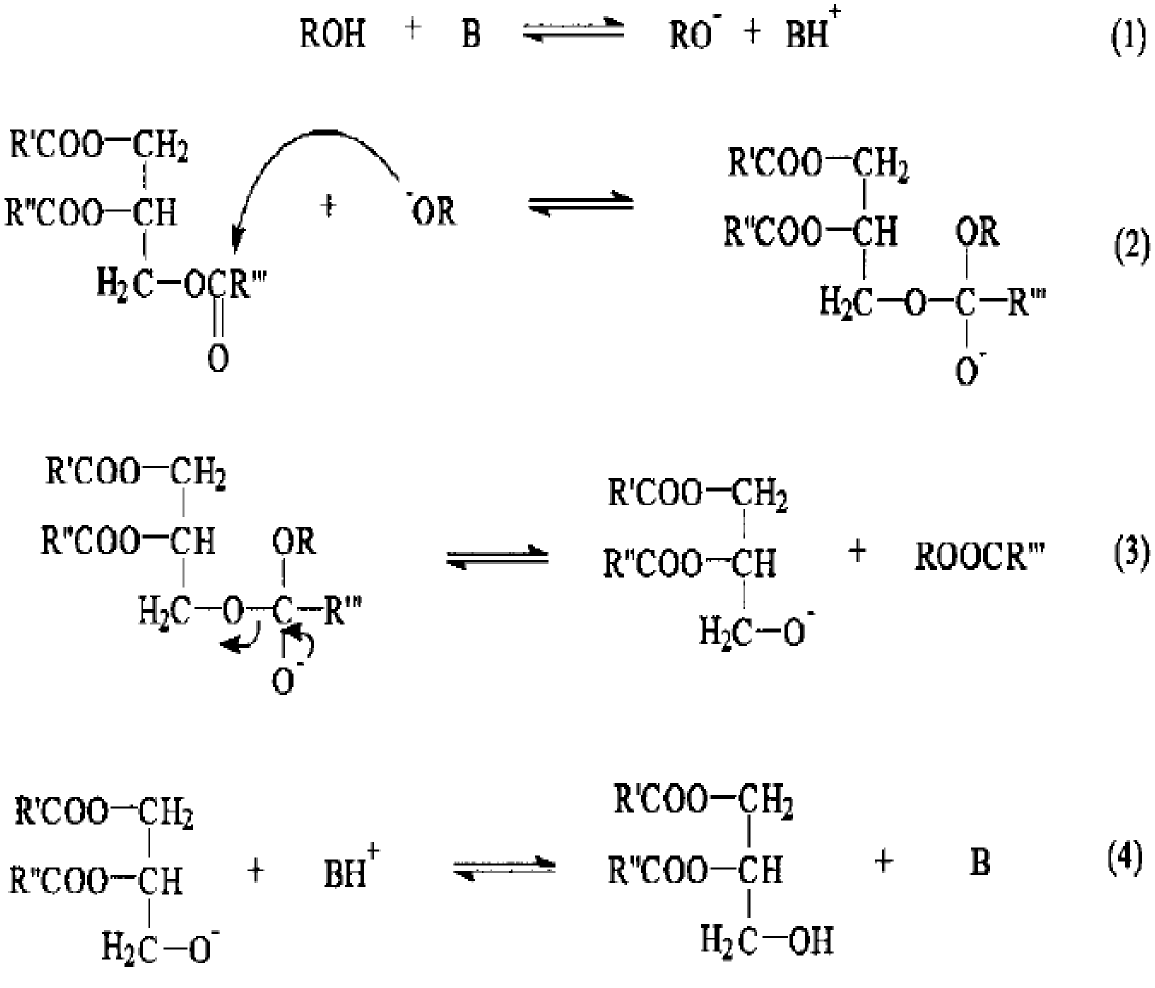


Figure 2.8. Transesterification mechanism in the presence of water using CaO as catalyst.

Due to easy preparation and low-cost researcher focus attention on MgO and CaO catalyst. Huaping et al., (2006) obtained 93% yield using CaO as catalyst. Di serio et al, (2006) achieved 92% yield by using MgO as catalyst. Dossin et al., (2006) used MgO as catalyst in batch work reactor and found that satisfactorily at ambient condition. Magnesium oxide is identified as good homogeneous catalyst for transesterification of ethyl acetate with methanol (Dossin et al., 2006).

2.10.2. CaOZnO Catalyst

The combination of Cao and ZnO (CaOZnO) catalyst in palm kernel oil transesterification is studied. The mixture of CaO and ZnO has small particle size which result in large surface contact area as compared to individual oxides. Ngam charussrivchai et al., (2009) used CaOZnO catalyst with Ca/Zn ratio 0.25 for the transesterification of palm kernel oil and obtained greater than 94% yield at reaction temperature 60^{o}C, methanol to oil ratio 30 and reaction time 60 minute. CaOZnO catalyst is used for the transesterification of sun flower seed oil and 90% yield is obtained (Alba-Rubio et al., 2010). The CaO and ZnO are synthesized by co-precipitation method or impregnation method. Ngam charussrivchai C et al (2009), found that the catalyst synthesized by the co precipitation method result in higher yield (94.2%) compared to impregnation method (90%). The literature shows that the activity of reaction depends on Ca to Zn atomic ratio, it is synthesized between ratio ¼ to 4. At atomic ratio of 0.25 the CaOZnO catalyst produce 93.5% of esters which is larger as compared to another atomic ratio.

2.11. Enzyme catalyzed transesterification

The problem related to conventional catalytic process, like removal of catalyst, treat large amount of waste water and high energy requirement are solved by using enzymes. Enzyme do not form any soap like alkaline catalyst and without the need of washing they esterify both FFA and TAG in single step. These are biological catalyst and can catalyze different chemical reactions. They can be either used in free or immobilized form in transesterification that leads to the production of biodiesel (Haas et al., 2006). A wide range of enzymes such as lipase has been used for esterification (Fjerback et al., 2009). Lipase from fungi and bacteria are mostly used for process and they belong to group of hydrolytic enzymes which is also known as hydrolases.

The lipase catalyzed reaction is classified as (Vulfson, 1994).

1. hydrolysis 2. Synthesis a) esterification b) transesterification.

1) Hydrolysis

$R^1COOR^2 + H_2O \rightleftharpoons R^1COOH + R^2OH$

Esterication

$R^1COOH + R^2OH \rightleftharpoons R^1COOR^2 + H_2O$

Transesterification

Alcoholysis

$R^1COOR^2 + R^3OH \rightleftharpoons R^1COOR^3 + R^2OH$

Acidolysis

$R^1COOR^2 + R^3COOH \rightleftharpoons R^3COOR^2 + R^1COOH$

2.11.1. Immobilization of lipase

Immobilization of lipase is the state of arrest of the enzyme in region (Jegannathan et al., 2008). Immobilization provide number of benefits such as enzyme reuse, easy separation of product from enzyme (Peilow and Misbah, 2001). Many other properties are also improved such as chemical, thermal and mechanical properties making them to use in harsher environmental condition (Awang et al., 2007; Bhushan et al., 2008). Salah et al., (2007) obtained 25% conversion with immobilized lipase and 3% conversion with free lipase while butanolysis of acetic acid. General technique used for immobilization are 1). Adsorption 2) Entrapment 3) Cross linking 4) Encapsulation. Adsorption is simplest method; in this enzyme are attached to the surface by combination of Vander wall or electrostatic forces.

2.11.2 Effect of presence and absence of solvent enzyme based transesterification

Using enzyme as catalyst for biodiesel production of oil is tried in the presence and absence of solvent. Nelson et al., (1996) done methanolysis of tallow oil using hexane as solvent in the presence of Mucormehei lipase and obtained 77.8% yield. But many workers favour solvent free reactions. Furthermore, toxicity and inflammability of solvents, prevent the use of solvent enzyme based transesterification. Oznur et al., (2002) done transesterification of cotton seed oil using immobilized lipase and obtained 92% of yield in a solvent free medium.

2.12. Properties of Biodiesel

Biodiesel is produced in different countries, from different sources. So, it is necessary to set a standard of fuel quality for better engine performance (Balat and Balat, 2010). Many countries approved the standard for biodiesel such as Germany, France and United States (Meher et al., 2006). International biodiesel standards for testing are (ASTM 6751-3) or the European Union Standard (EN14214) (Atabani et al., 2012). Some of these properties include density, ash content, water content, sulfur content, glycerin, and oxidation stability. The physiochemical properties of biodiesel depend on fatty acid composition and type of feedstock.

2.12.1 Flash point

Flash point of fuel is defined as temperature at which fuel will ignite when fuel is exposed to spark. The flash point of biodiesel is higher than the base diesel fuel (Atabani et al., 2012). Thus, biodiesel and its blends are safer to handle and store (Atabani et al., 2012). The flash point of base diesel is 55-60^{0}C (Atabani et al., 2012) While that of biodiesel is more than

150^0C. The flash point limit is prescribed by ASTM D93is 93^0C and in EN ISO 3679 is 120^0C (Atabani et al., 2012).

2.12.2. Cloud point, Pour point and Cold filter plugging point

At low temperature solidification of fuel cause blockage of fuel lines, and filters leading to various problems. Cloud pointis the temperature at which cloud (wax crystal) appear in the fuel. When the fuel is cooled generally biodiesel has higher cloud point. Pour point is the lowest temperature at which fuel can flow. The cloud point and pour point are measured using ASTM D2500 procedure (Atabani et al., 2012). Biodiesel has higher pour point and cloud point compared to diesel (Atabani et al., 2012). It is proposed to specify pour point for diesel and specify cold filter plugging point (CFPP) for biodiesel. The CFPP indicates the limit of filterability at low temperature.The filter starts to plug due to crystallizing some component of fuel. At low temperature fuel gets thicker and may not flow properly affecting fuel supply in pump, injector and fuel lines. CFPP is measured using ASTM D6371 (Meher et al., 2006 and Moser and Vaughn, 2010).

2.12.3 Kinematic viscosity

Viscosity is the ability of the material to flow. Higher the viscosity the fuel it is difficult to inject and flow the fuel in fuel lines especially in winters. The kinematic viscosity of biodiesel is generally 10 – 15 times greater than diesel fuel because of its larger molecular mass and larger chemical structure. Improper viscosity may also lead to poor combustion, which results in excessive smoke with loss of power. If the viscosity of fuel is very low, it may not provide sufficient lubrication for the closely fit pumps and injectors. They can promote wear and cause leakage in injector pump which leads to loss of power as fuel supply is reduced. So, the viscosity must be in limit. The allowable limit according to ENISO 3104 (3.5-5.0mm2/s) and (1.9-6.0 mm2/s) in ASTM D445 (Atabani et al., 2012).

2.12.4. Density and Specific gravity

Density is the mass per unit volume. Biodiesel is slightly heavier than petro diesel (specific gravity is 0.859 compared to 0.850 for diesel fuel). For making biodiesel blended biodiesel should be added at the top of diesel fuel. If biodiesel is put at the bottom, then diesel is added it will not mix. The oil with high density contains more energy (Atabani et al., 2012). Density is measured according to EN ISO 3675/12185 and ASTM D1298. Density is tested at reference temperature 15^0or 20^0C (Atabani et al., 2012). Specific gravity is the density of component compared to the density of water. It is used to make mass to volume conversions, calculate flow and viscosity properties.

2.12.5. Cetane number

Cetane number is the ability of fuel to auto ignite quickly when injected. Higher the cetane number means the fuel has better ignition quality. Higher cetane number indicates shorter time between the initiation of fuel injection and the ignition. This results in higher combustion efficiency and smoother combustion. Biodiesel has higher cetane number than diesel fuel (Moser and Vaughn, 2010). The cetane number of diesel specified by EN ISO 5165 is 51.0 min and ASTM D613 is 47min (Atabani et al., 2012).

2.12.6. Microalgae species selection

There are different strains of algae which can be grown in various conditions like freshwater or brackish (Esteban and Finlay, 2003). Some critical parameters for selecting algae species for biodiesel production as biomass and lipid productivity, lipid and oil content, qualitative and quantitative fatty acid and lipid composition (Huerlimann et al., 2010). In addition to these, biodiesel produced should have high cetane number, suitable density, and viscosity, low emissions, which meet the International standards (Gopinath et al. 2009). Xia et al., (2013) worked on seven species of algae and among them *Scenedesmus obtusus* XJ-15 was screened and cultivated it in two stages. The maximum biomass productivity was 212.1 mg $L^{-1}d^{-1}$ that is further improved by 26.1% adding NaCl in the medium. Leesinga et al., (2013) similarly cultivated photoautotrophically and heterotrohically three different chlorella microalgae species and obtained that under heterotrophic conditions maximum biomass is obtained as compared to autotrophic condition. Hsieh and Wu, (2009) cultivated chlorella species in different modes such as batch, fed batch and semi-continuous mode and finally obtained highest lipid yield 0.139 g $L^{-1}d^{-1}$ in semi continuous mode. Ji et al., (2013) has grown microalgae in waste water to produce low cost lipid, optimum yield was obtained when supplied with 15 % CO_2. Moreover, it was observed that nitrogen and phoshphorus are completely consumed within four days. In an average lipid content in microalgae mostly varies from 1-70 %, but it can reach 90 % of the dry cell mass with different nutrient limited media (Li et al., 2008). Reported few microalgal species that produce high oil content includes *Neochoris oleoabundans* (Yoon et al., 2015) *Neochloris oleoabundans* produces 80% TAGs and most of its fatty acids are saturated carbon chains lying in between 16-20, which make it appropriate for production of biodiesel by transesterification (Tornabene et al., 1983). Recently Li et al., 2008, has confirmed total lipid content upto 40 % obtained in nitrate starvation conditions and 0.133 kg m^{-3} day^{-1} total lipid productivity in *Neochloris oleoabundans*. Heterotrophic cultures were found to be superior to photoautotrophic ones as it allows higher lipid yield and growth. For heterotrophic growth natural source of carbon such as glucose is supplemented that increases cost of biodiesel generation, which contribute the 80% of the aggregate cost making it unfeasible (Li et al., 2007). To decrease the algae cultivation expenses when grown heterotrophically, starch hydrolysates can be used as source of carbon because of their less cost and waste product of industries (Cheng et al., 2009).

Cane molasses is one of the cheap carbon source to be used for algal cultivation, which is the by-product of sugar refinery. It comprises of around half (w/w) of absolute sugars (fructose, sucrose and glucose), protein, metals, water and vitamins (Jiang et al., 2009). After treatment of molasses, it becomes reasonable source of carbon for various microalgal species when grown heterotrophically.

Microalgae provide various special varieties of renewable fuels that include methane produced from algal biomass by anaerobic digestion (Spolaore et al., 2006) bio hydrogen (Kapdan et al., 2006), and biodiesel from algal oil (Gavrilescu and Chisti, 2005). Biodiesel is currently produced from numerous sources that include edible oil, corn oil, canola oil, and animal fat (Felizardo et al., 2006). The typical method of biodiesel production is transesterification, not a new method but known from earlier nineteenth century (Meher et al., 2006). Transesterification reaction rate and biodiesel yield depend on numerous parameters; for example, the type of catalyst used and the molar ratio of oil to alcohol (Meher et al., 2006). Transesterification reactions are catalyzed by basic and acidic catalysts but base-catalyzed reaction occurs at a faster rate almost 4,000 times the latter, however has less yield (Meher et al., 2006). For production of biodiesel from microalgae, same transesterification technique is utilised (Belarbi et al., 2000). Literature study shows that majority of work has been carried on production of biodiesel from edible and nonedible oils such as karanja, jatropha, mahua, and various other feedstocks. *Neochloris oleoabundans* is recognized and considered as a promising alga for renewable biofuel production (Li et al., 2008). The quantity of lipid in this microalga can get extended up to 50% when grown in nitrogen-limited condition (Gouveia and Oliveira, 2008). Microalgae can grow photoautotrophically and heterotrophically by utilizing different carbon sources (Chen and Chen, 2006). The carbon source, i.e., glucose, was tested for the growth of *N. oleoabundans* (Perez et al., 2011). Lot of work has been done on algae growth optimization but no study was conducted on biodiesel production along with its optimization from *N. oleoabundans* and its physicochemical characterization.

2.12.7. Engine performance and emissions testing

The important parameter for diesel engine performance are Torque, BSFC, BP, BTE and it was observed that power output and torque of engine running on biodiesel blends decreases in contrast to petro diesel because of low heating value of biodiesel. The following are the few literatures available on using algae oil methyl esters in CI engine.

Biodiesel can be produced from various feedstocks, like waste cooking oil, microalgae etc., which after transesterification form esters and glycerol (Ozcanli and Serin, 2011). Biodiesel produce lower exhaust emission compared to petro diesel (Yasar et al., 2011). Biodiesel can be used directly (in pure form) in compression ignition (CI) engine or in blended form with slight or no modification. Fuel properties of biodiesel changes with different blends of biodiesel with diesel, because biodiesel has different fuel properties compared with petro diesel (Alptekin and Canakci, 2009). Biodiesel blended with diesel results in reduction of Particulate matter (PM), Hydrocarbon (HC) and Carbon monoxide (CO) emissions but slight increase in NOx emission (Ozcanli and Serin, 2011; Keskin et al., 2008).

Various studies show the use of biodiesel in an internal combustion engine (Saribiyik et al., 2010; Ozcanli et al., 2012). However, many modifications are recommended while using biodiesel, NOx emission can be reduced by retarding injection timing (1^0 to 5^0) or by use of a catalytic converter. Use of B100 requires modification in injection timing and fuel pump (Meher et al., 2006).

2.13. Engine performance

Various researchers mentioned that biodiesel runs satisfactorily in diesel engine up to B20 blend. However, many researchers obtained good combustion characteristics with B100 biodiesel due to oxygenated nature of biodiesel. To evaluate the performance of engine fueled with biodiesel and its blends following parameters are used.

2.13.1. Brake power (B.P): It is the power available at the output shaft. It is the rate of doing work. It is the product of angular velocity and torque. So, to find power torque (T) and speed (N round per minute) is required. Thus, torque is measured with dynamometer and speed with a tachometer.

However, indicated power (I.P) is more than shaft power because some energy is used to overcome friction. Thus the difference between I.P and B.P is known as frictional power. So mechanical efficiency is defined as the ratio B.P to I.P.

$$B.P = \frac{2\,\pi\,N\,T}{60} \quad Watt \qquad (2.1)$$

Literature survey shows that engine power drops with the use of biodiesel because of the low heating value of biodiesel (Xue et al., 2011). It is expressed in terms of brake specific fuel consumption (BSFC) in Kg/kW-hr. The power and torque produced by the engine running on biodiesel are 3-5% less as compared to engine fueled with diesel (Dwivedi et al., 2013). However, some results show fluctuation. Nidal H. et al., (2014) found that heating value of almond biodiesel is 41.76 MJ/KG, which is 14% lower than the LHV (lower heating value) of diesel. Hansen et al., (2006) used B100 biodiesel and observed that torque loss for it is 9.1% compared to D2 diesel at fixed rpm (round per minute) due to variation in LHV (lower heating value) and their high density and kinematic viscosity.

Biodiesel blends are prepared by mixing petrodiesel with biodiesel in suitable proportions and mostly denoted by "B" factor. It is found in various ranges B2, B5, B20, B30, B50, B70, B80, B100 (Nair et al., 2013). Three methods are used for blending, splash mixing method, in line mixing method and injection mixing. Many researchers found that with an increase in biodiesel content power will decrease (Xue et al., 2011). Aydin et al., used cottonseed oil and found that torque decreases with increase in the concentration of biodiesel blends because of the high viscosity of biodiesel and low heating value as compared to diesel. This trend was observed in all the engines like single cylinder four stroke engine and six-cylinder DI (Direct injection) diesel engine. However, some researchers have reported that biodiesel up to B20 blend does not follow above trend. The power increases up to B20 blend and reach maximum and then decreases with increase in biodiesel content. For pure biodiesel (B100) the power produced is minimum (Xue et al., 2011). It has been observed that B100 biodiesel has solvent effect and release accumulated impurities in the vehicle's fuel system which clogs filters and requires filter replacement (Nair et al., 2013).

2.13.2. Brake specific fuel consumption: It is the amount of fuel consumed to develop brake power (Dwivedi et al., 2013).

$$\text{Brake specific fuel consumption(BSFC)} = \frac{\mathrm{m_f}}{\mathrm{B.P}}\left(\frac{\mathrm{Kg}}{\mathrm{KWhr}}\right) \qquad (2.2)$$

M_f = mass of fuel consumed in Kg/hr.

B.P = brake power in KW

Few authors reported that brake specific fuel consumption (BSFC) of biodiesel is higher than diesel due to lower heating value and high viscosity. Many researchers said that increase in specific consumption of fuel is equal to the loss in calorific value compared to diesel (Ozsezen et al., 2009; Choi and Oh, 2006; Hasimoglua et al., 2008; Armas et al., 2010). Hosimoglua et al., (2008) found that increase in specific fuel consumption is 13% compared to diesel while a decrease in calorific value is 13.8% which is almost similar. Karthikeyan and Srithar, (2011) worked on this area and found that specific fuel consumption increased by using biodiesel due to low calorific value. However, some researchers obtained that increase in fuel consumption in case of biodiesel is more than the loss in calorific value (Aydin H et al, 2010; Raheman H et al, 2004; Lujan J. M. et al, 2009). For instance, labeckas G. et al., (2006) reported that specific fuel consumption increases by 18.7% whereas the loss in calorific value is 12.5% which is lower than specific fuel consumption. Aydin H. et al, (2010) obtained that for B100 biodiesel fuel consumption increase by 18% whereas the loss in heating value is 8% only.

Few researchers found that increase in fuel consumption was less than the loss in calorific value (Yucesu H. S. et al., 2006; Sharma D. et al., 2009). Gumus et al., 2010 has obtained that for B100 biodiesel increase in specific fuel consumption was 4.8% while the loss in calorific value is 7.4%. Dorado et al., (2003) found no difference in fuel consumption for biodiesel and diesel.

2.13.3. Thermal Efficiency: It is the ratio of power output to the energy supplied by the fuel. It is named as indicated or brake thermal efficiency (Dwivedi et al., 2013).

$$\text{Indicated thermal efficiency} = \frac{\mathrm{I.P}}{\mathrm{m_f} \times \mathrm{C_v}} \qquad (2.3)$$

$$\text{Brake thermal efficiency} = \frac{\mathrm{B.P}}{\mathrm{m_f} \times \mathrm{C_v}} \qquad (2.4)$$

C_v = Calorific value KJ/Kg

M_f= Mass of fuel supplied Kg/Sec

Karthikeyan and Srithar, (2011) worked on brake thermal efficiency and found that there is no change in efficiency by using blends up to B20 and after that with an increase in blending BTE decreases, due to the low heating value of the fuel.

2.13.4. An additive effect on engine performance: Studies have reported that the additives such as kerosene, ethanol, methanol, Mg, and Mo improve the fuel properties which increase the performance and control NOx emission. However, the selective additives should be used in the engine (Shahabuddin et al., 2013). Keskin et al., (2008) found that mixing Mg and Mo-based additives with tall oil does not work properly. Xue et al., 2011 used Mg-based additives and found a positive effect on flash point, pour point and viscosity. Use of ethanol additives enhances the combustion performance. It has been found that use of additives rich in antioxidants with biodiesel reduces the NOx emission (Shahabuddin et al., 2013). Varatharajan et al., (Varatharajan and Cheralathan, 2013) studied the effect of antioxidant fuel additives on soybean and Jatropha biodiesel and found a decrease in NOx but increase in CO, HC, and smoke emissions. The National Renewable Energy Lab. (NREL) reported that NOx emissions are reduced by ethyl hexyl nitrate, di-tertiary butyl peroxide and tertiary butylhydroquinone (Shahabuddin et al., 2013).

2.14. Emissions

As compared to the engine running on pure diesel, engine running on biodiesel blends show significant improvement in greenhouse gases emission. Use of biodiesel blends reduce particulate matter (PM), Carbon dioxide (CO_2), Carbon monoxide (CO), with a slight increase in NOx emissions due to the presence of oxygen and undergoes complete combustion and improve performance (Chattopadhyay and Sen, 2013).

2.14.1. Particulate Matter (PM): PM is a mixture of liquid droplets and extremely small particles also known as particle pollution (Nair et al., 2013). PM includes acids, organic chemicals, dust particles and metals (Kumar, 2012). Studies have reported that PM emission from biodiesel blends are 30 - 47 % lower as compared to diesel (Nair et al., 2013). Wu et al., (2009) obtained the result of five pure biodiesel on DI engine with turbocharging and found that PM emissions are reduced by 53 – 69 % on average to diesel. Few authors reported that PM increases due to a higher viscosity which causes poor fuel atomization and combustion, so quality deteriorates (Kumar et al., 2010; Banapurmath and Tewari, 2008).

2.14.2. Hydrocarbon (HC): In biodiesel and its blends unburnt hydrocarbon emissions are less, because of oxygen present in fuel which lead to better combustion. Various researchers reported that exhaust emission of HC are lesser by 20-67% for biodiesel as compared to petrodiesel (Kumar, 2012). Many authors reported that with increase in biodiesel blend HC emission decreases (Xue et al., 2011). Godiganur et al., (2010) observed that with increase in biodiesel blends hydrocarbon was reduced linearly.

2.14.3. Nitrogen Oxides (NOx): NO and NO_2 are called as NOx. Its emission takes place at high temperature from by reaction of Oxygen and Nitrogen during combustion (Kumar, 2012). NOx is responsible for depleting ozone layer (Shahid and Jamal, 2011). Researchers observed that NOx emission increases with increase in blending and average increase is noted on average by 10-13% (Nair et al., 2013). However, in the literature (Lapuerta et al., 2008; Nabi et al., 2006) it is reported that NOx emissions are similar in biodiesel and diesel. Wang et al., (2000) used B35 blends of soybean and diesel on several vehicles and found that difference was not important. However, some literature reported that NOx emission is reduced using biodiesel (Puhan et al., 2005).

2.14.4. Carbon monoxide: CO forms in earlier stages of combustion before full conversion to CO_2. The CO emission from biodiesel is 48-50% lower than diesel (Nair et al., 2013). Krahl et al., (2003) found that with use of biodiesel CO emission was reduced to 50% compared to low sulphur diesel. Maximum reduction was observed by Raheman and Phadature (2004) nearly 73-94%. However some researchers observed less reduction in CO emission around 30% compared to diesel (Xue et al., 2011). It was observed that with increase in biodiesel content, oxygen content increases which result in lower CO emission (Xue et al., 2011).

2.14.5. Carbon Dioxide: Compared to diesel, biodiesel reduces CO_2 emission. It was reported that CO_2 emissions are reduced due to lower Carbon to Hydrogen ratio (Xue et al., 2011).

2.15. Technological issues in internal combustion engine and their solution:

It is essential to study the technical sustainability of biodiesel in compression ignition engine. There are various technical and environmental advantages of biodiesel over petrodiesel. Carbon deposit, cold starting, engine wear, storage, and clogging are some technological disadvantages. However, injection timing and fuel supply system are required to modify to run the engine on pure biodiesel.

Till now without any modification in existing Internal combustion (I.C) engine biodiesel is directly used having some physical and chemical properties like petrodiesel. Significant physical and chemical properties of various biodiesel are in Table 2.1 as compared to diesel. All the biodiesel has the required properties to run in diesel engine satisfactorily. The viscosity of biodiesel is more compared to diesel, at the low-temperature fluidity of fuel decreases, which affects the fuel injection system (Jain et al., 2011). However, Kojima et al., (2005) found that higher viscosity helps in reducing plunger leakage and increase fuel injector efficiency. Biodiesel has higher cloud point and pour point compared to diesel due to this biodiesel freezes at a higher temperature compared to diesel.

The engine fueled with biodiesel faces some problems that include fuel freezing at low temperature, less energy density, fuel degradation stored for a long period (Jain et al., 2011). The engine fueled first time with biodiesel blend face one more problem, has a layer of deposits in the fuel tank and hose, etc. Biodiesel blends remove those deposits which block fuel filters (Jain et al., 2011).

Biodiesel can be mixed with diesel at any level to create biodiesel blends. When pure biodiesel B100 is injected, combustion starts at the early stage with shorter ignition delay, due to this temperature of the exhaust gas in B100 is lower than diesel. So, injection timing needs to be adjusted (Canakci, 2007).

Table 2.1. Comparison of various properties of biodiesel with diesel. (Dwivedi et al., 2013)

Type of vegetable oil	**Pour Point (°C)**	**Density (kg/m$_3$)**	**HV (kJ/kg)**	**Viscosity (mm$_2$/s) Temp)**	**Cloud Point (°C)**	**Flash Point (°C)**
Linseed oil	-15.0	929.07	39307	27.2 (38°C)	1.7	241
Olive oil	--	918	--	--	--	--
Palm oil	--	910.1	--	--	--	--
Rapeseed oil	-31.7	920	39709	37.0 (38 °C)	-3.9	246
Sesame oil	-9.4	922	39349	35.5 (38 °C)	-3.9	260
Soybean oil	-12.2	997.5	39623	32.6 (38°C)	-3.9	254
Sunflower oil	-15.0	920	39575	37.1 (38 °C)	7.2	274
Tallow oil	--	820	40054	51.15 (40°C)	--	201
Jatropha oil	--	932	39700	51 (300C)	16	242
Diesel	-33.0	870.20	45343	2.7 (38 °C)	-15.0	52

Biodiesel at higher temperature is more prone to oxidation and forms products like aldehyde and alcohol insoluble, which causes injector fouling, forms deposits in the engine and other parts (Jain and Sharma, 2011). Biodiesel reacts with plastic pipes, so must be changed when pure biodiesel is used (Carraretto et al., 2004). Most of the studies show that 20% blends of biodiesel with diesel are run in the engine without modification. Thus, it would be appropriate to use blends rather than 100% biodiesel in existing engine. The solution to the problem of high viscosity, the high cetane number of biodiesel is a modification of engine.

The following adjustment are required, fuel pump, fuel filter and retardation in injection timing. Fuel pump generally faces a problem like corrosion of fuel injector, at low-temperature pump seizure due to high viscosity. To tackle these difficulties pump material should be more corrosion resistant (like aluminum alloy, iron-based alloy, etc.) (Jain et al., 2011). To reduce the cold weather problem, the heating system can be used which draw heat from engine radiator (Jain et al., 2011). To solve fuel filter problem, many researchers prescribed quicker fuel filter change which however increases cost. Jons et al. (2001) found that as compared to a diesel engine, for biodiesel fuel injector should be checked twice because of coking in biodiesel-fueled engine. The temperature of the nozzle has to be kept below 250^o C to avoid plugging and coke formation (Sgroi et al., 2005).

2.16. Modification of engine

Various researchers work on engine modification using different biodiesel in a different engine. Rodriguez P.R. et al. (2011) worked on diesel engine, using blends of palm and rapeseed oil biodiesel with diesel by modifying IVO – 18^o (inlet valve open) before TDC (top dead centre), IVC – 234^o (inlet valve closed) after TDC, while exhaust valve opening and closing at 120^o and 16^{o}after TDC respectively with compression 16:1 and observed that these blends have shorter ignition delay than diesel fuel. Bunce M et al.(2011) worked on 6 cylinder diesel engine, fueled with soy blends with the following modification, inlet valve opening and closing 20^o BTDC (before top dead centre) /200^o ATDC (after top dead centre), EVO (Exhaust Valve Open) – 220 BTDC, EVC (Exhaust Valve close) – 20^o ATDC and found that SFC (specific fuel consumption) and NOx emission are higher due to low calorific value and presence of oxygen content compared to biodiesel. The calorific value of various blend of biodiesel is shown in Table 2.2 Qi et al. (2011) used Soy biodiesel on four-stroke engine by modifying fuel injection system and found slight increase soot emission and BSFC whereas NOx emission slightly reduced due to an increase of exhaust gas recirculation rate. Aydin H et al. (2010) used single cylinder diesel engine having compression ratio 18:1, maximum torque 250 Nm, standard injection pressure 20 MPa and obtained that engine performance is increased with the use of ethanol blending with biodiesel and diesel. Oner C et al. (2009) experimented on four-stroke direct injection engine by modifying injection pressure (19.6 MPa) and compression ratio 18:1 and obtained that exhaust emission is reduced by using tallow methyl esters.

Table 2.2. Properties of fuel of Karanja oil and neem oil different blends (Jogdhankar et al., 2013; Jahagidar et al., 2011)

Fuel	**Calorific value KJ/Kg**	**Density Kg/m^3**
BK20Karanja oil blend	33400	837.85
BK40Karanja oil blend	32779	843.7
BK60Karanja oil blend	31199	849.55
BK80Karanja oil blend	30300	855.4
Neem oil	35125	970
Neem biodiesel	39000	920
N10 Neem oil blend	41313	840
N20 Neem oil blend	40225	858
N30 Neem oil blend	39938	872
Diesel (D)	42200	822

Karanja (K)	36120	861.25

2.17. Combustion in compression ignition (C.I.) engine: Diesel engine works on the basis of compression ignition. Compression raised temperature and pressure of air inside the cylinder when fuel injected atomize with air and autoignites. The combustion depends upon the air and fuel mixture. Generally compression ratio in CI engine is in the order of 12-24 to ensure high temperature. So, mixture autoignites (Shahabuddin et al., 2013). The combustion process in CI engine completes in four stages.

i. Delay period – a) Physical delay b) Chemical delay

ii. Uncontrolled Combustion

iii. Controlled Combustion

iv. After burning

i. Delay period: Delay period starts when the fuel is injected into the cylinder, this period is called preparatory period. During this period fuel vaporized and mixed with air but ignition has not yet started. The delay period is found different for both diesel and biodiesel (Shahabuddin et al., 2013). This is further divided into two sections: Physical delay and Chemical delay.

(a) Physical delay: The time between fuel injection and chemical reaction attainment is known as a physical delay. During this period fuel is vaporized and mixed with air so that it can auto ignite. The physical delay depends on viscosity, higher the viscosity greater is the physical delay.

(b) Chemical delay: After physical delay period free flame reaction start slowly and after some time fuel burns automatically and the time consumed in this is known as a chemical delay. It depends upon the temperature of the surrounding. Higher the surrounding temperature lesser is the chemical delay. (Mittelbach and Remschmidt, 2007)

ii. Uncontrolled combustion: During physical delay and chemical delay mere fuel droplet injected and a group of droplets burn together, this produced uncontrolled combustion. During this period pressure rise rapidly. The extent of pressure rise depends on the amount of fuel injected in this period. (Mittelbach and Remschmidt, 2007)

iii. Controlled combustion: After some period of uncontrolled combustion the temperature of the combustion chamber is so high that as soon as the fuel droplets enter in the combustion chamber it burns instantaneously and this is called controlled combustion.

iv. After burning: Diesel fuel is less volatile, there are some pockets of air/fuel mixture which will burn during expansion, known as after burning.

2.18. Different techniques to improve combustion in biodiesel.

To improve the combustion of engine when fueled with biodiesel many properties are needed to be improved because viscosity and mass density of biodiesel are higher after transesterification. As viscosity affects vaporization, atomization and fuel-air mixing, Lefebvre (1989) found that viscosity, surface tension and density affects the atomization in diesel engine. However, the density and viscosity is compensated by using turbocharge (Karabektas, 2009). Turbocharger ensure more air at higher temperature and pressure at the time of injection in the cylinder and provides better combustion (Ndayishimiye and Tazerout, 2011).$\mathrm{B.P} = \frac{2\pi NT}{60}\,\mathrm{Watt}$

Al-lwayzy and Yusaf [2013] examined the performance and emission of a 25.8 kW agriculture tractor engine using 20% blended biodiesel from *Chlorella protothecoides* microalgae(MCP-B20) and compared with petroleum diesel (PD). It was found that MCP-B20 can be used without any modification as fuel for tractors for commercial use. They conducted the PTO (Power Take Off) tests for the tractor engine at WOT (Wide Open Throttle) and HOT (Half Open Throttle), and demonstrated that the tractor power, torque and BSFC (Brake Specific Fuel Consumption) at MCP-B20 and PD are close and acceptable. Using MCP-B20, the emission of CO and $CO_2$was highly reduced. With MCP- B20, the values of power, torque, CO and CO_2emissions were significantly reduced and it was also shown using the ANOVA (Analysis of Variance) analysis for two points at maximum PTO torque and power.

Haik et al. [2011] used Ricardo E6 research engine, which is single cylinder variable compression indirect injection diesel engine. Five different types of fuels were studied i.e. base diesel fuel, raw algae oil, algae oil methyl ester (0.2), algae oil methyl ester (0.1) and algae oil methyl ester blended with diesel at 50/50 ratio. The engine parameters were variedly studied for all the five different fuels. It was concluded from the experimental study conducted on the oil extracted from two types of microalgae namely *Ankistrodesmus braunii* and *Nannochloropsis A. braunii* that its physical and chemical properties are close to diesel fuel. In comparison to raw algae oil or diesel fuel, the algae oil methyl esters produced less engine torque output and more combustion noise which can be improved by retarding the injection timing for the algae oil methyl esters. The combustion noise was decreased with decreasing engine compression ratio. Heat release rate was slightly higher for algae oil methyl ester than diesel. The better performance of the engine was obtained by using less methanol (10%) in producing algae oil methyl ester.

Makarevicieneet al. [2014] assessed the comparative studies for the engine tests, of fuel mixtures which contain 30% biodiesel fuel by volume (rapeseed oil methyl esters or microalgae oil methyl esters) and 70% by volume mineral diesel fuel. It was found that the quality of the fuel mixtures consisting of algal oil methyl esters and mineral diesel fuel matched with established fuel requirements. Algae oil methyl esters exhibit slightly lower calorific value, in comparison to rapeseed oil methyl esters, which is approximately 6% lower than mineral diesel fuel. The different calorific values affect engine operating efficiency and fuel consumption. It was also found that AOME (algae oil methyl ester) properties are comparable to the RME (rapeseed oil methyl esters). While running engine by replacing diesel with B30AME blends, the hydrocarbon was reduced by 10 % in contrast to B30RME that is very advantageous to the environment. Moreover, when the engine is running at full load, it was observed that smoke in the emission was 10 % lower for B30 RME as compared to B30 AME. Thus, B30 RME fuel is cleaner and pollutes less environment. The thermal efficiency factor (TEF) for B30 AME was observed 2.5 – 3% more in contrast to neat diesel used as a fuel, the TEF describe the energy utilization efficiency in the cycle. The smoke from engine exhaust and HC emissions was greatly reduced, which shows a large improvement in the environmental pollution. NOx emission for both the biodiesel blends is almost equal to the diesel fuel, while approximately 10% reported a reduction in CO emission for all the tested modes.

Chauhan et al. [2012] used 5%, 10%, 20% and 30% Jatropha biodiesel blends with diesel fuel to evaluate the performance and exhaust emissions, on an unmodified diesel engine. The investigations showed that Jatropha biodiesel blends and diesel fuel have similar engine performance. It was observed that BSFC and NOx emission of biodiesel blends are higher in contrast to diesel while HC, $CO_{2,}$ and CO, as well as BTE, were observed lower than neat diesel. Finally, they concluded that Jatropha biodiesel blends could be used directly in conventional CI engine without any alteration in the engine.

Agarwal et al. [2008] worked on different oils i.e. linseed oil, rice bran oil, mahua oil and linseed oil methyl ester (LOME)and analyzed their engine performance and emissions; experiments were performed on four-stroke single cylinder diesel engine. All the above-given oils were blended with diesel in different ratios. The author observed that direct use of vegetable oil brings operational and durability problems when used in CI engine for a long time, this was due to high viscosity, low volatility and higher polyunsaturated fatty acids of vegetable oil. However, when the oils were transesterified to LOME, such problems did not exist. Consequently, transesterification was observed as one of the best solutions that eliminated the above-given problems. They obtained that 20 % LOME blend with diesel was the optimum value that helped in reducing the smog density along with increasing the thermal efficiency of the engine.

Pali et al. [2014] explored the use of Kusum biodiesel blend for agricultural purpose engine and observed that BTE decreased from 3.8 % to 17 % with an increase in the biodiesel blend proportion. HC and CO were also observed reduced from 7 to 42 % at full load. However, NOx emissions increased up to 20% as compared to D100.

Qi et al. [2009] analyzed the effect of soybean biodiesel on engine performance, exhaust emissions, combustion characteristics and their effects were compared with diesel. Biodiesel and diesel have different properties, so these fuels show different characteristics at different engine load. At low load condition the peak HRR (heat release rate), peak rate of pressure rise, and peak cylinder pressure were higher for biodiesel than diesel. At higher engine load peak cylinder pressure was almost equal but the HRR and rate of pressure rise were higher for diesel. The crank angle for biodiesel blends at which peak values were observed was in advance in contrast to diesel. It was observed that biodiesel had shorter ignition delay, so combustion started at earlier conditions. Whereas, the power output for diesel and biodiesel was equal at all engine loads. The BSFC of biodiesel was higher due to lower calorific value. However the BSEC was almost equal. The different emissions such as CO, HC, NOx, and smoke were found to be decreased at full load condition.

2.19 Gap in Literature

Many studies are available on transesterification of edible oils, but very limited amount of work is found on the extraction of oil from algae and its transesterification for the production of biodiesel. In India, the high cost of edible oils prevents their use in biodiesel preparation. But algal oils are more suitable for biodiesel production. Awareness of the biodiesel extracted from algae is very less. A limited amount of work is available on the engine performance, and emission analysis on CI engine fueled with methyl ester of algae oil.

- Literature study shows that more than 50,000 microalgal species exist, but only a few are considered and analyzed yet, so a large number of algal species-rich in oil need to be explored and grown with cost-saving technologies.
- Efficient techniques to capture CO_2 from power plants need to be identified.

- The algal growth with varying nutrient conditions is not documented yet
- The municipal sewage and industrial waste are not explored fully as a suitable nutrient for algae growth.

2.20 Objectives of present work

1. To produce biodiesel from little-known microalgae having high lipid content using substrate like glucose.

2. To investigate the effect of temperature on growth of microalgae.

3. To investigate the effect of nitrate percentage on the growth of microalgae.

4. To measure the biodiesel properties.

5. To evaluate the performance and emission characteristics of the biodiesel in compression ignition engine using different blends of biodiesel extracted from algae.

6. To estimate the combustion heat release rate with diesel and biodiesel extracted from algae.

CHAPTER 3

Research Methodology

3.1 Introduction

The research strategy is a significant key aspect of acquiring a successful research with authorize results. It can be characterized from numerous points of view, for example, methodology, ways, strategies, and systems that are connected to fuse and accumulate all applicable data for the exploration.

This chapter describes how the entire research work was led and demonstrates the techniques by which algae was cultivated, lipid extracted, lipid quantification, biodiesel production, FAME composition of biodiesel, physio-chemical properties of biodiesel, biodiesel blends, and how engine performance and engine emissions were calculated.

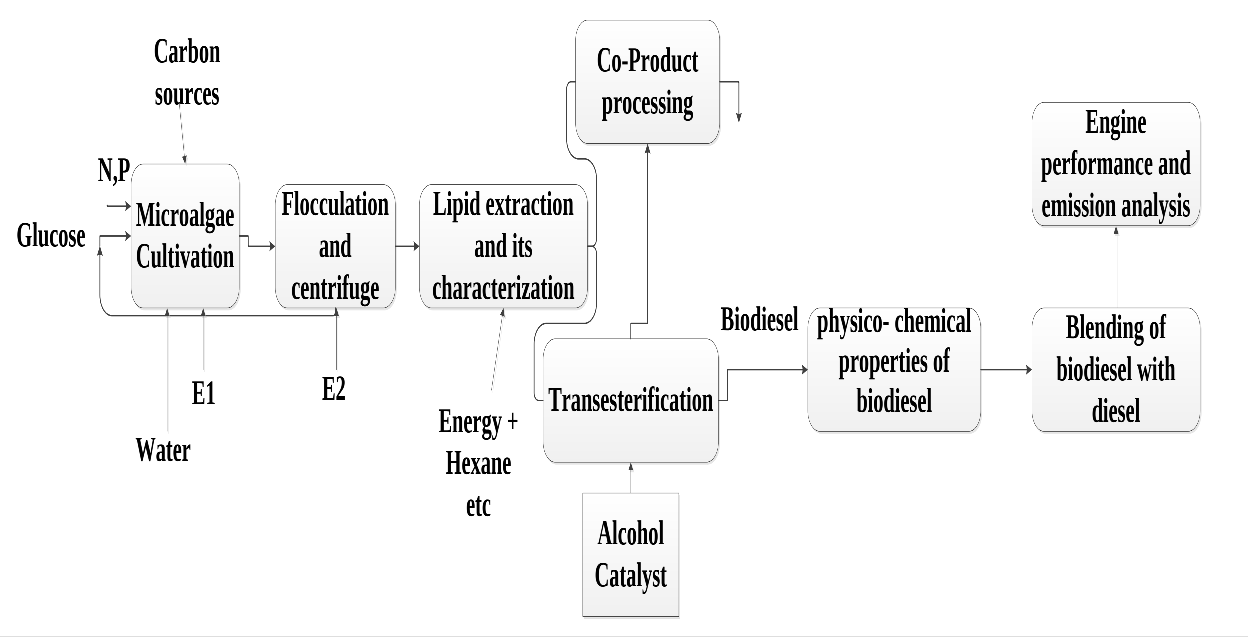


Figure 3.1: Flow chart of the research work.

The list of instruments used in research is also appeared in this Chapter. Moreover, it also covers the heat release rate and mass fraction of fuel burnt calculations. Furthermore, this unit also calculates the different uncertainties and accuracies of measurement. To accomplish the objective of the present work, a few organized events are completed. In view of the research work, the research strategy is proposed as appeared in Figure 3.1. Where, N shows the nitrogen, P phosphorus and E1 and E2 are the energy consumption at different stages.

3.2 Materials and Chemicals

The microalgal species *N. oleoabundans (Neochloris oleoabundans*), UTEX 1185, was procured from the University of Texas, Austin, USA. *Neochloris oleoabundans* also called *Ettlia oleoabundans* is a single cell species, initially found in Saudi Arabia. This alga has a size of 3-10 µm dia which highly depends upon the cultivation and surrounding conditions. It duplicates asexually from mother cell of large size greater than 10 µm, by discharging zoospores. This alga can be grown in different nutrient, light and temperature condition as well as in open pond and closed photobioreactor. *N oleo.* is stated to gather total lipids more than 50% of its dry algal biomass having 80% neutral triacylglyceride (Li et al., 2008). Moreover, according to Yang et al., 2013 among all the fatty acids formed, oleic acid (C18:1) was the major fatty acid obtained (Yang et al., 2013). According to Knothe, (2008) for biodiesel purpose oleic acid is the most favored and ideal fatty acid (Knothe, 2008). *N. oleo.*, thus, a best algae species for biodiesel. The investigation was performed in a biotechnology lab at Delhi Technological University, Delhi as shown Figure 3.2. Initially, Bristol medium was used to maintain a culture in small test tubes and after that scaled up to 2 L conical flask and then putting media into larger volume bottle of 20 L. The microalga was harvested by centrifugation and dried in sunlight to remove moisture. Chemicals and solvents used for extraction were purchased from Sigma Aldrich (India) and Merck Specialties Pvt. Ltd. (India).

Figure 3.2. Picture showing microalgae cultivation.

3.3. Lipid extraction method

Oil from microalga was extracted by solvent extraction method by using a Soxhlet apparatus and pure n-hexane as a solvent (Khola and Ghazala, 2012) shown in Figure 3.3. Dried algal powder (5g) was charged in apparatus and n-hexane (310 ml) was utilized to remove lipids. For oil extraction, the temperature was kept between 50 and 70°C. The oil extracted was measured gravimetrically, mixed with hexane (30 ml), and for later use it was stored in a sealed glass. Finally, the solvent was recovered by distillation process.

3.4. Esterification and transesterification processes

The algal oil obtained from *N. oleoabundans* had 9.2% free fatty acid (FFA), so a two-step reaction process was used for biodiesel production as explained below. Titration method was used to determine the acid value.

3.4.1. Acid-catalyzed esterification

In the beginning, the oil had 9.2% FFA which is more than the threshold limit (2%) for transesterification of oil using an alkaline catalyst. Thus, to lower the FFA content, the oil was pretreated with acid catalyst. So, 2 ml methanol having concentrated H_2SO_4 content of 3.3% (g/100 mL) was blended with 5 ml oil sample and heated at 70°C for 4 h with continuous stirring. The FFA content of oil finally reached 0.8% which is within the prescribed limit for alkali transesterification. Flow chart showing esterification and transesterification reaction steps Figure 3.4.

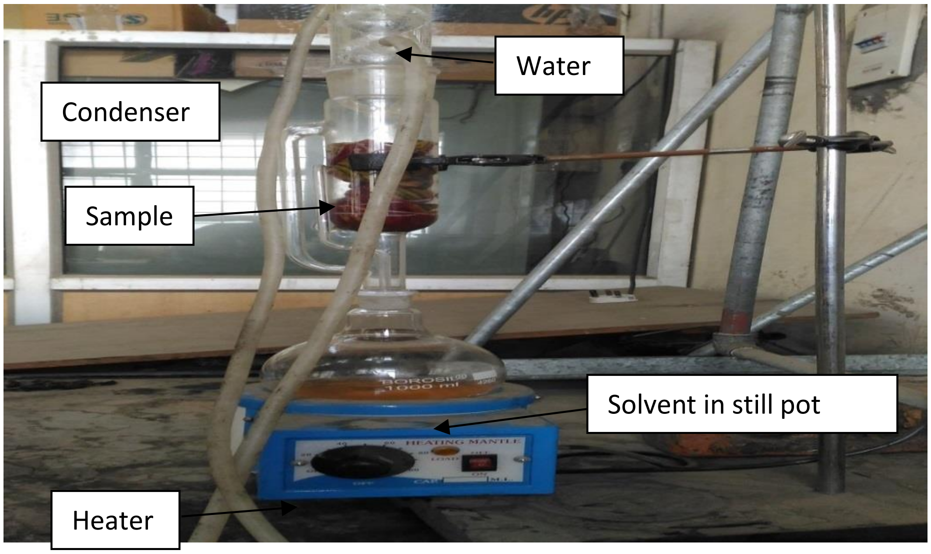


Figure 3.3. Soxhlet apparatus

3.4.2. Alkaline-catalyzed transesterification process

Pretreated algal oil was mixed with methanol in ratio (4.5:1,6:1 and 9:1) containing NaOH catalyst (0.5%, 0.75%, 1%,1.25) and heated up to 65°C on a magnetic stirrer at 150 rpm for 1 h. Using distilled water, the above sample was washed to get rid of alcohol and catalyst.

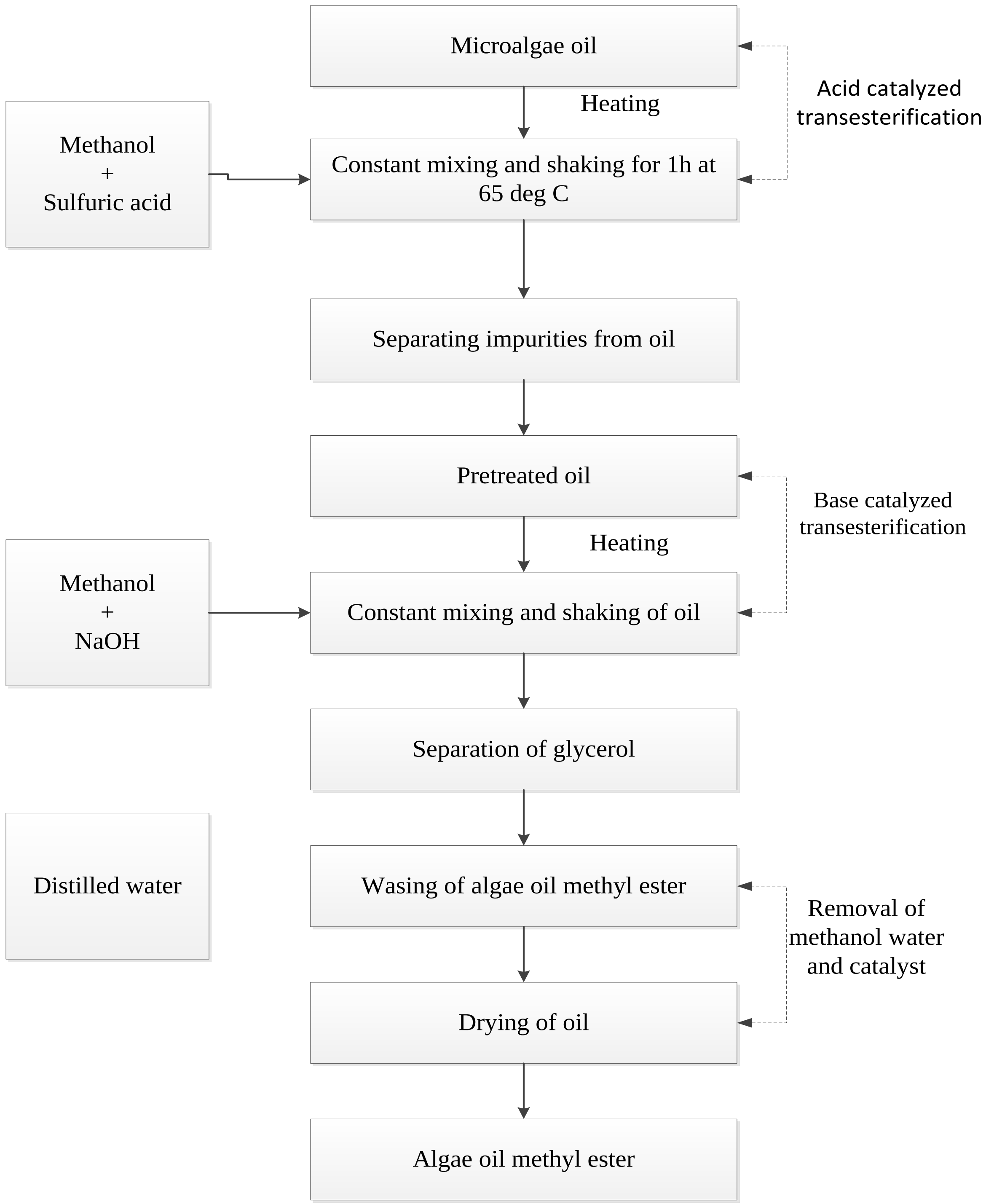


Figure 3.4. Flowchart of transesterification reaction

3.5 Physio-chemical properties analysis and equipment

The physio-chemical properties have enormous impact on the combustion of the diesel–air mixture, exhaust emission, quality and calorific value, ignitions qualities, engine starting in cold weather conditions (cloud point, pour point and cold filter plugging point). The properties related to wear of engine parts are lubricity, cleaning effect, viscosity. Materials used to manufacture the fuel system highly depends upon the properties. The properties such as viscosity, density, flash point, cloud point, pour point, cold filter plugging point, calorific value, and oxidation stability are measured according to some standards and shown in Table 3.1 it also shows the different equipment's used to find these properties.

Table 3.1: Property testing equipment's and test methods

Properties	**Measurement Apparatus**	**Test Method**
Density at 15°C (g/cc)	Stabinger Viscometer-SVM 3000 (Anton Paar India Pvt. Ltd.)	ASTM D1298
Viscosity at 40^0C (cSt)	Stabinger Viscometer-SVM 3000 (Anton Paar India Pvt. Ltd.)	ASTM D7042/D445
Flash point & Fire point (^{0} C)	Pensky-Martens closed cup apparatus (Normalab, France)	ASTM D 92
Cloud and Pour point	Cloud and Pour point tester - automatic NTE 450 (Normalab, France)	ASTM D 2500 and D 97
CFPP (^{0}C)	Automatic NTL 450 (Normalab, France)	ASTM D 6371
Calorific value(MJ/kg)	Parr 6100 calorimeter (IKA, UK)	ASTM D240
Oxidation Stability (h)	873 Rancimat (Metrohm, Switzerland)	EN 14112

3.6 Acid value calculation

Estimation of FFA (free fatty acid) content is essential since it chooses which sort of transesterification procedure to be utilized. If the FFA content is under 2.5 wt%, base catalyzed transesterification is utilized and on the off chance that FFA higher than 2.5 wt%, Acid-catalyzed transesterification is utilized. In this way, a technique for titration is utilized to decide the FFA content in oil. In this strategy, a solution of phenolphthalein indicator is prepared in which 0.05 g phenolphthalein is blended with 50 ml ethanol and 50 ml of water and afterward we mix this solution for a couple of moments and phenolphthalein indicator is ready. At that point, we make another arrangement in which 1 gram of NaOH is blended in 100 ml of refined water and afterward we dilute it by adding it in 900 ml water. This mixture is poured in the burette. At that point, we make another solution in measuring flask by blending 1 ml of algae oil in 10 ml of methanol. At that point we put a few drops of phenolphthalein marker in the above solution. After this we add 0.1% NaOH arrangement in oil alcohol phenolphthalein arrangement utilizing burette until the point when the arrangement in the measuring flask seems pink. Titration is thought to be completed when the pink color appeared. The FFA content is chosen by the measure of 0.1% NaOH utilized in titration. Therefore, acid value can be calculated using the equation.

$$AV = \frac{\mathrm{Mwt} * \ \mathrm{No} * \ \mathrm{Vo}}{Wt}$$

Mwt = Molecular weight of NaOH.

No= Normality of NaOH solution (0.1 N).

Vo= Volume of NaOH solution used in titration.

Wt ≡ Weight of oil sample.

3.7 Triglyceride characterization and quantification

Gas chromatography and mass spectrometry (GC - MS) characterize, separate and evaluate solution of many lipid types. TAG compounds are difficult to process in the GC – MS due to their large size. So, by transesterification process they are converted into small FAME (Fatty Acid Methyl Ester). FAMEs were prepared by adding 0.5 M methanol and 48.5 mg of sodium hydroxide in lipid tube. It was kept at 50^0C for 10 minutes with continuous stirring inside the water bath. To neutralize the reaction, 0.5 ml of Hydrochloric acid was added and washed with 5ml of hexane and 5 ml of deionized water. This solution formed two phases: an upper layer containing FAMEs with hexane and a lower layer containing glycerol with residual methanol, water, and other contaminants. The top layer was isolated and dried using anhydrous sodium sulfate. To analyze the FAMEs, gas chromatograph Agilent 6890 along with silica column was used shown in Figure 3.5. 5 µl of sample was injected, and flow of inert gas (He) was 2ml/min. Starting column temperature was kept at 120^0 C which further increased to 250^0 C and temperature of the injector is held at 250^0 C while FID temperature 270^0 C was maintained. Then peaks were characterized as shown in Figure 3.6 to estimate FAMEs quantities.

3.8 Multi gas analyzer

The emission analysis was done with the help of multigas analyzer (AVL digass 444) certified by ARAI. It helps in analyzing the CO_2, hydrocarbon, carbon monoxide, NO and O_2. With the help of NDIR gas analyzer, the emission hydrocarbon, CO, and CO_2 are detected while other emissions were measured electrochemically. Units of measurement of

CO and CO_2 are % vol. Whereas HC was measured in parts per million (ppm-hex) hexane comparable and NO ppm vol. The technical description of the multigas analyzer is shown in Table 3.2. The actual picture of multigas analyzer is shown in Figure 3.7. The data was communicated with EC (electronics communication) bus RS232C, which noted down the data over a period of 120 s with 20 s back to back pauses.

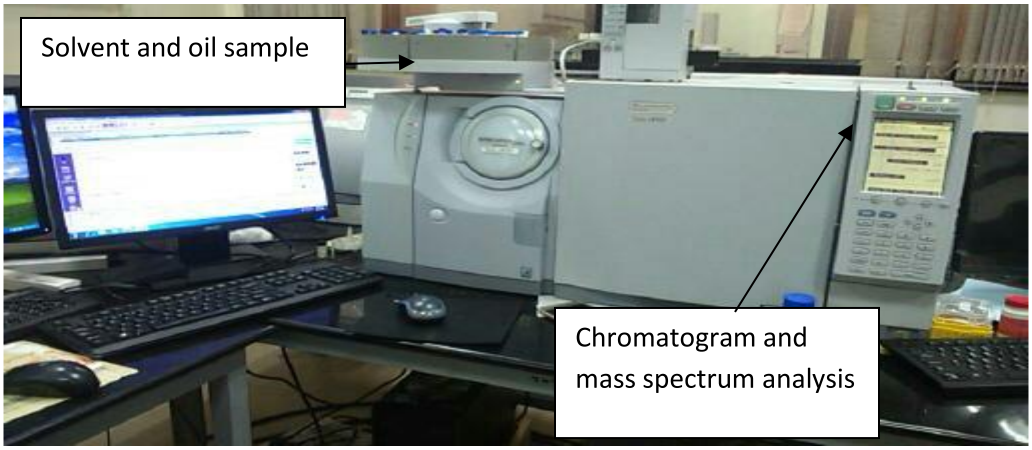


Figure 3.5. GCMS apparatus.

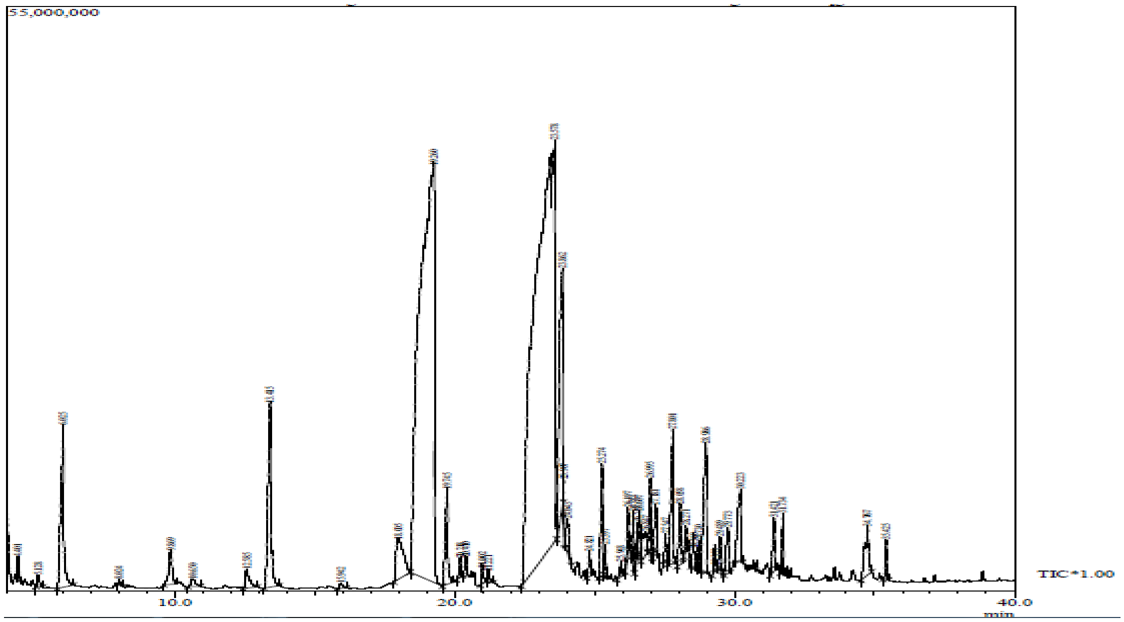


Figure 3.6. Chemical composition of algae oil methyl ester obtained from GCMS.

Which recorded the discharges over a traverse of 120s in 20 s back to back interims, which was more noteworthy than the instrument response period of 15 s, for each instance of the engine run. The gasses from the outlet of exhaust are tapped in to analyze before they entered to the analyzer are cooled. Before starting the experimentation, leak check was performed. In the beginning of experiments, it is calibrated with the analyzer and aligned with the suggested calibrated gasses.

Table 3.2. AVL DIX Emission diagnostic system Specifications

Measured Variable	**Measurement range**	**Resolution**	**Accuracy**
NO	0–5000 ppm	1 ppm vol.	<500 ppm vol.: ±50 ppm vol.
Opacity	0-100%	0.001	±1%
CO	0–10 vol. %	0.01 vol. %	<0.6% vol.: ±0.03% vol.
HC	0–20,000 ppm	<2000: 1 ppm vol >2000: 10 ppm	<200 ppm vol.: ±10 ppm vol P200 ppm vol.: ±5% of ind. val.
CO_2	0–20 vol.%	0.1 vol.%	<10% vol.: ±0.5% vol.

The sensor of the gas analyzer was fabricated by Selenium photocell have dia of 45 mm. Emission tests were carried out by inserting a probe into the engine’s exhaust tube by opening the ball valve. Before taking the emission test, a leak check was conducted in the digital gas analyzer, to discharge the residual gases by closing the probe’s nozzle manually. Engine emissions were analyzed by putting a probe into the exhaust pipe by turning on the valve. Before the beginning of the test, a leak check was led in the computerized gas analyzer.

Figure 3.7. Actual setup of multigas analyzer.

3.9. Data collection for engine performance

3.9.1. Brake thermal efficiency (BTE)

With a specific end goal to calculate the brake thermal efficiency (BTE), three significant values were recorded: I) Total time calculation for 20 cc of fuel utilization

ii) Calorific value (CV) of the diesel and biodiesel blends and

iii) Density of diesel and its blends. The equation used to compute the BTE, is given underneath:

BTE = (BP x 360000/(Vol. fuel flow rate/ hour x ρ x CV)). (3.1)

3.9.2 Brake specific fuel consumption (BSFC)

The equation utilized to determine the BSFC is specified under:

BSFC = (vol. fuel flow rate/hour *ρ/ BP) (3.2)

The both BSFC and BTE data was calculated with the help excel sheet by data obtained from data acquisition system utilizing the previously explained equations.

3.9.3 Heat release rate

It is very crucial to calculate heat release rate in order to find the combustion behavior. There are numerous models regarding heat release rate calculation available for example, heat release rate, pressure rise rate etc. For the current study equations from Heywood 1988 for heat release calculations were utilized. According to conservation principle, from first law of thermodynamics the energy equation can be written as $\partial Q - \partial W = dU$ (3.3)

Where ∂Q- heat release, $\partial W = PdV$ and $dU = mc_v dT$ by solving the equation heat release equation is given below.

$$\frac{\partial Q}{d\theta} = \frac{\gamma}{\gamma - 1} P \frac{dV}{d\theta} + \frac{1}{\gamma - 1} V \frac{dP}{d\theta} \tag{3.4}$$

Where

$\frac{\partial Q}{d\theta}$= rate of net heat release rate (J/^{o}CA)

P= cylinder pressure

V= volume of gas inside cylinder

θ = Angle of the crank (^{o})

γ = specific heat ratio

At the top dead center (TDC) the crank angle is usually found to be zero in an internal combustion engine. The combustion phenomena occur in CI engine between the start of injection and up to the completion of exhaust stroke that is 23^{o} before TDC and 40^{o} before BDC (bottom dead center). The reason for the information acquisition software is to obtain engine cylinder volume value at different crank angles. This is accomplished with the help of above-mentioned software (data acquisition). To confirm the accurate amount the CA required to be changed into combustion chamber volume utilizing engine parameters. Heywood , has given formulas to find the volume of the cylinder using CA for a slider-crank model (Equation 3.5).

In cylinder volume $V = V_c + \frac{\pi D^2}{2*2}(x + r - y)$ (3.5)

Where (V_c) is the clearance volume, D- Cylinder dia, x- Connecting rod length, r- is the crank radius, y- is the distance amongst the axis of the crank and the piston pin.r_c is the compression ratio. R is the ratio of x/r. $y = r\,cos\theta + \sqrt{x^2 - r^2 sin^2\theta}$

$$\frac{V}{V_c} = 1 + \frac{1}{2*1}(r_c - 1)\ [(R + 1 - cos\theta - (R^2 - sin^2\theta)^{1/2}]$$

Derivating V wrt to θ we get the value of $\frac{dV}{d\theta}$

3.10 Estimation of uncertainty

While determining any value, the outcomes always change from the actual quantities even having cautious experimentation. Table 3.3 demonstrates the accuracies and uncertainties related with numerous measurements. It was detected that all of the experiments performed showed higher accuracy. It was observed that the numerous measured properties like density, viscosity calorific value (CV), flash point, acid value, fatty acid profile etc., were observed thrice and the average value was taken for analysis. Obtained values were identical because the instruments use has high precision and are regularly calibrated. Hence, the uncertainties of the instruments used were very low. As shown in Table 3.3 the % uncertainties in these equipment’s were very low. For viscosity, density, calorific value and cold flow plugging point the percentage uncertainty was less than 0.3% and highly satisfactorily.

Table.3.3: Accuracies and uncertainties of measurements

Measurement	**Measurement technique**	**Accuracy**	**Range**
load	strain gauge	±0.1 Kg	0-50 Kg
Smoke	Photochemical	± 2%	0-100%
CO$_2$	Non-dispersive infrared	±0.2%	0-20% vol.
TUHC	Non-dispersive infrared	±2 ppm	0-20,000 ppm
NOx	Electrochemical	±15 ppm	0-4000 ppm
CO	Non-dispersive infrared	±0.2%	0-10% vol.
Pressure	Piezoelectric	±1 bar	0-345.5 bar
Exhaut Temperature	Thermocouple type-K	±1^{0}C	0-1200°C
Speed	Magnetic pick up	±1 rpm	0-2000 rpm
Crank angle encoder	Optical	± 0.2^{0}CA	0-720 °CA

Table.3.4: Calculated values of accuracies and uncertainties of measurements

Measurement	% uncertainty	Measurement technique
Time	±0.2	Stop watch
Manometer	±1	balancing column of liquid
Heat release rate	±6%	First law of thermodynamics
Engine power	±1.0%	Root sum square formula
Brake thermal efficiency	±1.5%	Root sum square formula
BSEC	±1.5%	Root sum square formula
Fuel consumption	±2%	Volumetric method
Air consumption	±1	Orifice meter

CHAPTER 4

Microalgae cultivation and harvesting

4.1 Introduction

Fossil fuels are unsustainable creating environmental pollution, because of urbanization and industrialization it is unable to meet the energy demand and is depleting. Therefore, it is essential to find alternative fuel. Renewable biofuels with low emissions are seemed to be the best alternative. First generation biofuels, for example, animal fat, soybean, palm oil, sugar cane are inadequate to meet the demand because these are the primary food crops, which further increase the risk of food security. On the other hand, the oil from non-edible plants are known as second-generation biofuels, but this again requires arable land to cultivate. Microalgae is known as the third-generation biofuel which does not require arable land to grow and are non-edible, so they do not compete with food crops. Some species of algae have high oil content and can multiply.

Despite, of these advantages production of biodiesel from microalgae is till now is in its initial phase of development. Thus, it is very urgent need to explore the algae cultivation, harvesting, lipid extraction techniques as well as biodiesel production techniques using an advanced method such as ultrasonic and microwave heating. Moreover, fuel properties of biodiesel are also needed to be explored, and microalgae oil methyl esters influence on engine performance and their emissions are also very important to be addressed in order to found it a suitable alternative to diesel fuel.

Currently, plant oils are the mainly used as the source of biodiesel, which cannot adequately meet the increasing demand of transport fuels due to less availability of farmland and high cost (Scarlat et al., 2008). In contrast to conventional plant oils, microalgae prove to be an efficient producer of biofuel in that they have faster growth rate and high oil yield (Hu et al., 2008; Pruvost et al., 2009). There are approximately more than 50,000 species of microalgae, but only 30,000 are analyzed and studied (Richmond A., 2004).

The objective of the present research is to check the feasibility of glucose as a carbon source for high lipid yield of *Neochloris oleoabundans*. This work shows the capability of glucose as the organic carbon source. Various C/N ratio of media are maintained and studied. It is characterized and quantified using GC/MS.

4.2 Microalgae strain and culture conditions:

The microalgae *N. oleo (Neochloris oleoabundans* - UTEX 1185) was taken from University of Texas, Austin, USA. At first, culture was set up in Bristol medium (James D. E. 1978) with pH 7.5 in a test tube and then scaled up to 5 L flask by putting media to larger volume flask after every 5 days. Before, the inoculation of algae all the samples of media was decontaminated with the help of ultraviolet light by putting it in laminar flow hood for 15-20 minutes. During scale-up period no external source of carbon is provided. The temperature of the culture was maintained at 28^0 C and for the growth 16:8 L/D light periodicity was provided with 40 W tubes with manual shaking couple of times in a day. For later use the initial media was prepared by adding glucose and varying the sodium nitrate amount in the Bristol medium to maintain low C/N (carbon/ nitrogen) ratio. Bristol medium composition is presented in Table 4.1.

Table 4.1: Bristol medium composition. James D. E. (1978), Sanchez et. al. (2013)

.Bristol medium	$NaNO_3$	$CaCl_2.2H_2O$	$MgSO_4.7H_2O$	K_2HPO_4	KH_2PO_4	NaCl
(in mM)	2.94	0.17	0.3	0.43	1.29	0.43

4.3 Cell mass estimation

The inoculated culture flask was kept for continuous shaking at temperature 30^{o}C at 180 rpm. After few days when the growth became constant, new fresh media samples were prepared by limiting $NaNO_3$ concentration as well as adding glucose as carbon source and kept in two Erlenmeyer flasks. This media solves the purpose of different nitrate concentration as well as the addition of carbon source. One more sample of media was prepared with normal Bristol medium nutrient concentration. Now all the media samples were transferred to a new flask, which occupied one-third of the total volume of the flask. Now, these flasks were autoclaved at 121^{0}C and 103.421 kPa pressure to steam sterilize them. Now all the samples are cooled and, inoculated with centrifuged algae cells under the laminar flow hood, and there an initial mass of cells was noted for later comparison. Every day for the next working day dry cell weight was evaluated by centrifuging the media and drying the cells. Now the growth curve was plotted for all the working days, and the total cell mass was noted after centrifugation drying the cells.

4.4. Harvesting of *Neochloris oleoabundans*

For microalgae *Neochloris oleoabundans* harvesting the 1 L algae culture was cultivated as given in the above culture condition. For harvesting, simple sedimentation, centrifugation was used. To harvest large size samples, combination of these two techniques was used and one flocculant was also used.

4.4.1 Sedimentation

In sedimentation algae particles in the media solution, settle down in the fluid when they were kept at steady state for a long time. They settle down because of the application of gravitational force on algal particles. For sedimentation process the above-prepared culture was added in different tubes, experiments were performed in triplicates. It was kept for 24 hrs. for settling down; supernatant media was removed. The biomass collected was kept in different preweighed tubes for drying and then weighed.

4.4.2 Centrifugation

Centrifuge is an instrument which rotates around fix axis, due to the rotation the force is applied to the algal particle, and they move radial outward. It works on the principle of sedimentation; the forces are due to a rotation that causes the algae to move radially outwards. The centrifuge used in the laboratory causes the algae denser biomass to settle down to the bottom of the tube. Separation of algal cells from the medium is a difficult task because of the smallest size of the algal cell. Mixtures are separated by spinning in a centrifuge. For centrifugation similarly, culture media was divided into three samples each and they were centrifuged at 3000 rpm for 5 minutes. After the completion of centrifugation, the supernatant was thrown, and biomass was kept in preweighed tubes for measuring biomass.

4.4.3 Flocculation

Harvesting of the algae depends upon the effective particle size. Chemical flocculants are added to increase the particle size so that another method like floatation and filtration are used to harvest the algae. In the suspension microalgae have negative charge that prevents them from self-aggregation. Addition of flocculants reduces or neutralizes the negative surface charge. Similarly, three samples were prepared as explained above and simultaneously ferrous sulfate an inorganic flocculant was added, and culture was kept for settling down. Similarly, supernatant was removed, and biomass was dried by transferring into preweighed tubes. The final biomass was measured by subtracting the weight of flocculants to get the final weight.

4.5 Direct transesterification

The conventional biodiesel production technique is a complex process that involves cell disruption followed by a collection of oil using different solvents and then transesterification of oil (Folch et al., 1957). So, oil extraction is high energy consuming because microalgal cell walls are firm and thick which are difficult to break. The study shows that when the algal biomass is wet, it negatively influences the biodiesel yield, so Hidalgo (2013) suggested direct transesterification method for oil extraction from wet algal biomass that involves two steps in a single reaction that is lipid extraction and conversion to biodiesel simultaneously (Hidalgo et al., 2013). Here one-step (direct) transesterification incorporates both esterification and transesterification of free fatty acids and triglycerides respectively. Thus, simplifying the biodiesel production process by eliminating the oil extraction step that includes a lot of oil loss. In direct transesterification process FAME yield highly depends upon the presence of a catalyst (Atadahi et al., 2013). So numerous studies have been conducted on different catalysts such as enzymes, homogenous and solid catalysts (Zabeti et al., 2009). Presence of moisture negatively affects the FAME yield when alkaline catalysts are used because of saponification (Demirbas, 2009). Therefore, more work is conducted on acid catalysts such as BF3 and H3PO4 (Bharathiraja et al., 2014). Biodiesel production by in – situ transesterification from dry microalgae using H_2SO_4 as a catalyst is getting more attention (Park et al., 2015) because dry algae react with methanol and H_2SO_4 where methanol solves the purpose of both solvent and esterification reagent. However, very less work was conducted on wet microalgae. Velasquez – orta et al., (2013) used H_2SO_4 as a catalyst for different moisture content in algal biomass and obtained maximum FAME yield of 73%. Effect of methanolic HCl was not addressed yet on wet microalgae. The present work examined the efficiency of methanolic HCl in in – situ transesterification of wet algae which act as both catalyst and solvent. Furthermore, effect of methanolic HCl was addressed when moisture content in the algae biomass was very high which was not investigated yet for wet microalgae. Finally impact of variation of methanol, reaction temperature and methanolic HCl was explored and optimized to get high yield. Which is explained in chapter 5. Cell disruption is the major requirement for the extraction of lipid from the microalgae. For the cell disruption after harvesting the microalgae microwave was used discussed in fifth chapter.

4.6. Investigation of main parameters and their interaction utilizing design of experiments (DOE)

DOE software was utilized to decide the fundamental factors during the algae cultivation, and to consider the interactions between factors. The software DOE (Stat-Ease, ver. 7) was used for the optimization purpose. In this work, RSM method grounded upon the Box-Behnken design (BBD) was utilized to optimize the culture medium in order to maximize the algal biomass. Although number of investigations are conducted on optimization of algae growth of different types of algae. In spite of a few investigations performed on there is not any extensive prediction model that demonstrates the relationship between laboratory investigations and predicted models. The algal biomass changes with different parameters, so all the parameters that affect yield were examined. Three factors; the glucose amount (0-10 g/L), $NaNO_3$ concentration (0.15-0.35 g/L) and temperature (24- 30 ^oC), alone and in combination, were examined utilizing RSM (response surface methodology). The optimized results were repeated the output of this work could improve the algal biomass production.

The regression coefficients of all the associated linear and quadratic equations were calculated, the importance of all the examined coefficients were explored utilizing the ANOVA (analysis of variance). All the work is performed in triplicates

4.6.1 Experimental design and optimization by RSM

The optimum culture medium conditions to attain the maximum biomass were predicted utilizing the statistical package Design-Expert, (Stat-Ease, version7.0.0). To examine the crucial relations and quadratic effects of three independent variables (glucose (A), $NaNO_3$ (B), and temperature (C)) on biomass production, the Box-Behnken design (BBD) was applied (Box and Behnken, 1960). The maximum and minimum values for glucose, $NaNO_{3,}$ and temperature percentage were set at 0-10g/L, 0.15-0.35 g/L, and 24-30 ^oC correspondingly (Table 4.2). The optimized responses was algal biomass (Y1). The thorough optimization investigation involved

15 arrangements, which included three duplicates for the mid-point (Table 4.3). The responses function (Y) divided into the linear, quadratic and interactive terms as shown in equation 4.1:

$$Y = \beta_0 \sum \beta_i x_i + \sum \beta_{ii} x_{ii} + \sum \beta_{ij} x_i x_j + \varepsilon \qquad (4.1)$$

Where β0, Bi, Bii, and Bij signify the constant, linear, quadratic and the cross-product coefficients respectively. Besides, the levels of the studied independent variables defined as Xi and Xj, while ε represents the residual error value.

Table 4.2. Level of independent variables, Glucose, and sodium nitrate concentrations in g L−1, the temperature in °C and the coded levels (X) in the Box- Behnken design

Independent variables	**Units**	**Symbol**	**Code levels (X)**		
			-1	0	1
A (Glucose)	g/L	A	0	5	10
B (NaNO3)	g/L	B	0.15	0.25	0.35
C (Temperature)	Deg C	C	24	27	30

BBD was carefully chosen because it needs smaller number of experiments as compare to CCD (central composite design) when there are three or four independents factors. BBD gives a polynomial equation for each run of algae biomass. This technique helps to examine a massive number of factors with a smaller amount of investigational studies paralleled to central composite CCD (Morowvatetal, 2015).

Table 4.3. Experiments for BBD of three independent factors glucose, sodium nitrate, and temperature and data of 15 trials.

Run	Factor 1 A:Glucose g/L	Factor 2 B:NaNO3 g/L	Factor 3 C:Temperature Deg C	Response 1 Biomass g/L
1	0.00	0.35	27.00	1.1
2	5.00	0.25	27.00	1.65
3	10.00	0.25	30.00	1.99
4	5.00	0.15	30.00	1
5	10.00	0.15	27.00	2
6	5.00	0.15	24.00	1.5
7	10.00	0.25	24.00	2.7
8	5.00	0.25	27.00	1.68
9	10.00	0.35	27.00	2.5
10	5.00	0.35	24.00	1.8
11	0.00	0.15	27.00	0.8
12	5.00	0.25	27.00	1.7
13	0.00	0.25	24.00	1.29
14	5.00	0.35	30.00	1.5
15	0.00	0.25	30.00	0.9

In RSM of Box-Behnken design, three factors with three level are chosen to find the impact of three studied independent variables, glucose (A), $NaNO_3$ (B), and temperature (C) on the algal biomass (Y1). The experiments planned, and the results obtained were presented in Table 4.3. The broad plan covered 15 experiments sets that were accomplished in any order, containing twelve factorial and three central points. Three experiments were repeated (runs 2, 8, 12) at the midpoint of the experiments planned to determine the pure error sum of squares. To predict the quantity of algal biomass (Y1), a predictable regression model prepared and displayed utilizing the second- order polynomial equation given below (utilizing coded factors):

Biomass (Y1) = Biomass = 1.68 + 0.64 * A + 0.20 * B -0.24 * C + 0.050* A * B -0.080 * A * C + 0.050 * B * C + 0.097 * A^2 -0.17 * B^2-0.053 * C2 (4.2)

In Equation (4.2), Y1 is the quantity of Biomass yield, A, B, and C are the coded independent factors of glucose, Sodium nitrate and temperature, correspondingly.

The Fisher-test investigation was used to calculate the statistical importance of the advocated regression model. Results of the ANOVA of all the trials were shown in Table 4.4. The achieved outcomes confirm that the above model is highly significant (P-value 0.0001).

Table 4.4. ANOVA table to analyze the effect of independent variables on the dependent variables and the regression coefficients, and the probability values of all the factors.

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**P-Value**	
Model	4.23	9	0.47	170.80	0.0001	Significant
A-Glucose	3.25	1	3.25	1180.84	0.0001	
B-NaNO3	0.32	1	0.32	116.22	0.0001	
C-Temperature	0.45	1	0.45	163.89	0.0001	
AB	0.010	1	0.010	3.63	0.1150	
AC	0.026	1	0.026	9.30	0.0284	
BC	0.010	1	0.010	3.63	0.1150	
A^2	0.035	1	0.035	12.53	0.0166	
B^2	0.11	1	0.11	40.29	0.0014	
C^2	0.011	1	0.011	3.81	0.1082	
Residual	0.014	5	2.753E-003			
Lack of Fit	0.012	3	4.167E-003	6.58	0.1348	not significant
Pure Error	1.267E-	2	6.333E-004			
Cor Total	4.25	14				

The result obtained from the software directly are explained in this section as well as are shown in Table 4.4. The F-estimation of 170.80 suggests that the model is noteworthy and significant. There is very small only 0.01% possibility that a "Model F-Value" this massive could happen because of noise. Estimations of "Prob > F" and below 0.0500 demonstrate model terms are significant. For this situation, A, B, C, AC, B^2, C^2 are significant model terms. The "Lack of Fit F-esteem" of 6.58 suggests the Lack of Fit isn't significant respect to the pure error. There is a 13.48% possibility that a "Lack of Fit F-esteem" this huge could happen because of noise. Not- significant lack of fit is great; we need the model to fit.

Table 4.5. Pareto analysis of variance of fitted model

Std. Dev.	0.052	R-Squared	0.9968
Mean	1.61	Adj R-Squared	0.9909
C.V. %	3.26	Pred R-Squared	0.9522
PRESS	0.20	Adeq Precision	43.472

To find the fitting of the model, R- Squared coefficient was calculated, displayed in Table 4.5, the value of R- Squared , Adjusted R- Squared (R-adj) and R^2 predicted) of 0.9968, 0.9909 and 0.9522, which demonstrate that the given model for biomass production fits to the test data obtained. Obtained data of R^2 and Adj R^2 are extremely large for every model, which infers that what fraction of simulated information is well-matched and to the promoter for a massive significance of the model. It was observed that, the "Pred R-Squared" of 0.9522 is in reasonable agreement with the "Adj R-Squared" of 0.9909. Moreover, adequate precision measures the signal to noise ratio. A ratio higher than 4 is desirable. The ratio of 43.472 indicates a suitable signal. This model can be used to navigate the design space.

Besides, the coefficient of variation (CV%) denotes the virtual dissemination of experimental data from the approximation of second order polynomial. Predicted R^2 shows that total mean is a predominant forecaster of a reaction than the current model and Adq Prec. Calculates the SN ratio, which ought to be more than 4 that is required and for this situation, the proportion is observed to be bigger than 43.47 alludes to a sufficient signal. The supply of residual data revealed by typical plots which demonstrate the contrast between the simulated information and predicted information of the response Figure 4.1, 4.2 and 4.3.

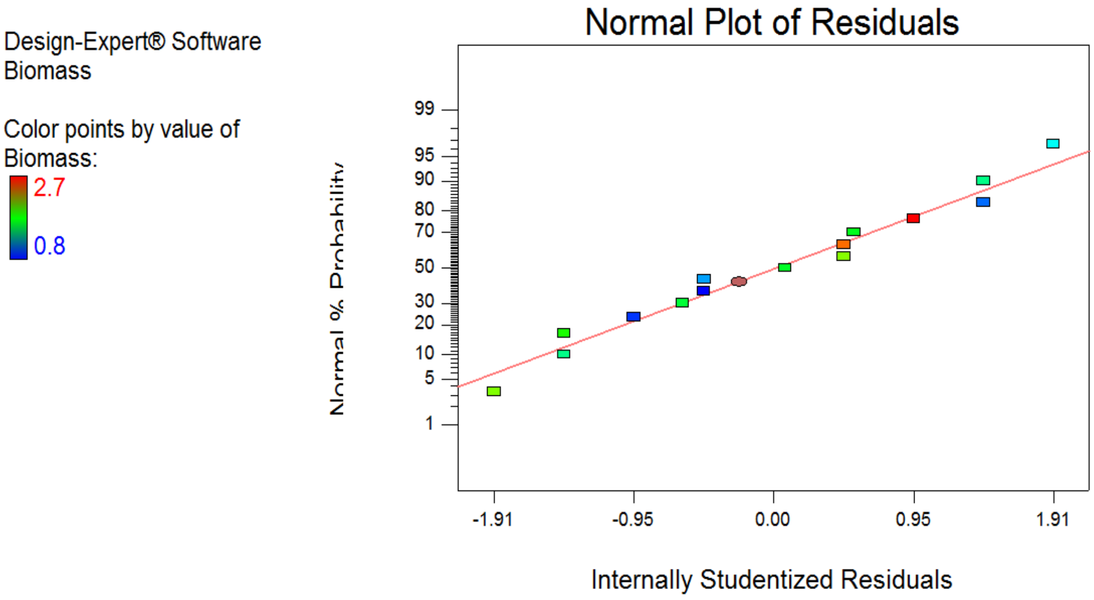


Figure 4.1. Plots of normal % probability for biomass production.

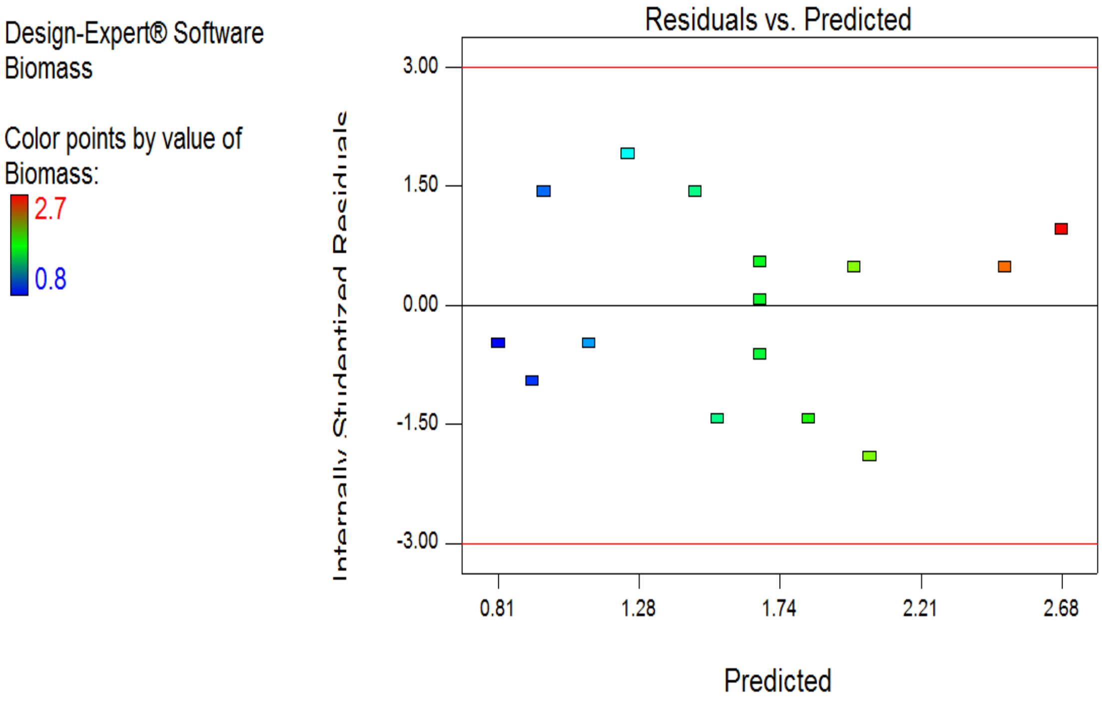


Figure 4.2. Plot of internally studentized results vs. predicted for biomass production.

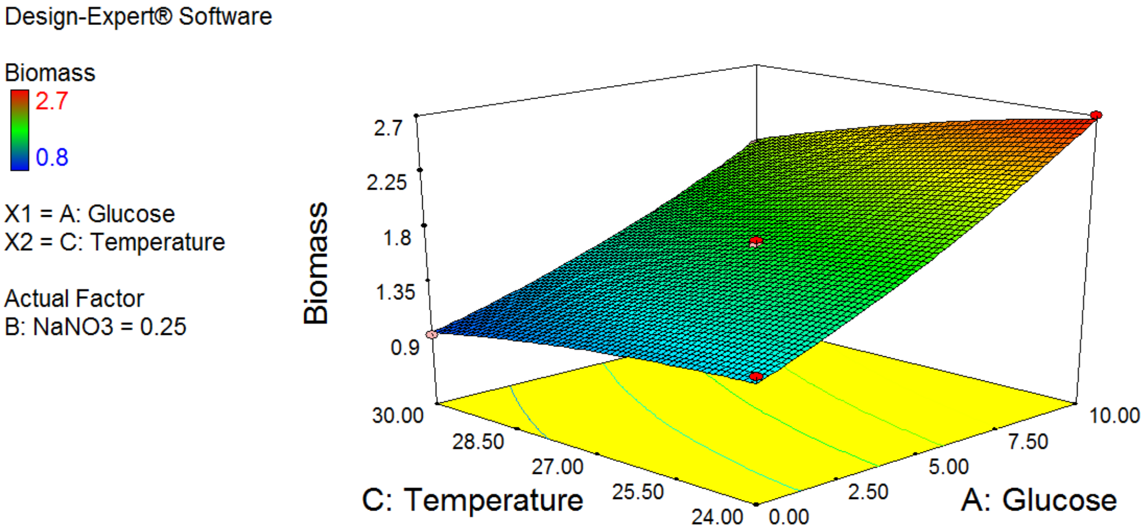
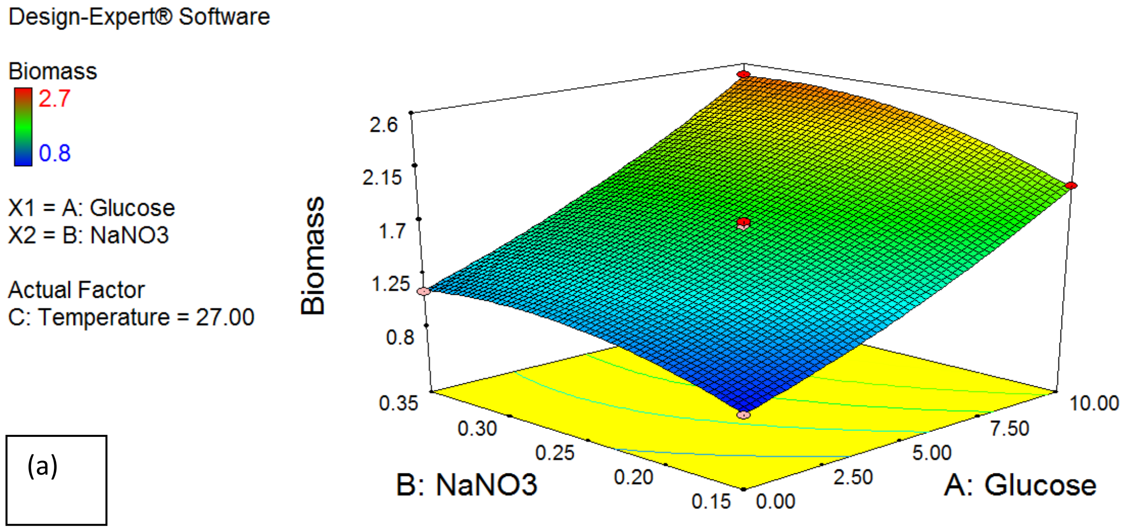


Figure 4.3. Plot of predicted values of biomass vs. experimental values.

4.6.2 Graphical interpretation of the response surface model.

To explore the impact of different independent variables on algal biomass (g/L) and to optimize each factor value, the 3D response curves were plotted, as portrayed in Figure 4.4 a-c. The 3D contours plots show the interaction of two factors while keeping one constant.



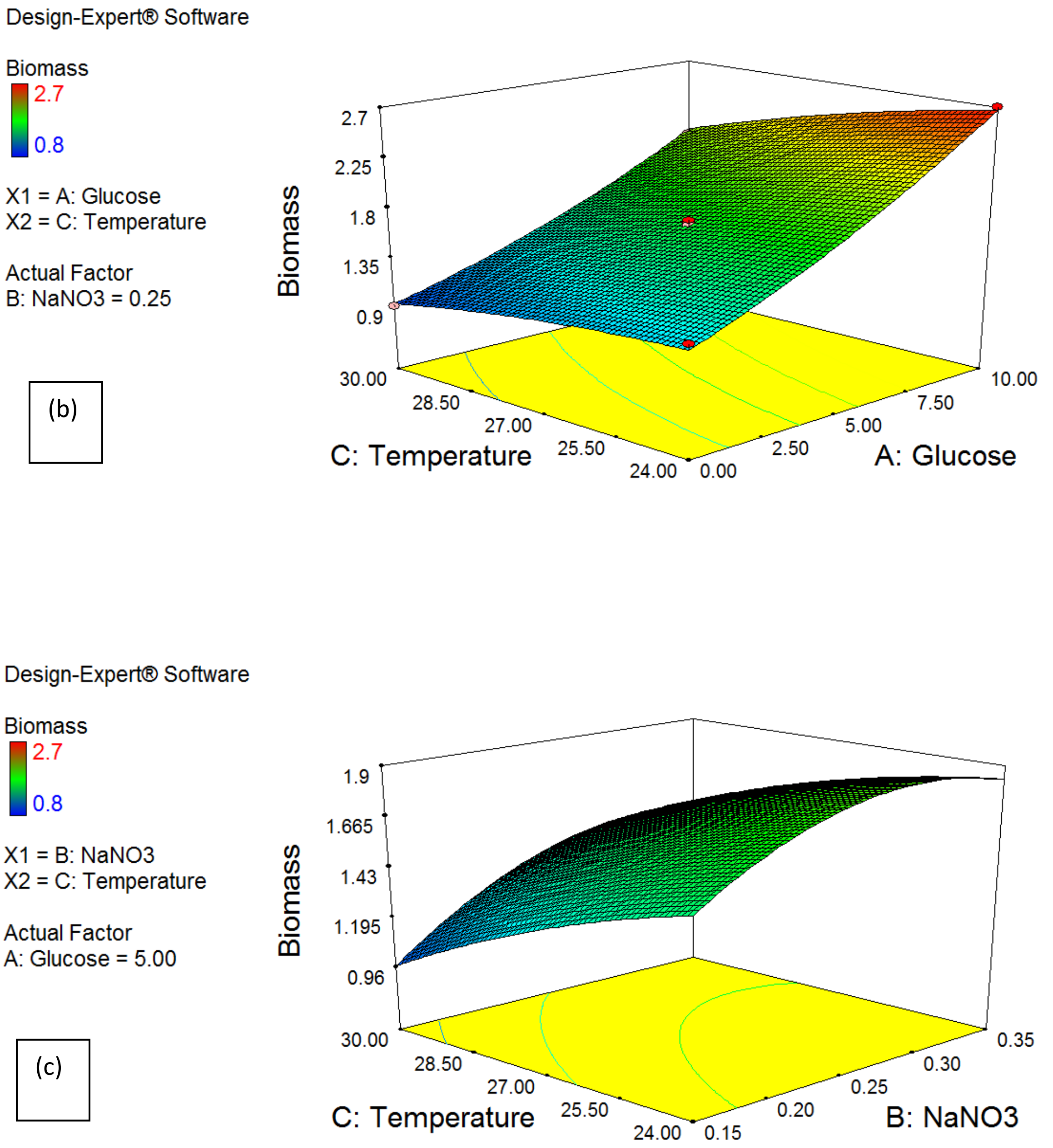


Fig. 4.4 (a-c). Response surface plots (3D) demonstrating the impact of three examined factors on algal biomass yield (g/L), while to analyzing the optimum parameters using the accompanying pair of parameters, by keeping one parameter constant at midpoint.The relation among the use of glucose and sodium nitrate focus on biomass yield shown as a;

connection amongst glucose and temperature biomass yield are presented in b; while exhibit the associations amongst the sodium nitrate and temperature.

As depicted in Figure 4.4 a-c the growth of algae highly depends upon the glucose concentration in the media, the biomass of algae increases by increasing the glucose amount. This can be described with the fact that during heterotrophic cultivation mode, the glucose is the favorite carbon supplement (Jiang and Chen, 2000). Moreover, *N. oleoabundans* can metabolize glucose easily. The mostly two pathways pentose phosphate and Embden-Meyerhof are given by which glucose is catabolized. Only *N. oleoabundans* can assimilate glucose in heterotrophic condition because of the presence of active enzyme while few do not assimilate glucose such as Prymnesium parvum (Neilson and Lewin, 1974). However, increasing the amount of sodium nitrate also enhance the growth of microalgae, but it decreases the lipid content in the microalgae. Temperature is another factor that effects the growth up to a great extent. At low temperature the growth obtained was high as compared to high temperature but it requires more days of cultivation.

4.6.3 Suggested solution 1

Keeping in mind the end goal to locate the suitable culture media conditions to improve the algal biomass, a few factors ought to be considered. Among them, the amount of two noteworthy components: carbon and nitrogen are the essential sources for preparing culture and maintaining at some temperature. Considering the responses, it is evident that the glucose, nitrate, and temperature significantly affected algae growth and its biomass. The outcomes come to an agreement with introduced data in Table 4.6.

Table 4.6. Range and goal of independent constraints

Name	Goal	Lower Limit	Upper Limit	Importance
Glucose	minimize	0	10	3

NaNO3	is in range	0.15	0.35	3
Temperature	is in range	24	30	3
Biomass	maximize	0.8	2.7	5

Table 4.7. Suggested solution by point prediction numerical technique

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Biomass	1.97	0.03	1.88	2.06	0.06	1.81	2.14

Table 4.8. Suggested solution according to desirability

Number	Glucose	NaNO3	Temperature	Biomass	Desirability
1	5.41	0.29	24	1.96	0.5470
2	5.37	0.3	24	1.95	0.5470
3	5.26	0.29	24	1.93	0.5468
4	5.43	0.29	24	1.96	0.5468
5	5.36	0.29	24	1.95	0.5467
6	5.54	0.3	24	1.97	0.5467
7	4.99	0.3	24	1.89	0.5453

The most common and famous method that is used to find the optimum output by adjusting the input values of independent factors is a desirability function for different responses. Desirability function range varies from zero outside the points of confinement to the main objective either to maximize or minimize. The achieve main objective the independent factors have five choices: none, maximize, minimize, target and in range. In this study, 'Numerical optimization tab of the design expert has been selected keeping in mind the end goal to locate the particular point that maximize the desirability as shown in Table 4.6 . The software looks to maximize the biomass yield. The objective looking for starts at an arbitrary beginning stage and continues up the steepest slant to a most extreme. There might be at least two maxima as a result of curvatures in the 3D diagram having different arrangement while looking at the desirability. Beginning the investigation to find the optimum value from the considered space enhances the odds of finding the best optimum value. (Amini et al., 2008).

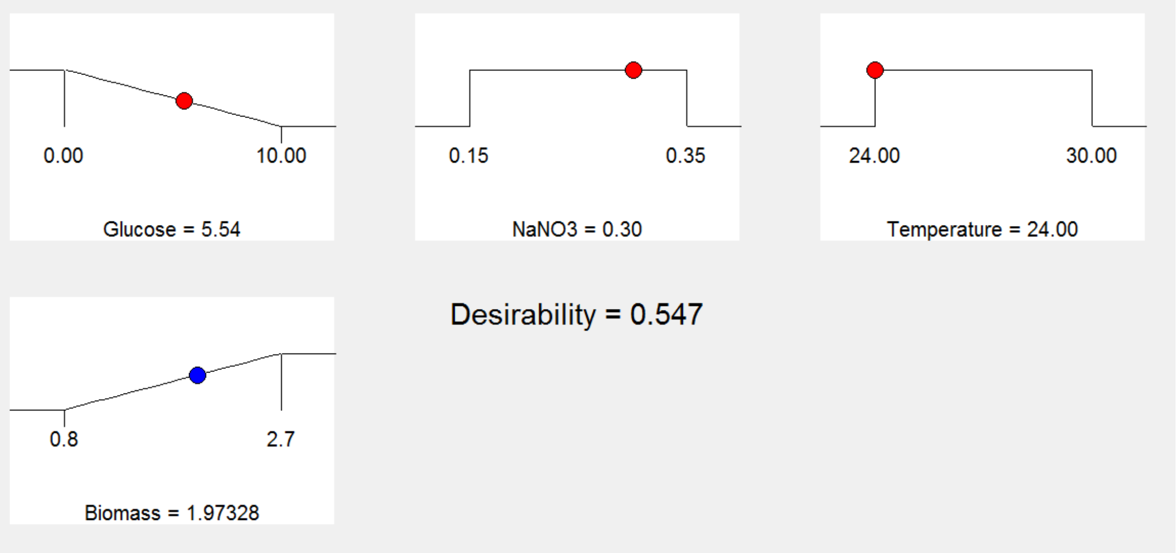


Figure 4.5. Desirability ramps for optimization of responses

The different desirability are then joined to give the complete desirability. The principal target of this work was to optimize the algal biomass g/L by changing the carbon source and culture medium nutrients. Subsequently, the objective to maximize the biomass was selected, with uppermost importance. Keeping in mind the end goal to maximize the algal biomass, for one case temperature and $NaNO_3$, the option 'in the range was chosen, while the amount of glucose chosen was minimum (Table 4.6) because it increases the cost of algae cultivation. The total 30 cycles for every optimization were selected and the value of epsilon was kept minimum in order to wipe out identical solutions . The values obtained from optimization solutions are demonstrated in Table 4.7. Seven arrangements were recommended by design expert. The desirability of first six solutions were same while the seventh solution have desirability value equal to 0.545. Solution number six was selected because it has maximum biomass 1.97 g/L at glucose and sodium nitrate concentration of

5.54 g/L and 0.30 g/L at temperature of 24°C. By comparing the solution six and seven where desirability value is different it was found that biomass can be increased by roughly 4.2% by increasing the amount of glucose by 11%, while keeping another factors constant as shown in Table 4.8. Figure 4.5 shows the ramps of the solution formed after optimization. The individual value of desirability for every factors and combined desirability is shown in Figure 4.6.

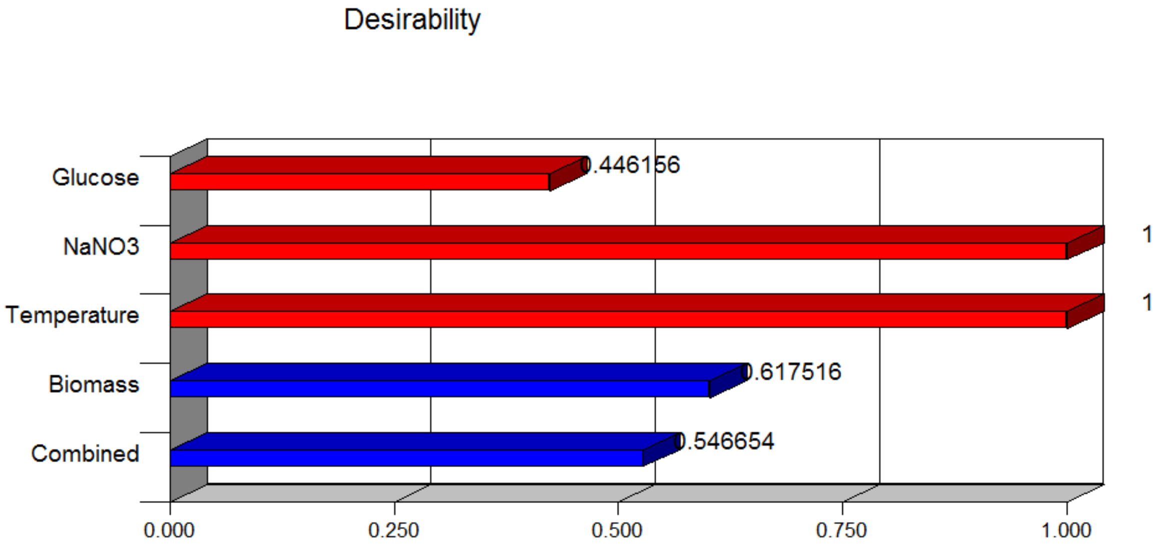


Figure 4.6. Bar graph illustrates the individual desirability of responses

4.6.4 Suggested solution 2

It was observed from the experiment that the lipid content highly depend upon the nitrate concentration in the medium at low concentration lipid contents are high. Moreover, temperature also play significant role in algal growth it was found that at 30°C the algae reach at peak value of biomass with in 9 days and harvested. So, one more solution was obtained from the RSM software the conditions for optimization were given in the Table 4.9. during this optimization the main goal was to maximize the biomass yield with high lipid content. so in this case the amount of glucose was again chosen to minium in order to reduce the cost of carbon sources and $NaNO_3$ was also selected minimum as already explained that lipid contents are higher at low nitrate concentration. Moreover, temperature in this case was taken equal to target value which is taken as 30^{o}C. the values obtained from the software are demonstrated in Table 4.9. in this condition only one solution was recommended by the software at desirability of 0.5 and the above given solution was selected in which the maximum biomass was 1.59 g/L at 28^{o}C. when this solution was compared with above solution it was concluded that despite of less biomass the lipid content were more and the maximum biomass was also obtained within 9 days as compared to the above suggested solution. So, all the experiments were conducted at this suggested solution. The desirability ramps and bar graph for the solution is given in Figure 4.7 and 4.8.

Table 4.9: Range and goal of independent constraints

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Glucose	minimize	0	10	1	1	3
NaNO3	minimize	0.15	0.35	1	1	3
Temperature	target = 30	24	30	1	1	3
Biomass	maximize	0.8	2.7	1	1	5

Table 4.10: Suggested solution according to desirability

Solutions

Number	Glucose	NaNO3	Temperature	Biomass	Desirability	
1	6.19	0.22	28.42	1.598091	0.513327	Selected

Table 4.11: Suggested solution by point prediction numerical technique

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Biomass	1.598	0.027853	1.526492	1.669689	0.059407	1.44538119	1.7508

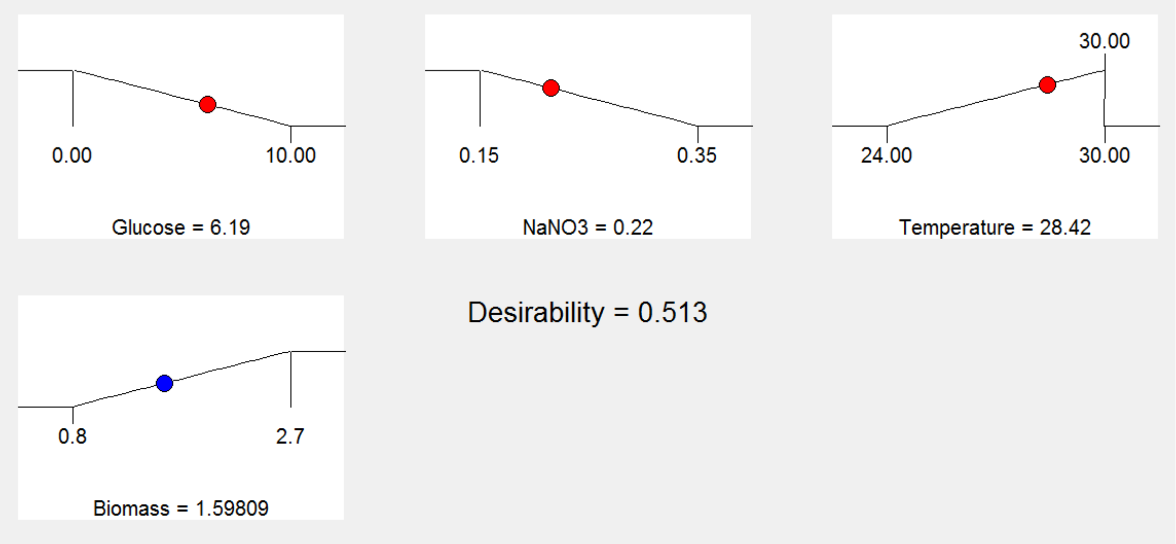


Figure 4.7: Desirability ramps for optimization of responses

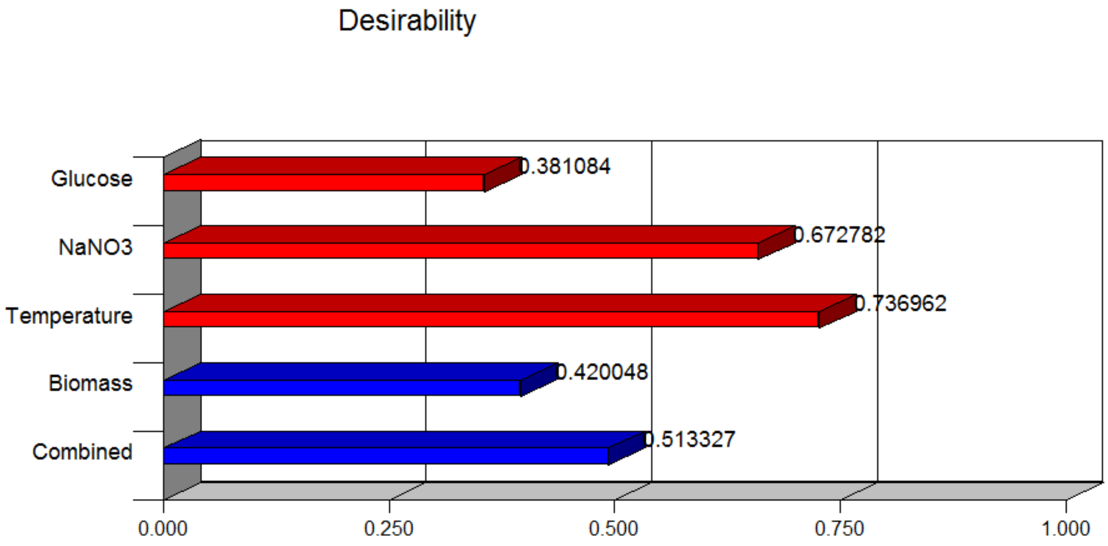


Figure 4.8: Bar graph illustrates the individual desirability of responses

4.6.5 Model validation and experimental confirmation

To show the validation of optimizing value obtained from second-order polynomial equation 4.2 using design expert (Stat-Ease ver. 7). The maximum biomass 1.97 g/L was calculated at the given optimum conditions, glucose 5.54 g/L, sodium nitrate 0.29 g/L and temperature 24^{0}C. At recommended culture conditions approximately 2.5 folds higher biomass was obtained as compared to the standard Bristol culture medium. To validate the model, the experiments were conducted in triplicate at the optimum parameters. As shown in Table 4.12 the average algal biomass 1.79 and 1.42 was obtained by both the methods which was almost equal to the predicted value by design expert, that shows the validity of polynomial equation 4.2. The predicted values and experimental values are similar this shows the adequacy of the predicted model using RSM, and it was successful in optimizing culture medium for the growth of *N. oleoabundans.* Here we affirm the strength of this software for increasing the biomass of microalgae.

Table 4.12: Optimized Process Parameters for microalgae *Neochloris oleoabundans*

Suggested solution	Optimized value of input factors			Biomass Predicted value g/L	Biomass experimental value g/L
	A	B	C		
1	5.54	0.29	24	1.97	1.79 ± 0.17
2	6.19	28.42	28.42	1.59	1.42 ± 0.11

4.7 Results of different harvesting methods

As explained above for harvesting of algae sedimentation, centrifugation and flocculent method was used. The 1L prepared solution was divided into 12 tubes, 25 ml in each tube. Further, these tubes are treated according to the type of harvesting method as explained in above section. As illustrated in Table 4.13 the biomass obtained from different harvesting methods. It was clear from the Table 4.13 that the best method for harvesting algae is the use of flocculant. While sedimentation and centrifugation method combination as well as centrifugation method approximately equal values are obtained. While the lowest biomass was obtained in sedimentation process this may be because within 24 hours the biomass could not settle down some biomass may remain suspended in the solution.

Table 4.13: The algal biomass obtained in different harvesting methods**.**

S. No.	**Harvesting method**	**Amount of biomass (mg) mean** ±SD
1	Sedimentation	25.9 ± 3.3
2	Flocculation	40.5 ± 1.8
3	Centrifugation	32.43 ± 2.6
4	Sedimentation + Centrifugation	32.53 ± 2.5

CHAPTER 5

Biodiesel production using ultrasonic reactor and magnetic stirrer

5.1. Introduction

The biodiesel production from microalgae is still in its initial phase to improve the production on an industrial scale; it is essential to use advanced techniques. Thus, this chapter discusses the biodiesel production from microalgae using ultrasonic reactor and results were compared with the simple conventional magnetic stirring method.

5.2 Ultrasound assisted biodiesel production

5.2.1 Principle

Ultrasound waves are the sound waves that have a higher frequency than the frequency at which human hears, which is about 20 kHz. This type of sound waves give energy to the immiscible liquid and produce powerful vibrations, which creates “cavitation” bubbles. When these waves pass through the fluid, the bubbles collapse, leads to an abrupt contraction of the liquid, thus enhance the mixing in the region of collapsed bubbles. In cavitation, the bubbles are formed in low vapour region, usually at phase boundaries. With intense energy action on the fluid, reaction time decreases with increase in the cavitation of the mixture; such reactions are completed somewhat above the atmospheric temperature.

Ultrasound is described by its frequency (k/Hz) along with intensity (W/cm^2). At high frequency the sonotrode vibration increases, which produce tiny bubbles, it further increases the surface area for emulsification of methanol and oil. Investigations were performed by different researchers within the frequency range of 24 to 1300 kHz to optimize the yield of biodiesel. Moreover, with an increase in intensity, the probe movement increases because with an increase in frequency the magnitude of vibration increases. Consequently, the emulsification efficiency increases, and ultrasound wave travel more distance in the immiscible fluid. Apart from intensity and frequency it is essential to optimize the power input requirement for transesterification. A large number of trials were performed by various researchers in the range of 20- 24 kHz.

During the beginning of transesterification reaction, vigorous mixing is essential to produce adequate interaction among the microalgae oil and alcohol. Ultrasound provides the convenient way to mix the two immiscible fluids, it increases the mass transfer to a great extent at micro-level, consequently the reaction rate increases.

The transesterification reaction utilizing ultrasonic reactor completes within a short time, and fewer amounts of catalyst and methanol are needed for this type of reaction. The ultrasonic reactor can be used for a large number of feedstocks, can utilize ethanol or methanol. A catalyst such as H_2SO_4, NaOH, KOH, and enzymes can also be used. Enzyme activity does not affected by the use of ultrasonic reactor.

5.2.2. Experimental Set-up

The schematic diagram of single frequency horn type reactor is shown in Figure 5.1 and 5.2 shows the actual equipment's. In this type of reactor, the horn is connected with the transducer that creates ultrasonic irradiation in the mixture. For ultrasonic transesterification, an ultrasonic homogenizer with power output 50 W having a frequency range of 27–30 kHz was used. The beaker was kept at the bottom and transducer ought to be dipped in the alcohol and oil mixture up to 2 cm. The given quantity of oil was poured into the beaker and mixture of methanol blended with NaOH was added to it. The immiscible fluids were mixed using ultrasonic cavitation to increase the rate of reaction.

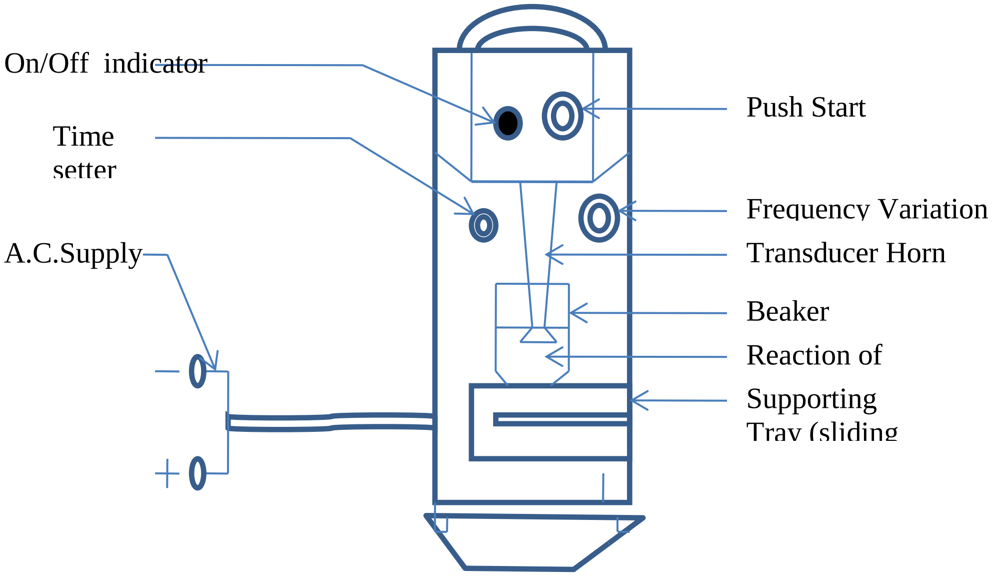


Figure 5.1. Schematic diagram of ultrasonic horn type reactor

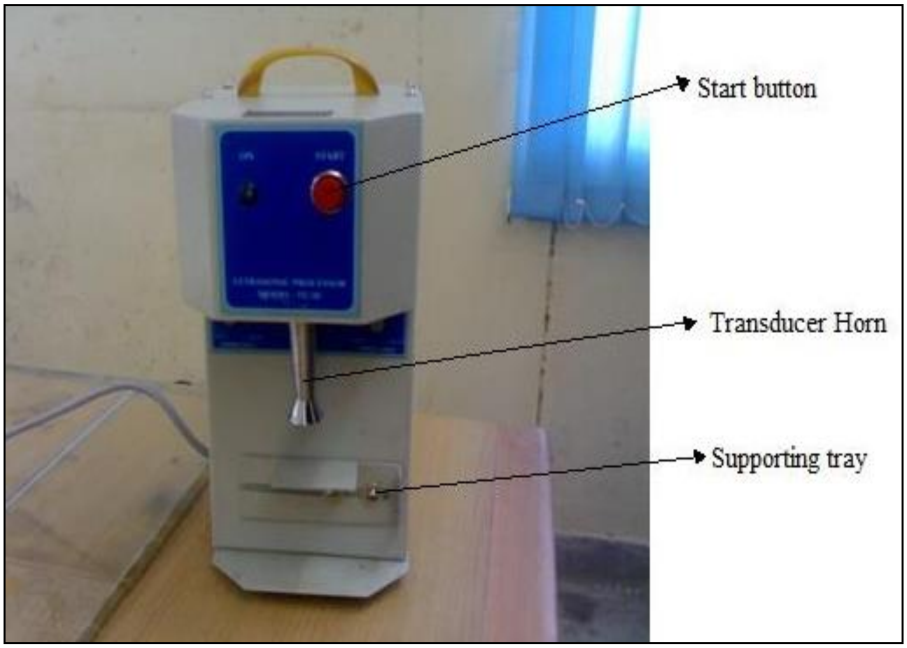


Figure 5.2 Photograph of ultrasonic horn type reactor

5.2.3. Experimental procedure for ultrasonic cavitation

The experiment was performed to optimize the biodiesel yield (%) and time. Algal oil sample of 50 g was taken for both ultrasonic method and mechanical stirring method to compare the results. The mixture of algal oil, methanol, and catalyst were brought in contact with probe of ultrasonic reactor (model TU-50). The experiment was performed at 28.5 kHz frequency.

5.3. Mechanical stirring method

In this method, the oil and methanol were mixed using a magnetic capsule which was dipped in the mixture. Rotation of the capsule forms a whirl in the fluid, which creates a disturbance at the phase boundary between the two fluids. It has a temperature controller that controls the temperature between 60-70^{o}C, and speed controller controls the speed of the stirrer. On this instrument hot plate act as a heating source to heat the two-phase mixture. The transesterification reaction takes place when a measurable amount of alcohol mixed with catalyst was transferred to the glass beaker and then the magnetic capsule is used to mix the solution running at specific rpm.

5.3.1 Experimental set-up and working procedure

The transesterification reaction to convert algae oil to its methyl ester was performed in a magnetic mechanical stirrer, the setup of which is shown in Figure 5.3. The temperature was measured with the help of a thermometer. The experiments were performed to optimize the biodiesel yield and time. Microalgae oil 50 g was poured into 250 ml beaker and heated above 110^{o}C to remove the moisture content from the oil to prevent soap formation. The sample of oil further cooled to 65^{o}C to proceed for further step. Then the mixture of methanol and catalyst (NaOH) was prepared and stirred correctly to dissolve the NaOH completely. Three different oil to methanol ratios were selected for experimentation, i.e. 1:4.5 and 1:6 and 1:9 while catalyst was taken in 4 different weight percentage of oil which is 0.5%, 0.75%, 1% 1.25% as shown in Table 5.1. The above-prepared mixture was added to algae oil sample. The two-phase mixture was obtained in which oil does not mix with methanol because of high density difference. In this prepared mixture magnetic capsule was used to mix the fluid with the help of a stirrer. After the completion of reaction, it was kept for gravity separation for 2 to 3 hr. Two layers were formed; top layer contains methyl ester, and bottom layer consists of glycerol. Algae oil methyl esters were separated and kept for purification. To remove the impurities such as catalyst, water is heated up to 60°C and added to the algae oil methyl ester and held for gravity separation. Water has higher specific gravity due to which it was settled down at the bottom. Finally, the moisture content in separated methyl ester was removed by heating this methyl ester sample. During the process, the excess methanol in the mixture was extracted by distillation process.

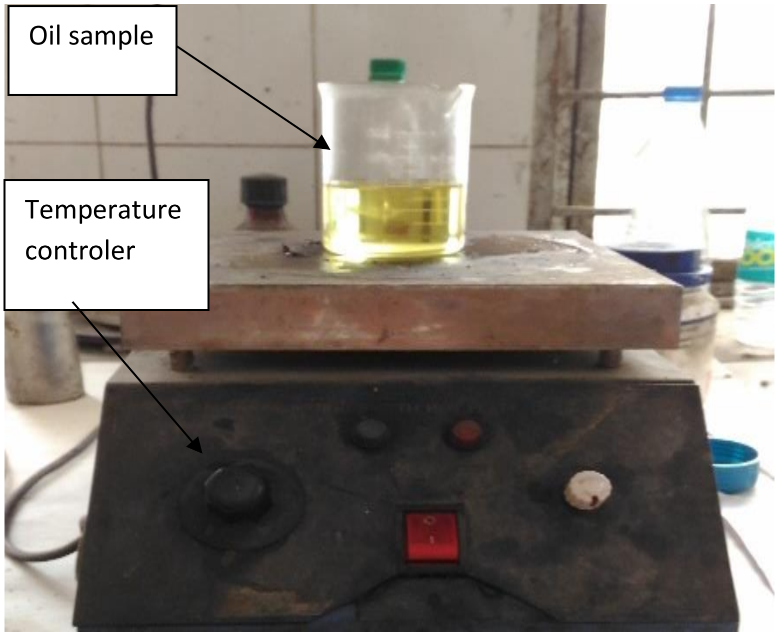


Figure 5.3: Photograph of Magnetic Stirrer

Table 5.1. Range of different factors for transesterification.

Molar ratio (methanol/oil)	Quantity of microalgal oil (g)	0.5 %	0.75 %	1.0 %	1.25%
6:1	50	0.25	0.375	0.5	0.625
4.5:1	50	0.25	0.375	0.5	0.625
9:1	50	0.25	0.375	0.5	0.625

5.4 Comparison of ultrasonic cavitation (US) and magnetic stirrer (MS) method at different molar ratios

Figure 5.4 shows algal oil methyl ester yield at 4.5:1 ratio by both ultrasonic (US) and magnetic stirrer (MS) method. It was found that using ultrasonic reactor 79% yield was obtained within 20 minutes of reaction. However,using magnetic stirrer 83% yield was obtained in 80 minutes. Thus, it was concluded that using ultrasonic reactor the time of reaction decreases due to intensive mixing of immiscible fluids. Figure 5.5 shows the biodiesel yield by US and MS method at 6:1. This is the optimum molar ratio at which 97% yield is obtained within 30 minutes at catalyst concentration of 1% using ultrasonic reactor. While using magnetic stirrer 90% yield was obtained at 80 minutes. Moreover, 91% yield was also obtained within 20 minutes at 6:1 molar ratio. It is also reported that biodiesel yield trend increases with an increase in reaction time similar trend obtained in this work as shown in Figure 5.4, 5.5 and 5.6. Thus, it is concluded that using ultrasonic reactor for transesterification reaction the reaction time decreases, and a constant value is obtained after 30 minute as shown in Figure 5.4, 5.5 and 5.6. In present work highest yield of biodiesel was obtained at catalyst concentration 1% and molar ratio 6:1 for both ultrasonic reactor (US) and mechanical stirring method (MS). The maximum yield 83% was achieved within 20 minutes by ultrasonic cavitation technique at molar ratio 9:1. However maximum yield 89% was obtained after 80 minutes by the conventional mechanical stirring method at molar ratio 9:1. As shown in Figure 5.6 lower yield was obtained at molar ratio 9:1 as compared to molar ratio 6:1 at catalyst concentration of 1% with ultrasonic reactor as well as magnetic stirrer method, this is due to the excess amount of alcohol to oil ratio, that helps in reversing the direction of reaction as the reaction is reversible. While on the other hand, with magnetic stirrer method low yield 83.2% was obtained at molar ratio 4.5:1 using MS method because this molar ratio is not sufficient to complete the transesterification reaction. Thus, from all the figures it is concluded that molar ratio 6:1 is the best ratio at which maximum yield was obtained for both ultrasonic and magnetic stirrer method at catalyst concentration of 1%. Biodiesel yield highly depends upon the molar ratio in this work molar ratio was varied from 4.5:1 to 9:1. Initially, when molar ratio was increased from 4.5:1 to 6:1 the increase in biodiesel yield was obtained. So, it was concluded that excess alcohol was needed to complete the reaction in forward direction. Generally, 3 moles of alcohol and one mole of oil is required at chemically correct ratio to complete the reaction. However, as this is a reversible reaction so large amount of methanol is needed to complete the reaction in forward direction. But, when the alcohol content exceeds the certain optimal value the reverse of reaction was observed, because at higher molar ratio it become difficult to remove glycerin from the solution as excess availability increases the solubility of solution. Increase in solubility of glycerin in solution drive the chemical reaction in backward direction thus decreasing the biodiesel yield as well as increasing the cost of production of biodiesel

(Barnwal and sharma., 2004). So, in this process molar ratio 6:1 was the optimum condition as explained above. It can be clearly discovered from the Figure 5.4, 5.5, 5.6, that the time required to complete the transesterification reaction using ultrasonic reactor is extremely low as compared to magnetic stirring method at all the given molar ratio. This phenomena is observed because of the cavitation, this phenomena mix properly the blend of oil with methanol thus increase the rate of reaction and complete the reaction at faster rate. The maximum yield was obtained at 1% catalyst concentration.

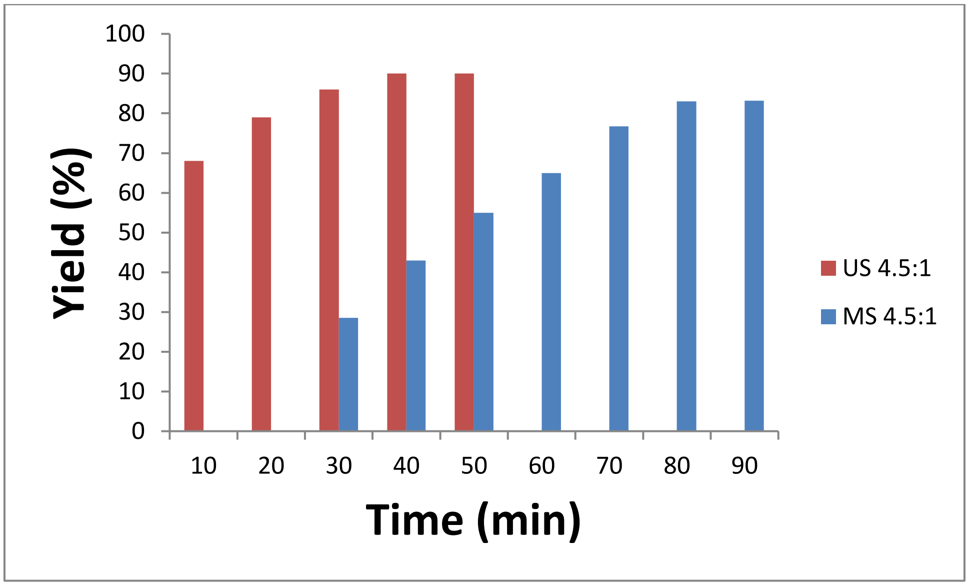


Figure 5.4 Comparison of yield (%) by different methods for 1% catalyst

Thus, from above figures, it was clear that transesterification reaction highly depends upon time and molar ratio as well as method of stirring (US or MS). It is evident that using sonication for stirring the yield increases and time of reaction decreases as compare to the conventional method. But in this case catalyst amount does not change and remain same for both the techniques. The similar trend of optimum molar ratio 6:1 and catalyst 1 wt% of the oil has been reported in research work for vegetable oil using conventional transesterification technique (Mustafa et al., 2010).

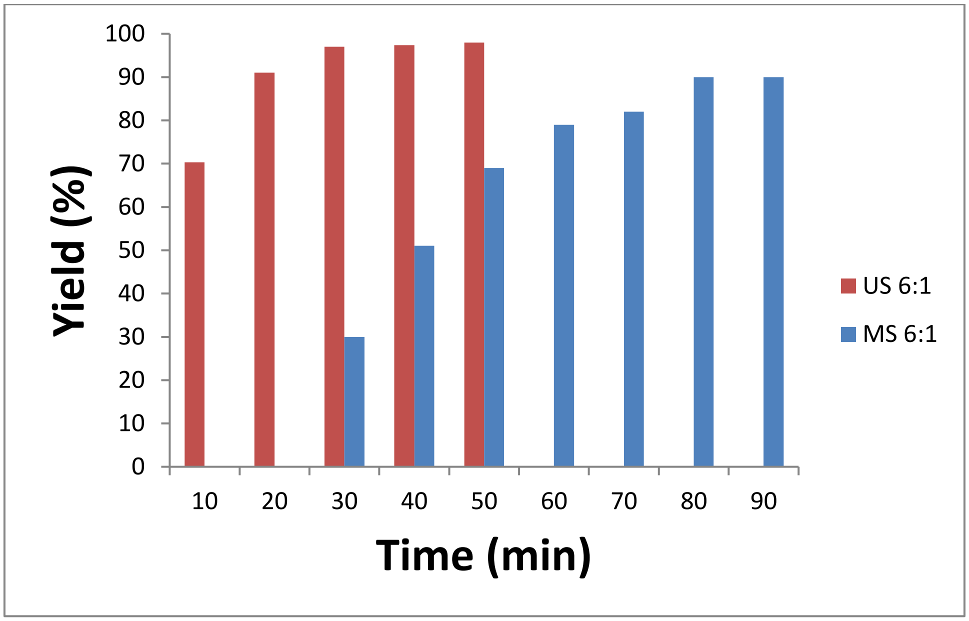


Figure 5.5: Comparison of yield (%) by various techniques for 1% catalyst

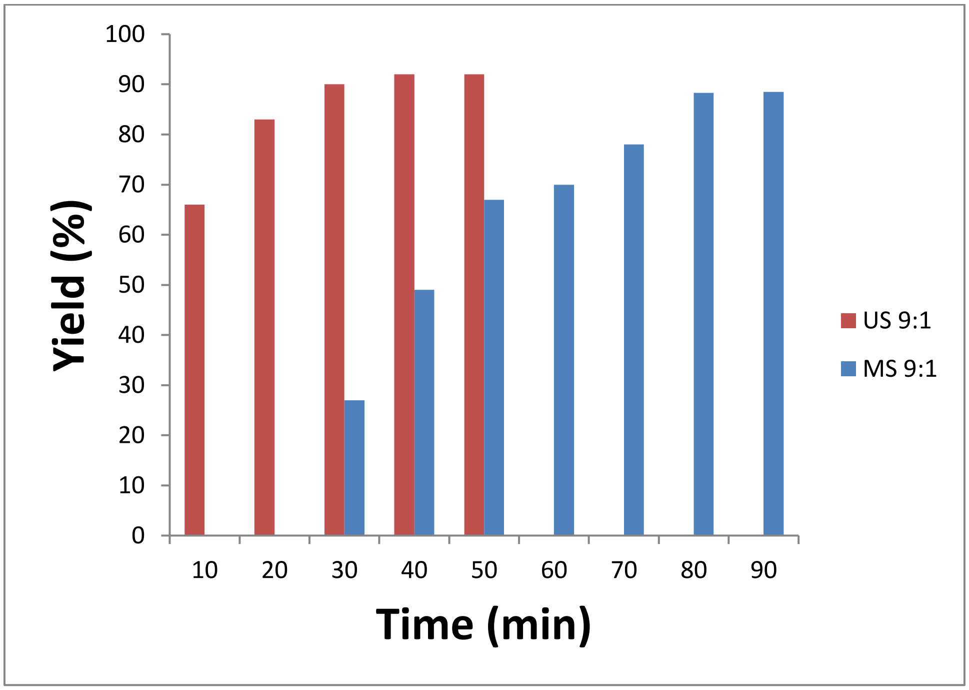
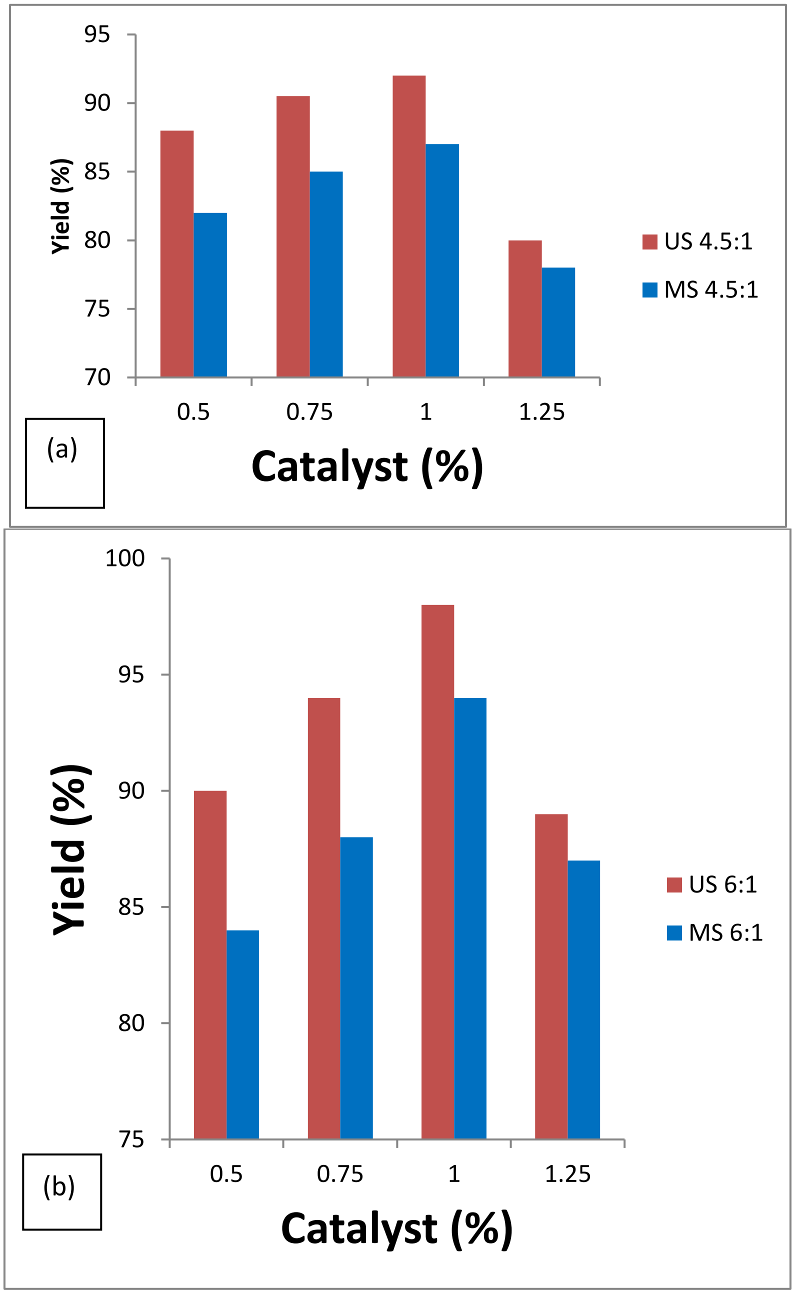


Figure 5.6: Comparison of yield (%) by different methods for 1% catalyst

5.4.1 Yield variation wrt catalyst for molar ratio 4.5:1, 6:1 and 9:1

Catalyst helps in increasing the rate of reaction. An excess amount of catalyst does not allow to mix the solutuion completely because of excess viscosity thus consumes a large amount of power to mix. However, when the amount of catalyst was not adequate than it is tough to obtain the optimum yield of biodiesel. So, it is essential to investigate the optimum catalyst concentration so that maximum yield can be obtained. Thus, in this case, maximum biodiesel yield was collected at 1% catalyst concentration. The variation of biodiesel yield with variation in catalyst concentration is demonstrated in Figure 5.7 (a-c). Microalgae oil biodiesel yield utilizing ultrasonic stirring method has highest yield at 1% catalyst concentration and lowest at 0.5% catalyst concentration. Similar trend using magnetic stirrer method was obtained. It is very clear from the Figure that 0.5% and 0.75% of catalyst concentration is not sufficient to increase the rate of reaction. Hence the maximum biodiesel yield is obtained at 1% catalyst concentration at 6:1 molar ratio. Figure 5.7 (a-c) illustrates the yield wrt to catalyst concentration; it is conferred from the Figure 5.7 (a) that at 4.5:1, similar results are obtained, and maximum yield was again at 1% concentration of catalyst. Moreover, it was also observed that while using ultrasonic reactor 94 % yield was obtained at 0.75% catalyst concentration when molar ratio was taken as 6:1. Thus it was concluded that for ultrasonic reactor, 0.75% catalyst concentration was sufficient for the completion of reaction. But in case of magnetic stirrer at 0.75% catalyst concentration comparatively lower yields were obtained. So, for magnetic stirrer 1% catalyst is sufficient to carry out the reaction. Additionally, it was observed that the yield start decreasing with increase in catalyst concentration beyond 1%. Sultana Shazia et al., 2014 studied the effect of NaOH concentration between the range of 0.1-0.9 wt% and obtained that yield increases with increase in catalyst concentration from 0.1-0.5%. The yield decreases with further increase in NaOH concentration and reduced to 50% with 1.5% NaOH concentration. This is because with increase in the concentration of catalyst, soap formation will take place and reduce the yield with increase in viscosity.



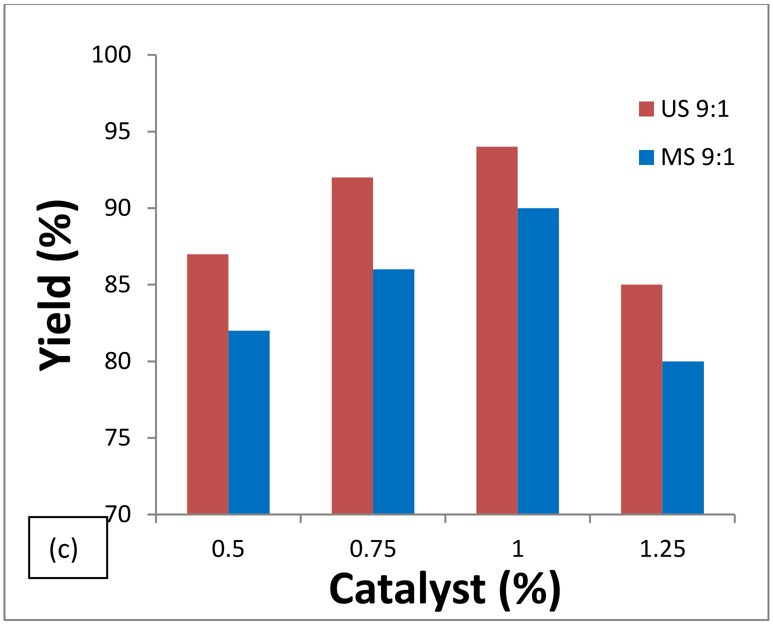


Figure 5.7 **(a-c)** Comparison of yield data for microalgae oil using ultrasound and conventional stirring method for molar ratio 4.5:1 6:1, 9:1wrt catalyst.

5.5 Direct transesterification of wet algae.

For direct transesterification, Lepage & Roy (1986) method was adopted and modified. The solution was made by adding solvent (methanol: chloroform 3:1) and wet algae biomass. Further, in the above solution 2 to 12% methanolic HCl was added to the glass tube and sealed with a cap. This Methanolic HCl was prepared according to Sukhija and Palmquist, (1988). For 5% methanolic HCl, 10 ml of acetyl chloride was added drop by drop to 100 ml methanol. Glass tubes were placed in water bath at 90^{0}C for 80 minutes to complete the reaction. After that, samples were taken out and cooled to room temperature. To neutralize the reaction 0.9%, NaCl was added. FAMEs were collected by adding 3 ml of hexane to solution and heating at 90^{0}C for 20 min. Finally, the solution was centrifuged at 2000 rpm to separate biomass. Upper layer containing FAME was collected and quantified by GCMS. Biodiesel yield depends upon various parameters such as moisture content in algae and time. Table 5.2 shows the optimization parameter range. For measuring the effect of different parameters microalgae biomass with constant moisture content 20% was taken. All the work was done in triplicate.

Table 5.2 Range of parameters for direct transesterification optimization.

Different parameters	**Range**
Moisture content (%)	0-50
Solvent/ Algae biomass ml/g	3.5-10.5
Methanolic HCL (%)	2-12

5.6 Effect of amount of moisture present in algae and its impact on biodiesel yield

Algae was harvested using centrifugation method which was 19 wt% dry. To remove more moisture additional heating was done. The percentage of moisture content was calculated using equation 1 (Kabutey et al., 2011). To check the effect of moisture, different percentage of moisture was taken.

$$MP(\%) = \frac{P_i - P_f}{P_i} \times 100 \tag{5.1}$$

5.7 Results and discussion

5.7.1. Effect of different moisture levels on FAME yield.

Wet algal mass 200mg (equivalent to dried algal mass) was directly transesterified using methanolic HCl and solvent. The maximum FAME yield 0.43 g was obtained when algal biomass had 0% moisture content and utilized for comparison with different percentage of moisture. Figure 5.8 shows the FAME yield by varying moisture content for different methanolic HCl amounts. Methanolic HCl amount was varied from 1.5 ml to 4ml; maximum

FAME yield was obtained at 3ml at all moisture level. As the moisture content increases in algal biomass, there was a significant decrease in biodiesel yield as shown in Figure 5.8. The result indicates that moisture in algal biomass affects negatively on the biodiesel yield. However, increase in methanolic HCl results in a higher yield. Biodiesel yield is low at higher moisture content because as transesterification reaction is a reversible reaction presence of moisture content hydrolyzes the FAME in reverse direction producing methanol and free fatty acids. Moreover, the presence of moisture in the biomass does not allow the oil to come in contact with solvents and thus inhibits the oil to become a part of the reaction. Therefore more amount of methanolic HCl results in a higher yield, as it enhances the oil contact with methanol. It was observed that at 4 ml methanolic HCl the FAME yield is same as at 3ml so using 4 ml solution is a wastage of money and uneconomical.

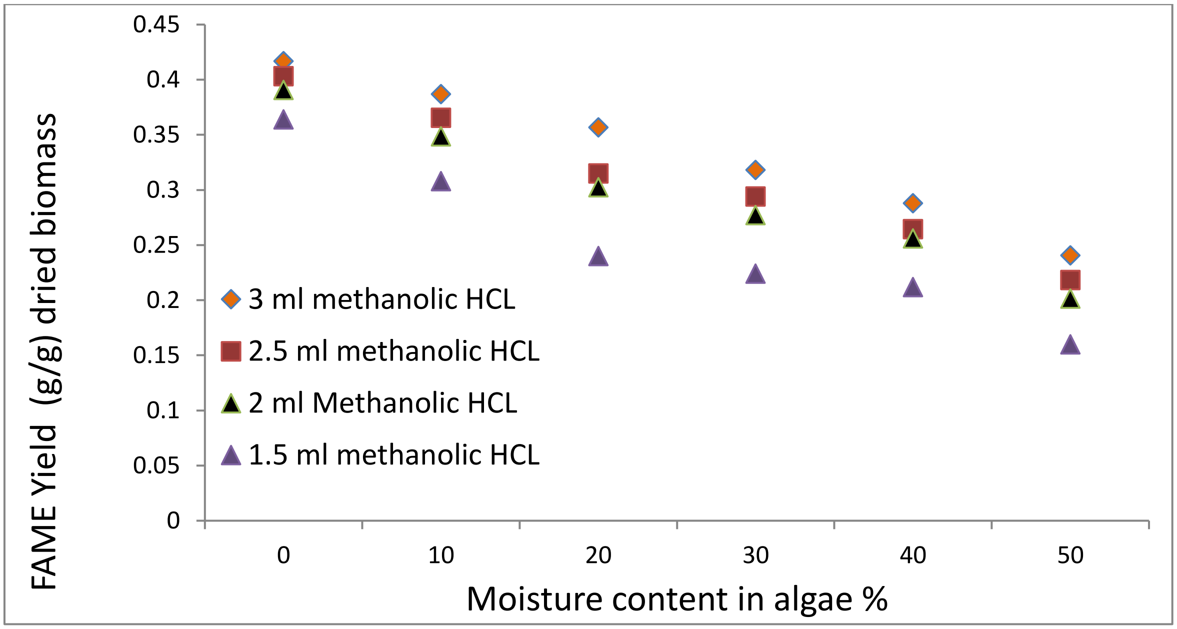


Figure 5.8: FAME yields of wet microalgae with different methanolic HCl amount at different moisture contents with a fixed amount of solvent (7.5 ml).

5.7.2. Optimization of different extraction parameters

For optimization purpose, the moisture content in the algae was set at 20%, and yield was analyzed with an increase in the percentage of methanolic HCl, and additional amount of solvent (methanol/ chloroform) effect was examined. The methanolic HCl percentage ranged from 2 to 12% and prepared according to Sukhija and Palmquist, (1988). The maximum yield 0.352g was obtained at 8% methanolic HCl as shown in Figure 5.9. In in-situ transesterification, with methanolic HCl, it solves the purpose of both solvent as well as a catalyst. HCl act as a catalyst while acetyl chloride act as a solvent so due to increase in the percentage of methanolic HCl, the effect of catalyst HCl enhances which results in higher yield. But beyond 8% methanolic HCl, that is at 10%, adverse effect was observed, and yield was reduced because of the formation of undesired and unidentified derivatives. To determine the effect of solvent, on the fatty acid methyl ester yield, the solvent amount was varied from 3.5ml to 10.5ml, and optimum volume of solvent was 7.5ml/g of algae biomass as shown in Figure 5.10. It is clear from the above data that FAME yield has a direct relation with solvent. When the solvent is low the yield was low because the presence of water in a small volume of solvent makes the lipid and solvent contact harder, resulting in lower yield (Cao et al., 2013). However, when the solvent volume was more than the optimum value, it results in a lower concentration of lipids in solvent and decrease in yield.

5.7.3 FAME and biodiesel yield at optimal condition

All the extraction parameters at optimal condition (3ml methanolic HCl 8%, 7.5 ml solvent 90^0C temperature, and 80 min time) was used for direct transesterification of wet microalgal biomass having 20% moisture. The biodiesel yield obtained at optimal condition was high which found to be 84%.

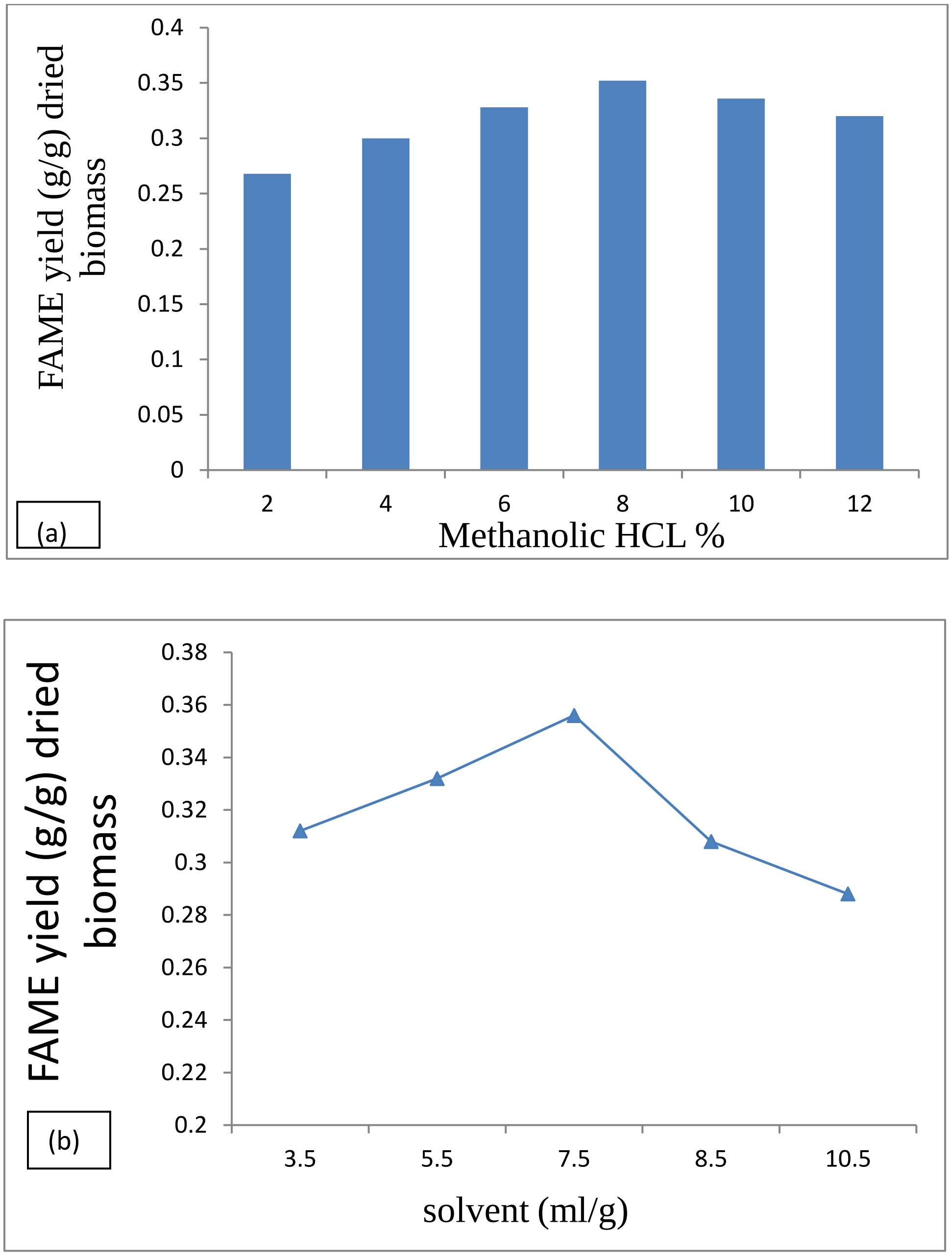


Figure 5.7 (a-b) The FAMEs yield obtained by changing extraction parameters during one step transesterification of *Neochloris oleoabundans* microalgae species (a) the methanolic HCL range from 2 to 12% (b) the solvent volume from 3.5-10.5 ml/g;

5.7.4 Comparison of conventional and microwave assisted -transesterification method

For microwave assisted lipid extraction method 4 g dry algae was mixed with water in the ratio of 1:2 (on wt. basis) and paste was prepared. Then this mixture was microwaved at 600 W power with 10 seconds rest period after 30 seconds operation for 5 min to break the cell wall of microalgae. Lipids were extracted according to the above optimized condition using methanolic HCL and centrifuged to separate the upper layer containing lipids, then measured gravimetrically. It was found that 0.46 g/g lipids were obtained using this method. But this method of biodiesel production is highly energy intensive. Microwave provides energy to reaction solvent through dipole or interfacial polarization and ionic conduction that superheat the solvent. Combining the charged conduction and dipole polarization mechanism is considered as an interfacial polarization (Refaat, 2010). The amount of lipid extracted depends upon the penetration ability of electromagnetic wave into the cell wall. Cell wall of algae will disrupt when the temperature of water in the cell wall exceeds boiling point causing great pressure gradient which results in disruption of the cell wall, thus moving out the lipid and mixing with the solvent (Bhattacharya and Basak, 2006).

The work shows that with microwave assisted direct transesterification 90% yield could be achieved in less time. The biodiesel yield was increased by the use of microwave as compared to conventional direct transesterification method. This was due to disruption of hydrogen bonds caused by molecular dipole rotation in microwave. In microwave heating electromagnetic waves take part in reaction at molecular level resulting in enhancing the solvent diffusion, thus increase in lipid yield.

CHAPTER- 6

EXPERIMENTAL ENGINE SET UP

6.1. Introduction

Laboratory engine setup and test procedure for the engine performance and emissions testing is discussed in this chapter. Also, the results obtained from engine testing of microalgae biodiesel were presented. The performance of algae biodiesel was compared with petrodiesel. Four stroke engine having one cylinder was used for investigation having cooling water system which was coupled to a dynamometer. Cylinder pressure and crank angle were measured by various instruments attached to the engine setup. Engine indicator interfaced signals to a computer that plot P-V and P-θ diagram. Airflow, fuel flow, load, and temperature were interfaced to computer through different sensors. The engine parameters including brake specific fuel consumption (BSFC) and brake thermal efficiency (BTE) were investigated. To assess performance, engine software "Engine soft LV" was utilized and for testing of smoke opacity, AVL DIX was used. From literature review, it was concluded that very less work was conducted on engine performance using microalgae biodiesel blends. So, studying this will bring helps in increasing the value of biodiesel research.

6.2 Engine setup of single cylinder Kirloskar engine

The experimental investigations were performed on a one cylinder, four stroke engine, attached to eddy current dynamometer, having attachments which measure pressure of the cylinder and the crank angle. P-θ and P-V diagram were plotted with the help of a computer. Different sensors are used to estimate the amount of fuel flow and air flow as well as temperature and load. Panel box of the engine has instruments like manometer, engine indicator, process indicator, transmitters for fuel and air flow measurements. Rotameters measures the cooling water flow. The setup has a mechanism by which compression ratio can be varied from 12-18. Using this setup engine performance parameters can be measured for instance BP (brake power), indicated and brake thermal efficiency, frictional power, mechanical power, brake and indicated mean effective pressure, fuel consumption as well as volumetric efficiency. Data acquisition software “Engine soft LV” was utilized for engine performance analysis. A sensing device was used to find the injection pressure of diesel and biodiesel blends, signals from the sensing device were sent to the computer. The picture and schematic diagram of the engine are demonstrated in Figure 6.1 (a and b). Table 6.1 shows the engine specifications.

Table 6.1. Kirloskar diesel engine specification

Engine type	1 cylinder, 4stroke, water cooled
Rated Power	3.5 kW at 1500 rpm
Cylinder Volume	661cc
Compression Ratio	18
Dynamometer	Eddy current, water cooled
Piezo Sensors	Range 5000 PSI
Crank Sensor	Resolution 1 Deg
Load Sensor	Load cell, type strain gauge,
Software	“Engine soft”, Engine Performance analysis software

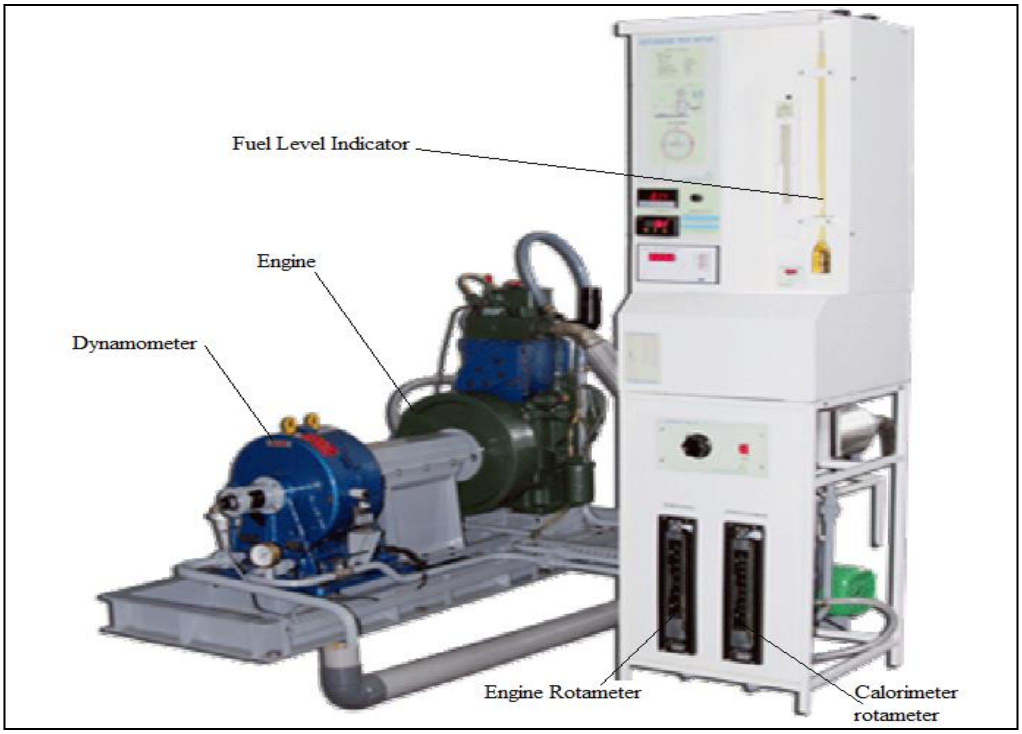


Figure 6.1(a): Actual experimental set-up of Kirloskar diesel engine

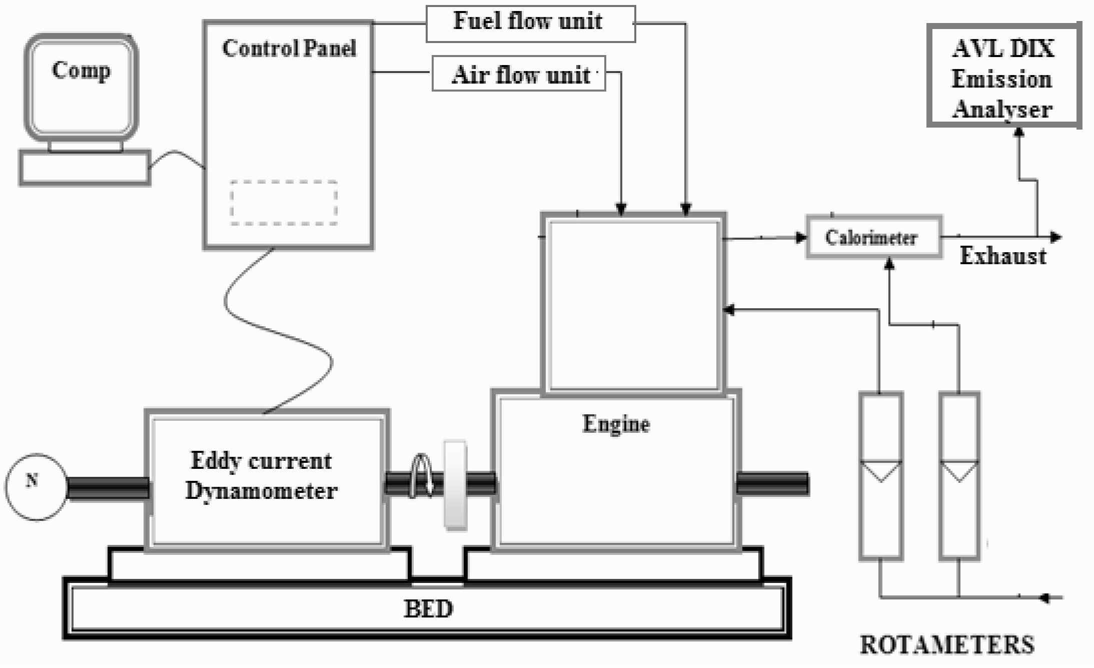


Figure 6.1(b) Schematic Diagram of the Experimental set-up

6.3. Smoke meter

It works on the principle of light extinction. The exhaust from the engine was passed through a long tube which has asolar cell at one end while another end has a light source. The smoke level is indicated directly with the amount of light passed through the column. The smoke concentration is calculated as a ratio of electricity output from solar cell when smoke is flown over it to the electricity output when atmoshpheric air is flown over it. It is calculated with the help of Beer-Lambert law.

$P= e^{-CA}$

$C= mbQ$

P= fraction of light transmitted

A= Light Path

The C = absorption coefficient of the obscuring matter/Length and n is the number of soot particle/ volume and Q is the specific absorbance per particle.

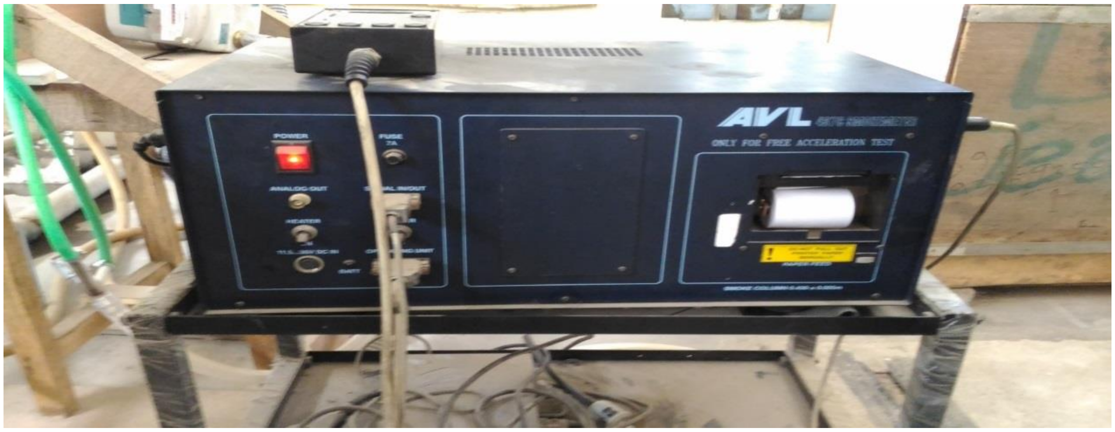


Figure 6.2. A.V.L. smoke meter

6.4 Results and discussion

6.4.1. Comparison of physicochemical properties of microalgae oil biodiesel and diesel

Fatty acid profile and investigation of biodiesel properties are vital steps in order to choose the optimal feedstock for biodiesel production. The fatty acid composition and lipid content may be significantly affected by different culturing systems. But the common fatty acid structure of microalgal oil comprises basically of palmitic, linoleic, oleic, and stearic acid (Knothe, 2009). Fatty acid composition of algal biodiesel was analyzed by gas chromatography and mass spectroscopy (GC-MS). It was found that total saturated fatty acid represented almost 31% while unsaturated fatty acids were almost 67.9%. Table 6.2 shows that palmitic acid is the second chief component which comes after oleic acid. The total polyunsaturated composition was high, i.e. 21.4%, while monounsaturated composition was 2.2%.

Table 6.2. FAME of *N. oleoabundans* oil

Type of fatty acids	*Neochloris oleoabundans* oil (%)
Palmitic (16:0)	28
Palmitoleic (16:1)	2.2
Hexadecadienoic acid (16:2)	0.7
Stearic (18:0)	3
Oleic (18:1)	39
Linoleic (18:2)	19
Linolenic (18:3)	7
∑ Saturated fatty acids	31
∑Unsaturated fatty acids	67.9

Microalgal oil and its biodiesel characterization was done according to standard approaches. The density of the oil after transesterification is within prescribed limit and close to diesel fuel. Pour point, and flash point are within prescribed range as shown in Table 6.3. The calorific value of *N. oleoabundans* biodiesel obtained was (38.6 MJ/kg) similar to that of the petro-diesel. The characteristics of algal methyl ester ascertained in present work are in great accordance with other research work (Gouveia et al., 2009). Thus, *N. oleoabundans* methyl ester can be utilized as a substitute to diesel fuel.

Table 6.3. Physical and chemical properties of AOME (algae oil methyl ester)

Properties	**Biodiesel**	**Diesel**	**ASTM**
	AOME		**Limit**
Density at 15^0 C (g/cc)	0.865	0.824	.86 -.90
Viscosity at 40^{0}C(cst)	4.359	3.11	2.52– 7.5
Flash point (^{0}C)	96	51	Min 130
Cetane no	63	50	47 min.
Pour Point (^{0} C)	-6	-35	-31
Calorific Value (MJ/kg)	38.6	44.45	Min. 33

6.4.2. Performance and emission characteristic of various blends of microalgae oil methyl ester on a Kirloskar engine

6.4.2.1. Brake Thermal Efficiency (BTE)

It is defined as the ratio of brake power (BP) to the energy supplied by fuel in the engine. Figure 6.3 demonstrates the BTE V/S BP for pure-diesel and its different blends of diesel with microalgae oil B10, B20, B30. With the increase in load the BTE values increases, this was because the heat loses decreased at full load as more power was generated. From Figure 6.3 it was found that the average BTE values for 10%, 20%, and 30% *Neochloris oleoabundans* methyl ester blend were 27.80%, 28.18%, and 26.38% compared to diesel fuel which was about 27.55%. An average decrease in BTE of B30 blend compared to diesel fuel was found as 4.24%. While there was increase in BTE for biodiesel blend B10 by 0.9% and for blend B20 by 2.28 %. Lower heating value and higher viscosity may be the reason for reduction in brake thermal efficiency for B30 blend. Whereas, for blend B10 and B20 increase in BTE was due to efficient combustion of blends because of the presence of excess oxygen in these blends as compared to diesel. According to Pal et al 2010, in comparison to B10, B30, and diesel, the B20 blend shows slight increase in BTE due to higher oxygen content causing proper combustion and slightly lesser heating value than that of diesel, similar trends were obtained by Agarwal et al.2015. In present work similar trends were obtained.

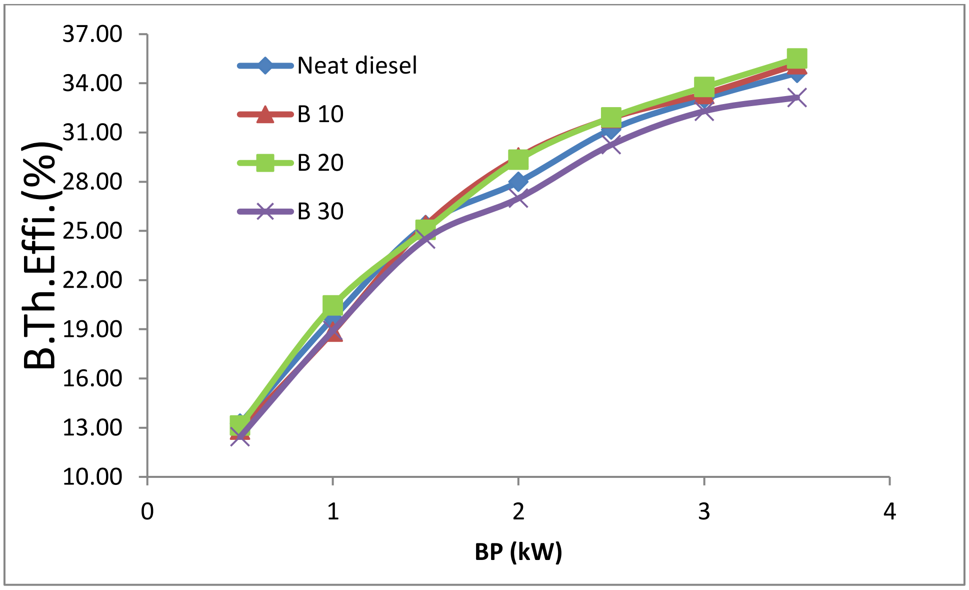


Figure. 6.3. Variation of brake thermal efficiency with brake power

6.4.2.2 Variation of brake specific energy consumption w.r.t. brake power

Volumetric consumption of fuel is the one of the significant parameter to analyze the engine performance for different fuels. Therefore, BSFC is used as conventional parameter to analyze the volumetric fuel consumption. In any case, numerous scientists recommend that BSFC isn't a trustworthy parameter when the heating value and densities of fuels differ significantly proposing BSEC is a more solid appraisal technique for correlation of volumetric fuel consumption. BSEC is the Joule of energy required to produce 1 kW power.

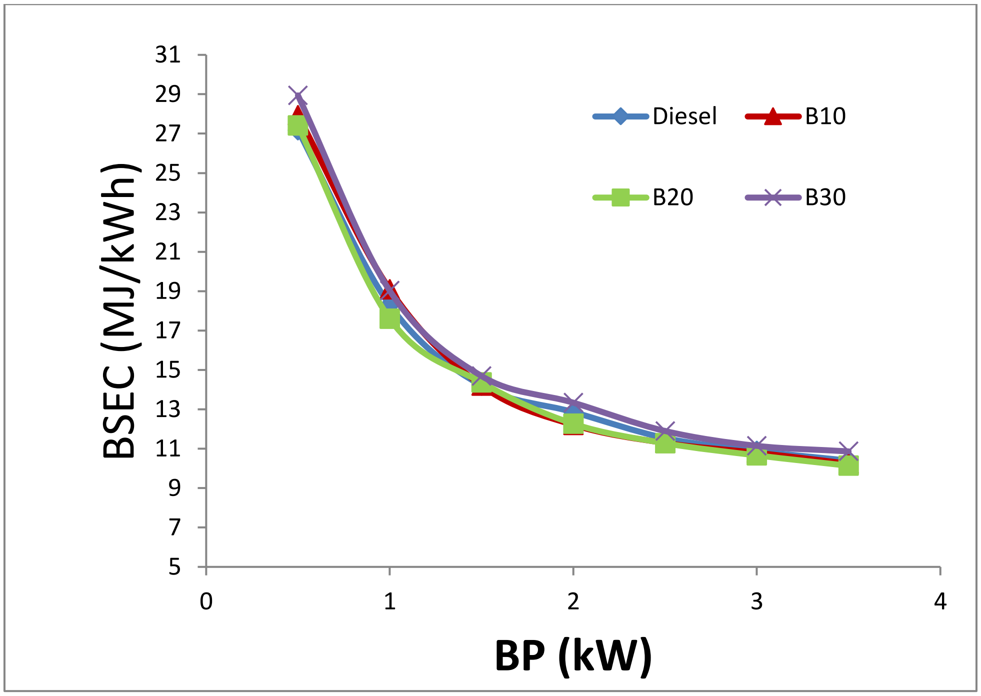


Figure 6.4 shows the variation in BSEC with BP

Figure.6.4 shows the variation in BSEC with BP for pure diesel and different blends of microalgae oil methyl ester B10, B20, B30. BSEC is the one of the significant parameter that helps in comparing engine performance where fuels have different heating values as well as the weight density. It was found that BSEC for B10, B20, B30 and pure diesel at full load were 10.24 MJ/kWh, 10.12 MJ/kWh, 10.86 MJ/kWh and 10.4 MJ/ kWh. At low load condition, the BSEC of different blends B10, B20, B30 and pure diesel were higher than at full load condition. The low BSEC at 10 and 20% biodiesel content is due to the availability of excess oxygen which cause proper combustion of the fuel. On the other hand, B30% blend have higher BSEC that may be due to the lower heating value of algae oil methyl ester blends and higher densities which decrease the mixing velocity of air/fuel mixture, leads to improper combustion and decreases the combustion efficiency, thus it requires more joule of energy to produce 1kW of power.

6.4.2.3 Unburned hydrocarbon emission (UHC)

The partial burning of the fuels is the principal reason that increases the unburned emission hydrocarbon. Figure 6.5 shows the formation of unburned hydrocarbon emission with the brake power. It was found that with an increase in the brake power the emission of HC increase, this is owing to the availability of comparatively less oxygen to complete the combustion when significant amount of fuel injected in the cylinder at higher engine load. It was also conferred that *Neochloris oleoabundans* biodiesel blended fuels presented lesser HC emissions in contrast to diesel. At higher load, pure diesel has utmost HC emission (75 ppm) whereas B30 blend showed lowest (57 ppm). The 10%, 20%, and 30% blends showed an average reduction of 8%, 14.6%, and 24% respectively compared to diesel fuel. An inability to reach the ignition temperature of the fuel to be oxidized or a lack of oxygen has been reported for the presence of UHC in the exhaust gases. It was observed that HC emissions were lower at partial load condition and increased at higher load conditions due to relatively less oxygen available for the reaction when more fuel was injected into the engine. Moreover, it was found that AOME blended fuels exhibited lower HC emissions as compared to diesel. The oxygen content in biodiesel has been shown to pre-oxidise the air-fuel mixture leading to a reduction of UHC emissions.

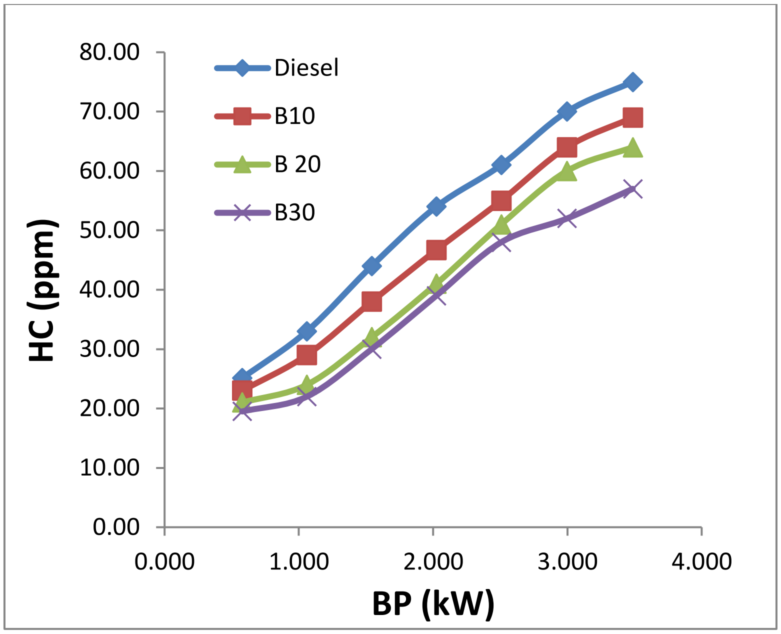


Figure 6.5. Variation of unburned hydrocarbon emission with brake power

6.4.2.4 Carbon monoxide emissions (CO)

Figure 6.6 shows lower CO emission at partial loads. At higher loads emission of carbon monoxide were higher, irrespective of fuels. Under the no-load condition, in-cylinder temperatures were more moderate leading towards incomplete combustion. However, with increasing load, the temperature rose due to fuel injected into the cylinder. At higher temperatures, improved fuel burning reduced the CO emissions. Interestingly, beyond 60% loading, higher fuel injection into the engine led to an incomplete combustion and increased CO emissions. Figure 6.6 shows that at higher loads or full load, AOME and its blends revealed lower CO emissions as compared to the diesel fuel.

This explains that at higher loads the oxygenated AOME biodiesel enhanced combustion and reduced CO emission than diesel fuel. Figure 6.6 shows the variation of carbon monoxide (CO) emission with brake power for blends of *Neochloris oleoabundans* biodiesel and diesel.

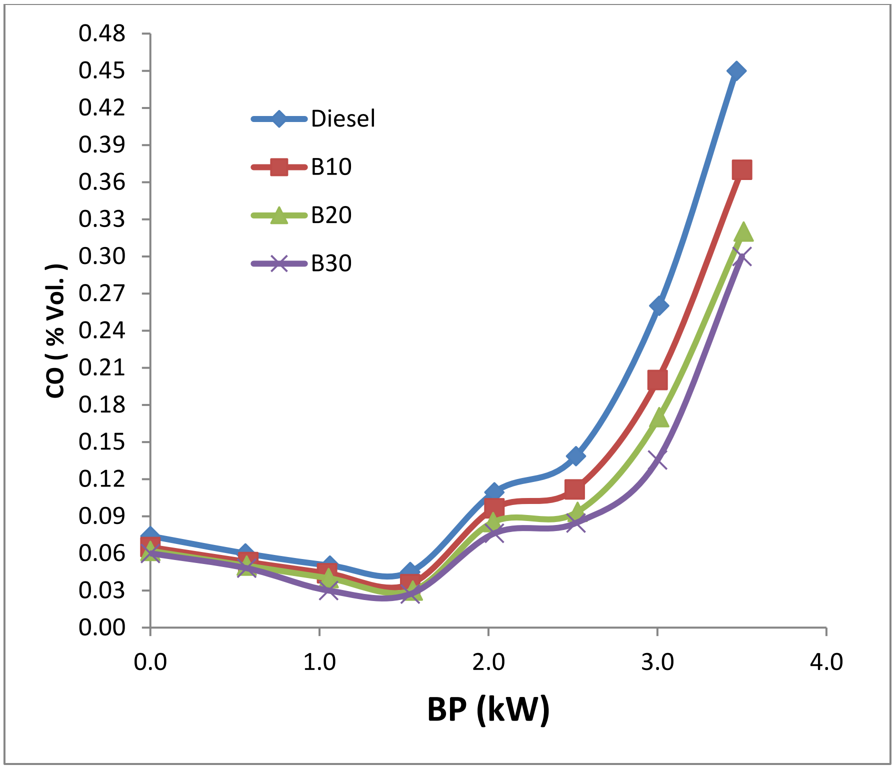


Figure 6.6. Variation of unburned hydrocarbon emission with brake power

All biodiesel blends emit lesser carbon monoxide emission as compared to diesel. This is attributed to an increasing in-cylinder temperature at higher engine load and the presence of oxygen in biodiesel fuel, which improves fuel combustion and consequently, reduces CO emissions (Ambarish Datta, Bijan Kumar Mandal,2016). The oxygen in biodiesel prevents the possibility of fuel-rich zones formation and also creates a more homogeneous distribution, which leads to complete combustion. These results were in agreement with results of previous researchers (Ong et al.2014; Chuah et al.2015; Khiari et al. 2016) even higher reduction levels (up to 87%) were recorded by Ozsezen et al. (2009).

6.4.2.5 Smoke opacity

Figure 6.7 illustrates the smoke opacity of microalgae oil methyl ester and diesel fuel. It was observed from the Figure 6.7 that as the load increases the smoke increases for all the blends.

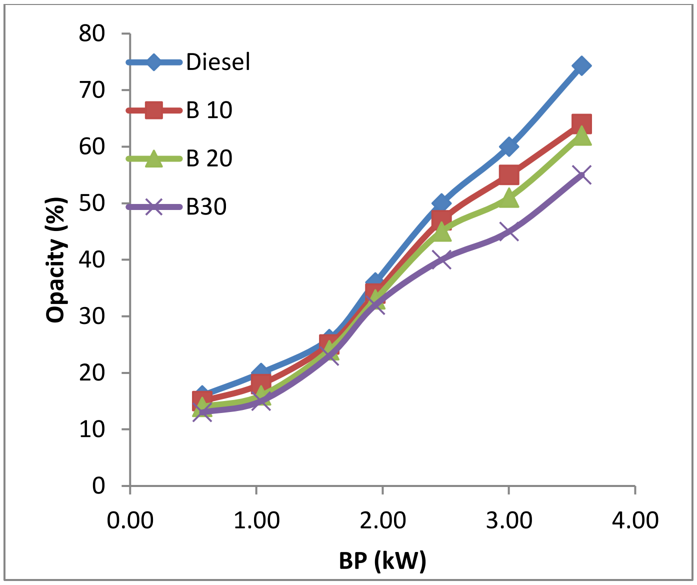


Figure 6.7. Variation of smoke opacity with brake power

This basically depends upon the A/F ratio, an excess amount of oil was injected at higher loads, some of the fuel remained unburnt and released as it in the exhaust (Sadhik Basha et al., 2014). It was further observed that at full load the smoke of blends was less compared to diesel. This is mainly due to the availability of excess oxygen in biodiesel blends (Tree et al., 2007).

6.4.2.6 Nitrogen Oxides (NOx): NO and NO_2 are called as NOx. Its emission takes place at a higher temperature from by reaction of Oxygen and Nitrogen during combustion (Kumar, 2012). NOx is responsible for depleting the ozone layer (Shahid and Jamal, 2011). Researchers observed that NOx emission increases with increase in blending and average increase is noted on average by 10-13% (Nair et al., 2013). As shown in Figure 6.8 it was observed that NOx emission of all biodiesel blends is higher than the diesel fuel. NOx formation inside the combustion chamber depends upon the cylinder temperature the higher is the temperature of cylinder more will be the NOx formation. All the biodiesel blends have extra oxygen as compare to biodiesel which raises the temperature of a cylinder and finally increase the NOx content in the exhaust. It was observed that for B30 blend total increase in NOx emission was recorded 14% at full load.

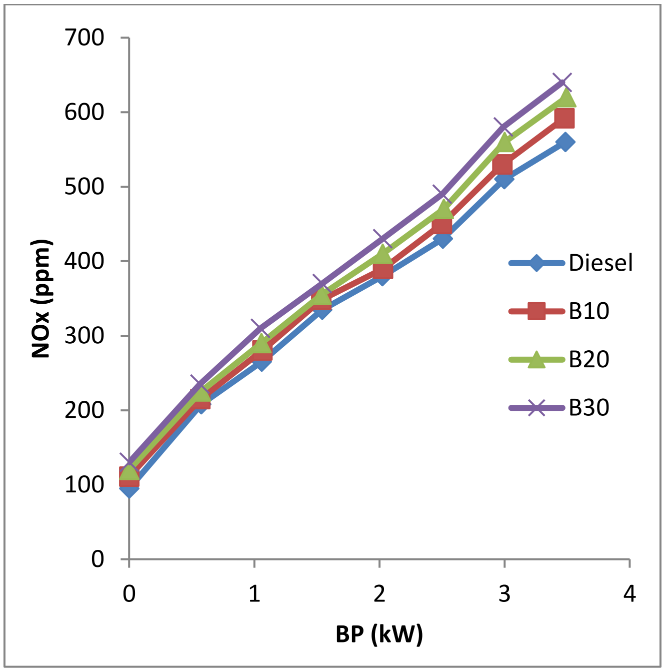


Figure 6.8. Variation of unburned hydrocarbon emission with brake power

6.5 In-cylinder pressure

The pressure analysis is one of the best successful approach to investigate the combustion and emission in diesel engine because the output is straightforwardly depends upon the cylinder pressure. In this work, the pressure with respect to the crank angle (CA) was recorded. It is the one of the significant parameters by which we can analyze the combustion behavior in engine. It helps in finding the start of combustion (SOC), maximum pressure of cylinder and ignition delay. The Pθ graph in Figure 6.9 gives the pressure rise with respect to crank angle for diesel and biodiesel blends (B10, B20, B30) at different loads. It was observed that the peak pressure of B20 blend is higher than the diesel. The peak pressure of B10 and diesel are almost equal. While blend B30 has the lowest peak pressure. The peak pressure of B20 blend is 63 bars. It was observed that after B20 blend with increase in proportion of blend in diesel the peak pressure drops. This peak pressure drop was due to large molecular weight fraction and high viscosity which affect the combustion of biodiesel blends.

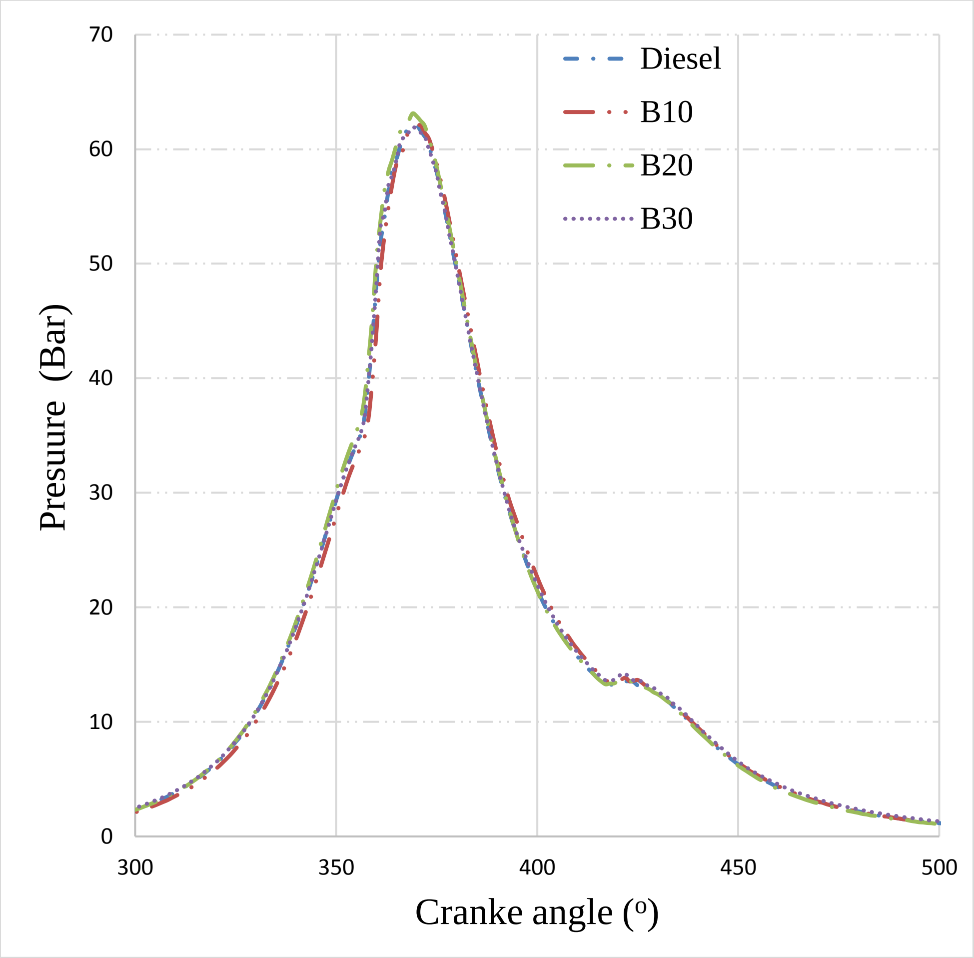


Figure 6.9: In-cylinder pressure for AOME fuel blends

6.6 Heat Release Rate (HRR)

The HRR is a quantitative explanation of the combustion pattern in the cylinder. Heat release rate study helps in estimating the combustion behavior of diesel engine. The P-θ angle varies due to the combined effects of combustion, volume change. To know the impact of the combustion procedure alone, it is required to relate all the parameters to the pressure and consequently separate the impact of the combustion only. This technique is called as the heat release analysis. This analysis gives the complete understanding of the combustion behavior that occurs in the combustion chamber. To calculate heat release rate, equations are derived from the first Law of thermodynamics which are already explained in third chapter. Figure 6.10 represents the net heat release rate for B10, B20, B30 and diesel. For all the fuels HRR curve has similar trend that is, firstly it has shown premixed combustion and then diffusion combustion. At full load premixed combustion phase was shorter and diffusion combustion phase was longer. Maximum heat release rate was observed near TDC for B10 and B20. For blend B10 and B20 maximum heat release rate is higher than the diesel during premixed combustion. While Blend B30 and diesel have higher heat release rate during diffusion combustion. This is because B10 and B20 blend shows improved combustion because of the availability of excess oxygen in the biodiesel which increase the air fuel mixture preparation. Whereas, for biodiesel blend B30 it has higher density and viscosity which make it difficult to vaporize and thus air fuel atomization is less. So, the mixture will burn in the diffusion phase. It was observed that HRR is not same for all the blends of fuel. Moreover, the combustion initiation for blend B10 and B20 is approximately same. The B20 blend HRR was found to be maximum among all the biodiesel blends and it further decrease with increase in biodiesel blend. The B20 peak value of HRR 59 J was obtained. The lower HRR of B30 biodiesel blends was due to higher viscosity and poor air utilization in contrast to B10 and B20. Variations were observed at the start of injection and combustion of both diesel and biodiesel blends. B30 and diesel are ignited little bit away from TDC as compared to B10 and B20, this shows thee is a ignition delay for all the tested fuels.

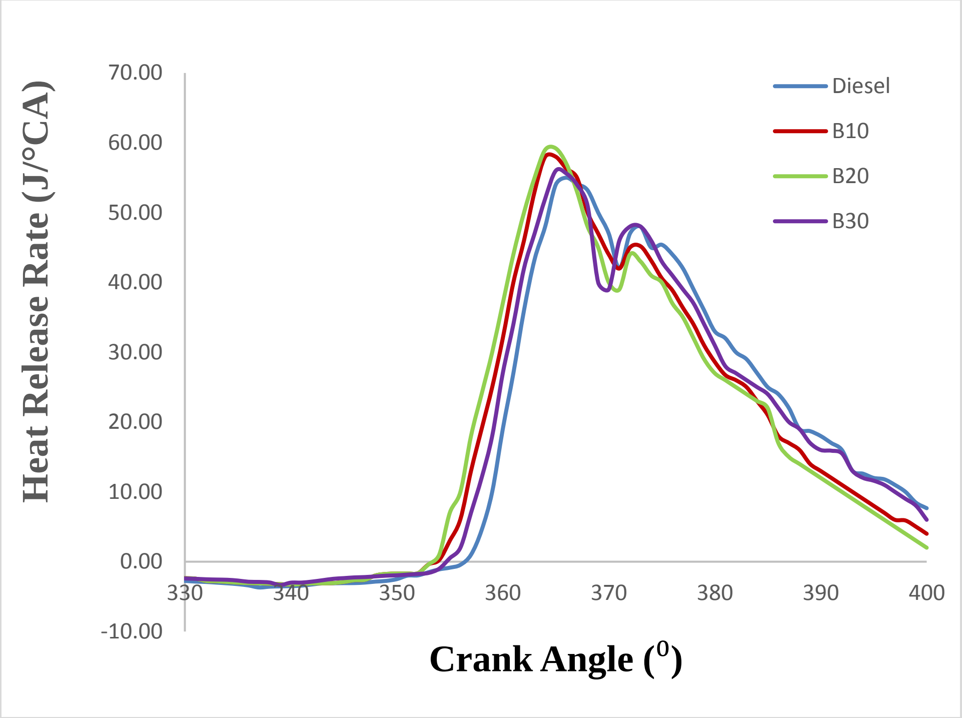


Figure 6.10: Heat release rate diagram for algae oil methyl ester Fuel Blends

6.7 Pressure rise rate

Pressure rise rate (PRR) in the engine shows the noisy operation of the engine. It depends upon the fuel quality as well as the heat release in premixed combustion. It indicates the load on the various parts of cylinder such as cylinder head. The greater the value of PRR the more will be the load on the parts, and that may cause maximum damage to the parts. From all the tested fuels diesel has maximum pressure rise rate, this is because of longer ignition delay due that excess amount of fuel is accumulated in the cylinder that leads to sudden combustion and pressure rise rate. Pressure rise rate with respect to different crank angle is shown in Figure. The PRR for diesel, B10, B20, B30 were 5.12 bar/^{o}C, 4.83 bar/^{o}C, 4.70 bar/^{o}C, 4.54 bar/^{o}C as shown in Figure 6.11. The lower pressure rise ratio for all the biodiesel blend shows that that the engine operation is less noisy than the diesel fuel.

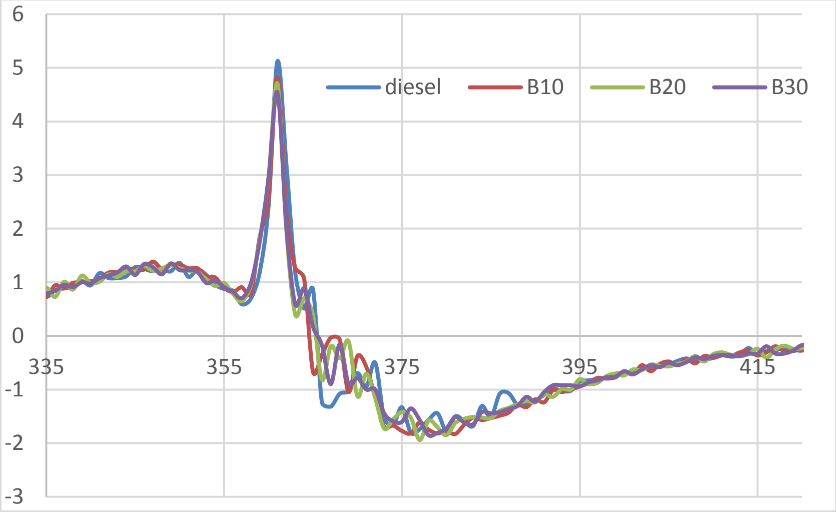


Figure 6.11: Pressure rise rate for all fuel blends.

6.8 Mass fraction burnt (MFB)

In every cycle of engine mass fraction burnt is a quantity that varies from 0-1. That explains the release of energy with respect to crank angle. The mass fraction burnt is calculated according to Rassweiler and Withrow method (published in 1938. This method assumes that the total pressure rise ΔP divided into two parts i.e, pressure rise due to volume change and combustion.

$\Delta P = \Delta Pc + \Delta Pv$

Where ΔPv can be calculated from polytrophic process of constant n

$$\Delta \mathrm{Pv} = P_i\left(\left(\frac{V_i}{V_{i+1}}\right)^n - 1\right)$$

$$\Delta \mathrm{Pc} = P_{i+1} - P_i\left(\left(\frac{V_i}{V_{i+1}}\right)^n\right)$$

As in diesel engine the combustion process never takes place at constant volume, thus the increase in pressure due to combustion is not depends straightly on the MFB. So, ΔPc because of combustion must be referenced to top dead center (tdc) volume.

Therefore ΔPc*= ΔPc*(Vi/Vtdc)

Thus, mass fraction burn can be calculated according to Rasweiler and Withrow technique

$$MFB = \frac{\sum_{O}^{i} \Delta \mathrm{Pc} *}{\sum_{O}^{N} \Delta \mathrm{Pc} *}$$

Where O is the start of combustion (SOC) and N is the end of combustion. The change in MFB as the crank angle varies for different biodiesel blends as well as for pure diesel at full load is shown in Figure 6.12. It was found that with increase in biodiesel blends up to B20 blend the MFB get increased. This is because of the availability of excess oxygen in biodiesel blend as compare to diesel, which increases the rate of combustion. The crank angle at which 10% mass fraction burnt is called as start of combustion (SOC) and at 90% MFB is called as end of combustion (EOC). Subsequently, distinction between crank angle at EOC and SOC gives the combustion duration. The MFB helps us to calculate ignition delay as well as the combustion duration. So, for the biodiesel blends and diesel ignition delay (ID) and combustion duration (Cd) were calculated from MFB. Thus, the ignition delay (ID) start from the angle at which fuel was injected to the crank angle at which the MFB value was 0.05. Similarly, the crank angle MFB of 0.05 to 0.95 given the Cd. As shown Figure 6.12 the combustion duration using biodiesel blend for blend B10 and B20 was less than the diesel and B30 blend. Using, B30 blends shows higher Cd as compared to diesel fuel. The difference in the crank angle at the start of fuel injection and start of combustion is known

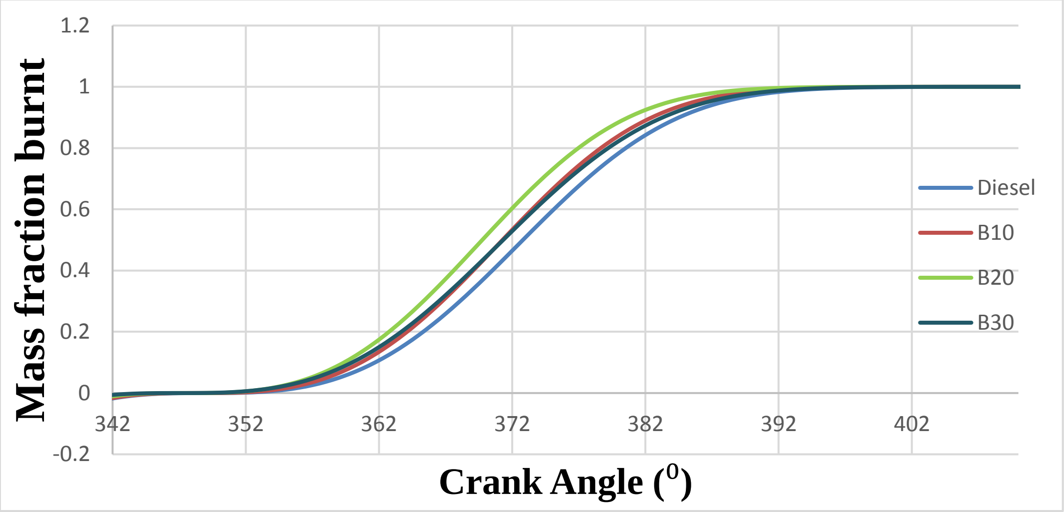


Figure 6.12: Mass fraction burnt for all tested fuels.

as ignition delay. During ignition delay the air fuel mixture preparation takes place this is divided further into physical and chemical phases. During physical delay the fuel drops mixes with air. Ignition delay depends upon the engine design, operating parameters as well as type of fuel used. Based on the crank angle, ignition delay is determined with the following equation: Ignition delay (CA) = (CA)5% MFB - (CA) at which fuel injected. The ignition delay in case of diesel fuel is maximum which is equal to 12^o CA. Ignition delay for B10, B20, B30 is less than diesel by 0.5^o CA, 1.5^{o}CA and 2^{o}CA. At higher load ignition delay get reduced, while the combustion duration increases. In comparison to diesel all the biodiesel blend s gives advance SOC and shorter duration. It is because of excess oxygen molecule in fuel increase oxidation and reduces the Cd as well as the high cetane value. From the figure it is observed that at B30 blend ignition delay is reduced but the combustion duration is more as compared B10 blend. Ignition duration for B10, B20, B30 and diesel were 28, 27, and 30.

CHAPTER 7

CONCLUSION

7.1 Conclusions

The primary objective of the work displayed in this thesis is to explore microalgae *Neochloris oleoabundans* as a potential source of biodiesel with the specific goal to utilize it in CI engine as fuel. Despite immense potential of microalgae biodiesel, few of the microalgae species are not studied yet, and biodiesel yield is not optimized yet. In addition, CI engine performance utilizing microalgae methyl ester blends and emission testing are not conducted due to the less amount of biodiesel production. The work in the thesis has covered a wide gap in the research of microalgae. In this thesis work was conducted from the growth to the biodiesel production. Engine emission and performance testing analysis also significantly fill the research gap of microalgae oil. Different fuel properties were tested for example viscosity, pour point and calorific value all these are within the prescribed limit of different standards. The knowledge of microalgae oil methyl ester properties increases our knowledge about algae oil and its potential to fulfill the demand of world. Engine emission testing was conducted on Kirloskar 4 stroke engine. From the thesis number of the conclusion are drawn.

1. *Neochloris oleoabundans* is the one of the best and appropriate feedstocks for biodiesel production.
2. Chemical flocculation is the best algae harvesting technique for biodiesel production, by which 95% biomass was obtained. In sedimentation harvesting it is difficult to harvest all the biomass some biomass remains suspended in the media and lost in the

supernatant. While centrifugation method is also suitable method of harvesting but it’s energy consuming device.

3. Using RSM the algal biomass yield was optimized, and maximum biomass 1.79 g was obtained.
4. Temperature highly affects the microalgae growth. It was concluded that at low-temperature 24^0C the maximum biomass was obtained when 10 g/L glucose was supplied, but the growth rate was very slow it takes 16-20 days to come in stationary phase. Whereas, at 30^oC the growth was fast, and it takes 8 to 10 days to go at stationary phase, but the algae biomass was less as compared to 24^oC.
5. Similarly, sodium nitrate concentration has significant effect on growth as well as lipid it was observed that at high concentration of sodium nitrate excess biomass was produced. However, at low concentration lipid yield was high.
6. Glucose is the one of the best carbon source that microalgae *Neochloris oleoabundans* can readily metabolize. At 10g/L maximum growth was obtained and when carbon source was missing the biomass yield was very low.
7. 91% biodiesel yield was obtained at 6:1 molar ratio by ultrasonic method within 20 minutes of reaction. However, magnetic stirrer method takes 80 minutes to complete the reaction.
8. For both Magnetic stirrer method and ultrasonic method the maximum yield was obtained at 1% catalyst concentration. However, at 1.25% catalyst yield reduced significantly.

9. In indirect transesterification method methanolic HCL was used as a catalyst. The optimum values obtained were 3 ml methanolic HCL (8%), 7.5 ml solvent, 90^{o}C temperature and 80-minute time.
10. When microwave was used for cell disruption of microalgae for direct transesterification than maximum 48-50% lipids were extracted. But this method consumes an excess amount of energy, but the rate of reaction increases.
11. All the fuel properties are within prescribed limit.
12. The engine emissions and performance were improved up to 20% blend because of the availability of oxygen in microalgae biodiesel blends.
13. All the engine emissions were reduced significantly except NOx emission.

7.2 Scope for future work

1. More microalgae species which are rich in oil should be analyzed and investigated.
2. In present work, direct transesterification was done at a small scale, but the pilot plant should be developed so that we can proceed towards commercialization of microalgae.
3. More lipid production techniques should be developed which eliminates the two-step transesterification process, thus reduces the cost of biodiesel production.
4. More cost saving carbon sources can be tested for the growth of algae.
5. As the NOx emission increases utlilizing algae oil methyl ester blends, so it is essential to work on NOx reduction methods.

PUBLICATIONS FROM THE THESIS

Published in journals:

1. Amrik Singh, Amit Pal, and Sagar Maji. Biodiesel production from microalgae oil through conventional and ultrasonic methods. Energy Sources, Part A: Recovery, Utilization, And Environmental Effects 2017; 39 (8): 806–810.
2. Amrik Singh, Amit Pal, and Sagar Maji. Biodiesel Production from Wet Microalgae Biomass through Direct Transesterification by Conventional and Microwave Radiation Method. International Journal of Materials Science 2017; 12 (2): 187-196.
3. Amrik Singh, Maji S. and Kumar Gaurav. Production of biodiesel from the algae: state of the art review. International Journal of Development Research 2014; 4 (12): 2685-2693.
4. Amrik Singh, Amit Pal, R S Mishra. Study on the Use of Renewable Fuels in a CI Engine. International Journal for Research in Applied Science & Engineering Technology (IJRASET) 2017; 5 (6): 2016-2022.

Published in international conferences:

5. Amrik Singh, Amit Pal, H Singh and S. Maji. Triacylglyceride's Transesterification for Biodiesel: A Review. In international conference on recent advances in mechanical engineering RAME-2016. ISBN: -978-194523970-0, PP- 47-58.
6. Amrik Singh, Amit Pal, D Nimker, H Singh and S. Maji. Engine Performance Parameter and Combustion Characteristics for Biodiesel: A Review. In international conference on recent advances in mechanical engineering RAME-2016. ISBN: -978-194523970-0, PP- 697-706.

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