

**Biodiesel production and characterization from indigenous
mixed algal biomass**

A thesis submitted for the partial fulfilment of the degree of

Doctor of Philosophy

by

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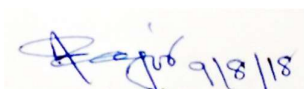
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CERTIFICATE

This is to certify that the thesis entitled 'Biodiesel production and characterization from indigenous mixed algal biomass' which has been submitted by Mr. Rachan Karmakar for the partial fulfilment of the degree of 'Doctor of Philosophy' in Energy and Environment from the School of Energy and Environment of Thapar Institute of Engineering and Technology, Patiala, India, is a record of the candidate's own independent and original research work carried out by him under our supervision and guidance. The matter embodied in this thesis has not been submitted, in part or full, to any other institute for the award of any degree.



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DECLARATION

This is to declare that the research work which is being presented in this thesis entitled 'Biodiesel production and characterization from indigenous mixed algal biomass' for the partial fulfilment of the degree of 'Doctor of Philosophy' in Energy and Environment from the School of Energy and Environment of Thapar Institute of Engineering and Technology, Patiala, India, is an authentic record of my own research work carried out under the supervision of Dr. Anita Rajor (Associate Professor, School of Energy and Environment, TIET, Patiala, India) and Dr. Krishnendu Kundu (Principal Scientist, Department of Biofuel, CSIR CMERI CoEFM, Ludhiana, India). Documents, embodied in this thesis, from other researchers' works are duly listed in the reference section.

The matter presented in this thesis has not been submitted, in part or full, to any other institute for the award of any degree.



Rachan Karmakar

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Tumi rabe nirabe, hridaye mama (You will, quietly, stay in my heart for ever)

- RABINDRANATH TAGORE

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ABSTRACT

Rapid urbanization and industrialization in 20th and 21st century brings the scarcity of fossil fuel resources. On top of that, these are the main causes of greenhouse gas emission and global warming. Research works on alternative fuels including biofuels are taking place throughout the world. Many of the resources and sources for energy production have been invented and discovered in last few decades. Biodiesel, one of the alternative and renewable source of energy, produced from different oils and fats has become prominent in these days. But it is economically incompatible with petroleum diesel. Algal fuel, on that perspective, is very new but might be one of the most promising fuel sources in near future. Algae's faster growth rate along with high oil content has drawn the attention of researchers worldwide. First world countries like USA is investing billions of dollars for the research on algal fuel. But in India, where the necessity is much more, very few research works are taking place. Researchers have proved that all three types of biofuels i.e. biodiesel, bioethanol and biogas can be produced from algal mass.

Algae, on the other hand, utilize CO₂ for their growth and can be minimized some sort of pollution. In Punjab, India, algae grow rapidly in several water-bodies. But all those are not used by anybody for any purpose. This biomass was used to produce biodiesel in this research work.

The algae were collected from pisciculture- ponds and a canal called Simlapuri Nahar. The growth parameters of these algae were optimized in terms of maximum yield of biomass with paramount oil content. It was found 4l/pond CDS, 30ml/l CO₂, 0.5m water depth, 5°C-10°C temperature and 'every 1.5h' mixing intensity are the optimized conditions for the growth of these algae. Almost 12kg of algae was produced from 5kg of algae and the oil content of the increased to almost 12% from 8.9% under optimized conditions. A statistical analysis proved that the most contributing parameters, for the growth of these algae and increase of oil content of the same, were the nutrient (cow dung slurry) and the temperature.

Experiment was done to find out a solvent with most capacity of oil extraction. An amount of 8.9% of algal oil was extracted by the mixture of hexane and acetone (1:1). The lowest quantity of oil was extracted, from dried algae, by methanol (3.4%).

In the transesterification reaction for biodiesel production, methanol to oil molar ratio of 6:1, catalyst concentration of 3%, reaction temperature of 60°C and reaction time of 60minutes were found to be the optimum conditions to get maximum FAME of 96% and minimum free fatty acid (FFA) content of 0.18%. A statistical analysis for the transesterification procedure also were carried out to find out the contribution of parameters. For, highest yield of biodiesel, catalyst concentration was found to be the dominant contributing parameter. On the other hand, for lowest FFA content, methanol to oil molar ratio exerted highest contribution.

To find out percentage of conversion of algal oil to algal biodiesel was calculated by means of the NMR graph of algal biodiesel. The conversion was found to be of around 96%. Therefore the yield of the biodiesel, from indigenous mixed algal biomass used in this experiment was almost 93%.

Important properties of the FAME produced like viscosity (3.12 mm²/s), cloud and pour point (-1 °C and -6 °C respectively), flash and fire point (153 °C and 158 °C), Carbon residue content (0.03%), acid number (0.36 mg of KOH/gm) were within the range of concerned limits of ASTM/ BIS standards.

It was found that apart from NO_x, the emission of other gases (CO, CO₂ and hydrocarbon) were lower for the combustion of this algal biodiesel than that of petro- diesel. While CO and HC emission were found to decrease with increasing engine load, the emission of CO₂ and NO_x increased with increasing engine load. Same incident was found to be occurred for the combustion of algal biodiesel- petro- diesel blends.

Therefore the biodiesel produced in this experiment was found to be very eco- friendly and engine- friendly.

KEYWORDS: Algae, Biodiesel, Solvent extraction, Transesterification, Fuel Properties, GHG emission

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ABBREVIATIONS AND CHEMICAL FORMULA

3D- Three dimensional

AAS- Atomic absorption spectroscopy

am- Anti meridiem

B10- Biodiesel blend with 10% biodiesel

B100- 100% biodiesel

B20- Biodiesel blend with 20% biodiesel

B30- Biodiesel blend with 30% biodiesel

C- Celsius

CDS- Cow dung slurry

CI- Compression ignition

CO- Carbon monoxide

CO₂- Carbon dioxide

CV- Calorific value

df- Degree of freedom

DO- Dissolved oxygen

FAME- Fatty acid methyl ester (biodiesel produced by methanol as alcohol)

FFA- Free fatty acid

Fig.- Figure

g- Gram

GDP- Gross domestic product

GHG- Green house gas

h- Hour

H₂SO₄- Sulphuric acid

ha- Hectare

HC- Hydrocarbon

HgSO₄- Mercuric sulphate
hrs- Hours
K₂SO₄- Potassium sulphate
kg- Kilogram
KH₂PO₄- Potassium dihydrogen phosphate
kJ- Kilo Joule
KOH- Potassium Hydroxide
l- Litre
m- Meter
M- Molar
mg- Milligram
min- Minute
MJ- Mega Joule
ml- Millilitre
MnSO₄- Manganous sulphate
MS- Mean of squares
N- Normal
NMR- Nuclear magnetic resonance
NO_x- Oxides of Nitrogen
OD- Optical density
PC- Percentage of contribution
pm- Post meridiem
ppm- Parts per million
s- Second
S/N- Signal to noise
SI- Spark ignition

SnCl_2 - Stannous chloride

SS- Sum of square

TKN- Total Kjeldahl nitrogen

TLC- Thin layer chromatography

CHAPTER 1

INTRODUCTION

This chapter introduces biofuels, biodiesel, significance and necessity of biodiesel, production procedure of biodiesel by transesterification reaction etc. It also includes a thumbnail sketch of algae and their importance as a feedstock of biofuel. Finally, it briefs the lacuna of this research field and all the objectives set for this experiment.

1.1. REQUIREMENT OF ENERGY RESOURCES

The power required to accomplish any work is energy. So, energy is the prime resource to run any machine like body of human being, engine of car, heater of oven etc. Need of energy resources has been increased many folds in today's world because of rapid industrialization, urbanization and automation of machines. Therefore, a swift depletion of energy resources (Robinson 1975) has occurred in last few decades. As a result, scarcity of these energy resources has cropped up since most of these resources are finite and non-renewable fossil fuels.

1.2. FUEL

Fuel is the material with stored energy to be released, generally, as a result of oxidation through combustion. Till date, the principal fuel, which are used to produce electricity, run vehicles, operate heavy duty machines in industries etc., are fossil fuels.

1.3. FOSSIL FUELS

Fossil fuels evolve very slowly and naturally in a long span of time (millions of years) as a result of anaerobic decomposition of naturally buried organisms. Therefore, these fuels are only available underground and at the same time they are very much non-renewable (Shafiee and Topal 2009). Fossil fuels are mainly available as coal, petroleum and natural gases. These fuels, especially coal and petroleum, cannot be used without purification. While the solid fossil fuel, the coal, is mostly used for the production of electricity, petroleum, the liquid fossil fuel, is used, widely, to run heavy or light vehicles. Petrol and

diesels are two important derivatives of petroleum which are, till date, the dominating fuels to be used in public and private vehicles. For their structural differences, different types of engines has been designed for both the fuels. While spark ignition (SI) engines are used for petrol or gasoline, compression ignition (CI) engines are used for diesel. The main difference between them is the way of the ignition takes place in both of them. In SI engine, ignition in the mixture of fuel and air is done by generating spark from a spark plug. Spark in CI engines is triggered by compressing the air and then injecting the fuel in the ignition chamber. Air, while getting compressed, gets highly heated up and thus the ignition occurs in the engine.

1.4. REQUIREMENT OF ALTERNATIVE FUELS

As it has been stated earlier, this is very evident that for their non- renewability, fossil fuels are getting depleted very drastically and the worldwide energy desideratum, on the other hand, is surging rapidly. As a result, the price of these fossil fuels are increasing and a huge gap, which is expanding with time between the demand and the availability of energy, is taking place. The situation is getting worse as it has been assumed that there will be no fossil fuel remaining on earth after the year of 2112 (Shafiee and Topal 2009) and on top of that the consumption of liquid fuels, in transport sector, will increase by 50% by the year of 2030 (B. P. Energy Outlook 2030 (January, 2011)).

It has also been observed that the environmental pollution has been surged worldwide after the commencement of industrial revolution in England (Hanlon 2016). A steep escalation of CO₂ pollution has been found to be engendered with increase in GDP with time (Fig 1.1).

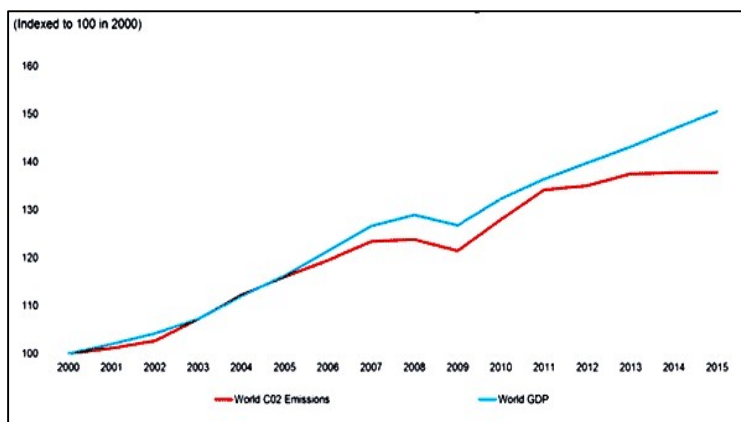


Fig 1.1: GDP and CO₂ emission with time (Source: IEA, energy related CO₂ emissions and industrial monitory fund, World Economic Outlook Database, April, 2016)

Apart from that fossil fuels have been found to be associated with pollution of mercury (Joensuu 1971), carbon monoxide (CO), unburned hydrocarbon (HC) and other greenhouse gases (GHG) (Smith et al. 1993; Riahi et al. 2011) and thereby the global warming (Hoel and Kvernodokk 1996; Steinberg 1999).

1.5. ALTERNATIVE FUELS

Therefore for this scarcity of natural resources, enhancement in oil price and increasing rate of emission of greenhouse gases (Singh and Srivastava 2016), the need of other energy sources and resources are taking place which should be renewable as well as eco-friendly. To overcome this situation, scientists have been involved, for a long time, in researches to innovate or discover such kind of energy source or resource. Researches kept going on to make the available energy technology smoother and bring new technologies into action.

Many of renewable resources have been presented by researchers in past years. Nuclear energy (Swain and Guttman 1983), solar energy (Bahnemann 2004), wind energy (Pena et al. 1996) and hydro- energy (Yang and Jackson 2011) are few of the examples of their outcomes. All these energy technologies has their own benefits and drawbacks. Biofuel produced from different resources is a comparatively new and promising technology for production of energy (Singh et al. 2014).

1.6. BIOFUEL

Biofuels are energy sources which are derived from different biological resources or feedstocks. As these fuels are having biological origin, they are renewable and biodegradable at the same time. Therefore, these are more eco- friendly options which may be a solution to the problem of non- renewability of fossil fuels.

While many of the alternative energy sources are used for the production of electricity, biofuels are mainly associated with transport sector. There are four dominant types of biofuels which are (i) biogas (Kundu and Bhattacharya 2007), (ii) bioethanol (Kundu and Bhattacharya 2006), and (iii) biodiesel (Kundu and Bhattacharya 2006).

Both bioethanol and biodiesel are the alternates to petrol while biodiesel can be interspersed with petro- diesel. Bioethanol can be derived from sources like peels and pulps of fruits (Reddy and Reddy 2007; Tiwari et al. 1986). On the other hand, biogas can be

generated from cattle- dung (Fig 1.2) (Singh and Singh 2017), kitchen and other organic waste materials (Goud 2017). While both of bioethanol and biogas can be produced from waste materials, either vegetable oil or animal fat are the requisite feedstocks for biodiesel production.



Fig 1.2: Biogas plant

1.7. BIODIESEL

Biodiesel can be produced, generally, by transesterification reaction of any triglyceride (vegetable or animal sourced fat and oil) and a typical alcohol of short chain like ethanol or methanol (Chaturvedi et al. 2013) in presence of a catalyst (Mohammed et al. 2016).

Structurally, oils are triglycerides (fatty acids glycerol) esters. Biodiesel production, by means of Transesterification reaction, eventuates as a result of exchange of an organic R group of oil with the R' group of an alcohol (Karmakar et al. 2017). In that way the glycerol of triglycerides gets separated and three molecules of fatty acid alkyl esters i.e. biodiesel are produced from a triglyceride molecule. Alkali catalysts are mostly used in transesterification procedure but they are extremely sensitive to free fatty acid (FFA) of oil. When the oil is high in FFA, the alkali catalysts along with those FFA gets involved in saponification reaction to form soap and inhibit transesterification reaction. Therefore for those oils, acid catalysed reaction is done first to reduce the FFA content and then it is followed by alkali esterification reaction.

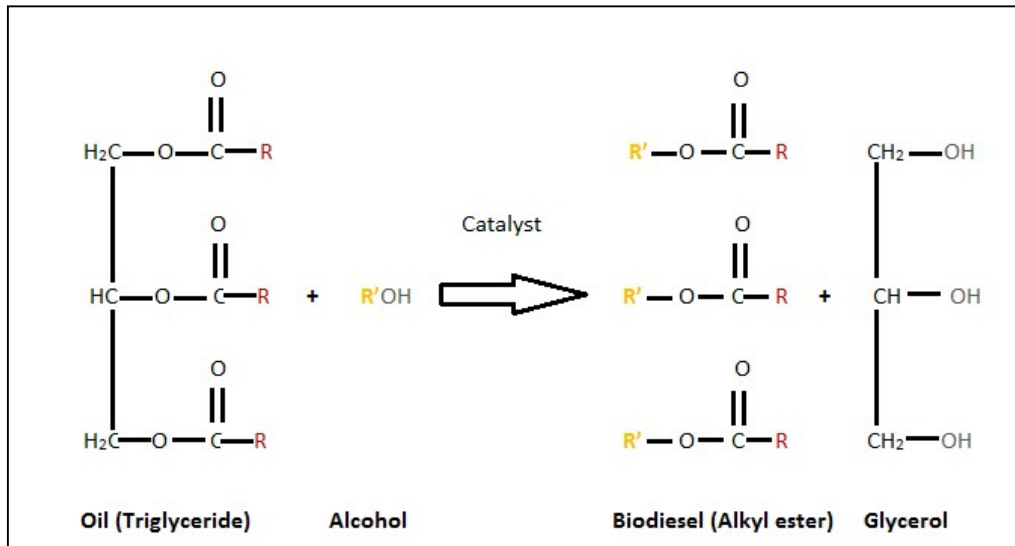


Fig 1.3: Transesterification reaction

1.7.1. REQUIREMENTS AND ADVANTAGES OF BIODIESEL

As because diesel engines play very salient role in energy and transport sectors for their thermal efficiency of high level and low market price (An et al. 2012), uninterrupted supply of diesel fuel or any alternate fuel is a matter to be considered with foremost importance. Therefore, for the sake of sustainable development, as the petro- diesel is expected to be exhausted completely from earth in next few decades, use of blends of petro- diesel and alike fuels is very much recommended for this time being, though, complete replacement of petro- diesel with alternative fuels would be the best option.

Till date, biodiesels have come out as the best alternatives to petro- diesel. As they are produced from either vegetable or animal fats or oils, they are obligatorily renewable sources of energy (Mekhilef et al. 2011; Demirbas 2007). It has also found that biodiesels are more environment- friendly fuels than the petroleum derived diesel (Mitra et al. 2009). Therefore, research on biodiesel can bring solutions to the problems of conventional fuels described in section 1.3, 1.4 and 1.5.

1.7.2. DRAWBACKS OF BIODIESEL

Though biodiesel produced from different types of animal oil and fat (Adewale et al. 2015; Bankovic-Ilic et al. 2014) as well as vegetable oil and fat (Aicantara et al. 2000; Gadge and

Raheman 2005; Baladhiya et al. 2016; Ragit 2011) are becoming popular in recent time, there are few problems associated with them which are as follows-

- (i) Food vs energy conflict: As biodiesels are produced from vegetable oil and animal fat, most of their feedstocks are found to be edible. Therefore, in a developing country like India (Kumar et al. 2013), research and use of those resources for the production of energy seems to be unethical. In an article of Seo et al. 2014, it was found to be reported that research and manufacture of biofuel is associated with effective increase in price of corn and sugar. As a result, a conflict of food versus energy (Parman et al. 2011) is taking place in India as well as all-over the world.
- (ii) Unavailability of crop- field: As the conflict between the usage of resource as food and energy production has commenced, the farmers does not allow to use the crop field to produce vegetables to be used in production of energy. In this way, the availability of field for production of not only edible crops but non-edible crops are also being declined (Cai et al. 2011).
- (iii) High market price: As a result of the problems described in section (i) and (ii), the market price of biodiesels are very high (Amano- Boadu et al. 2014). It is hard to carry on research, which can result in reduction of the price of biodiesel, without availability of feedstock. On top of that, the vegetable oils like jatropha, mahua etc., which are available for biodiesel production, are very costly. Therefore, the biodiesel produced from those triglycerides are expected to be of high market price.

Hence, a need for biodiesel produced from non- edible feedstocks is becoming an issue of utmost importance.

1.8. IMPORTANCE OF ALGAE AS RESOURCES OF BIODIESEL

Algae, which are mainly aquatic microbes, can be a solution to the problems with research and manufacture of biodiesel. Algae possess numerous advantages for production of biodiesel which are as follows-

- (i) Utilization of atmospheric carbon dioxide: Like other crops, algae utilize carbon dioxide for their photosynthesis. So, they can mitigate pollution of carbon

dioxide and it has been reported by researchers that increased supply of CO₂ enhances the growth of algae (Holbrook 2014; Dassey et al. 2014; Widjaja et al. 2009). In another article it has been demanded that biofuel from algae can fix 0.6% of atmospheric carbon dioxide (Ponnusamy et al. 2014).

- (ii) Requirement of less area for growth: Algae require much less (100- 200 times less) land area for its growth (Chisti 2007). Algal growth is seen in all parts of lithosphere and hydrosphere including non-productive barren lands (Costa and Moraise 2011).
- (iii) High growth rate- Algae grow very fast (Dauta et al. 1990; Chisti et al. 2007). Its growth rate is much faster than that of major biodiesel- crops like jatropha and palm. Photosynthetic efficiency is also more in microalgae (exceeds 10%) than that of terrestrial plants (less than 0.5%) (Li et al. 2008).
- (iv) High oil content: Its oil content is 10%- 20% higher than that of jatropha, palm and soybean (Table 1.1) (Chisti 2007; Chisti 2008; Meng et al. 2009).

Table 1.1: Oil yield and requirement of land area of crops and algae (Chisti 2007)

Crop	Yield of oil (L/ ha)	Requirement of land area (ha)
Coconut	2689	99
Canola	1190	223
Soybean	446	594
Jatropha	1892	140
Palm	5950	45
Corn	172	1540
Microalgae	58,700- 136900	2- 4.5

- (v) Usage of water for growth: While fresh water, the scarcity of which is of more concern (Chisti 2013), is an important desideratum for the growth of many plants, algae can be grown in waste and brackish water (Woertz et al. 2009; Ashokkumar et al. 2014; Christenson et al. 2011).
- (vi) Not used as food: On top of all points mentioned, algae, in India are not used as food. Therefore it solves the problem of food vs energy conflict.

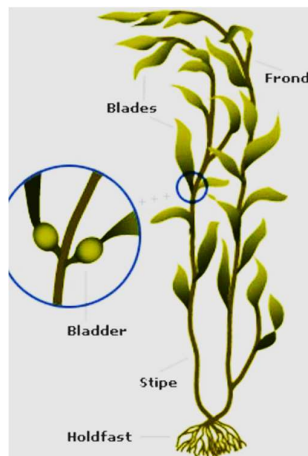
1.9. ALGAE IN BRIEF

Algae (Fig 1.4) are mostly eukaryotic, non- flowering microbes having chlorophyll for photosynthesis (Pelczar et al. 2001). Algae lack true leaves, roots, stems, and any vascular tissue. Most of the algae are single or multi cellular aquatic organisms.

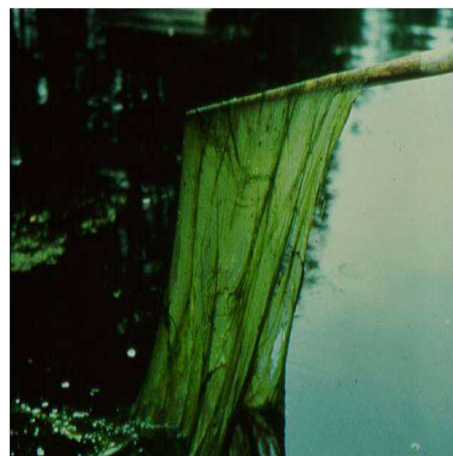
Some algae are filamentous (Fig 1.5 (b)) and some are of complex structures. Algae, with the most complex structures, are known as seaweeds (Fig 1.5 (a)). Although green photosynthetic algae (Chlorophyta) are dominant algae, some other types of algae like red algae (Rhodophyta) also exist.



Fig 1.4: Algae growing in a pond



(a)



(b)

Fig 1.5: (a) Seaweed and (b) filamentous algae (source: Google images)

Classification of algae at Division and kingdom level, is very tough. Some of the researchers assigned the classes of *Bacillariophyceae*, *Phaeophyceae* etc. to the division Chromophyta. At the same time according to some other researchers, they belong to their own divisions Bacillariophyta, Phaeophyta sequentially.

The general classification of algae is given in Table 1.2.

Table 1.2: Classification of algae (according to Cavalier- Smith)

Super kingdom	Eukaryota	
Kingdom	Plantae	
Super group	Members of the group	Endosymbiont
Primoplantae/ Archaeplastida	<ul style="list-style-type: none"> i. Chlorophyta ii. Rhodophyta iii. Glaucophyta 	Cyanobacteria
Excavata and Rhizaria	<ul style="list-style-type: none"> i. Chlorarachniophytes ii. Euglenids 	Green Algae
Chromista and Alveolata	<ul style="list-style-type: none"> i. Heterokonts ii. Haptophyta iii. Cryptomonads iv. Dinoflagellates 	Red Algae

1.10. SIGNIFICANCE OF THIS RESEARCH AREA

Use of resources with no use (Singh et al. 2016) and waste (Jamil et al. 2018) is becoming a key subject of research in current time. Therefore, from that perspective, biodiesel production from unused algae is a subject of high impact. Worldwide research on biodiesel production from different non- edible oil (Banković-Ilić et al. 2012) is expected to be fruitful.

Algae, on that context, are new but can become one of the most promising alternative resources for energy production. While the economic feasibility of most of the biofuels are very uncertain (Amano-Boadu et al. 2014), the biodiesel produced from unused algae can be a dominating alternative to petro- diesel.

1.11. GAPS IN THIS RESEARCH FIELD

A lot of literatures have come out on different aspects of algal biodiesel. Although in India, where the necessity of alternative fuel is huge and production of algae is abundant, very few research works have taken place on algal biodiesel. After reviewing relevant literature, following technical gaps have been observed-

- i. Most of the works done are specific in species. Oil from single species has been used in most of the related studies while it is known that monoculture is comparatively expensive and complex which causes the increase in biodiesel price.
- ii. There is no evidence of research work in India on unused algal biomass of mixed species which grow naturally and indigenously in waterbodies in India, especially in the state of Punjab.
- iii. Photobioreactor (Podevin et al. 2017) is the best tool for algal biomass production but its installation and operation charges are very high. On the other hand, any or few modifications and optimization of growth parameters can be very fruitful for algae-proliferation in open ponds. But studies on open pond culture are avoided mostly due to its less productivity.
- iv. Number of research works on the characterization of emission due to the use of algal biodiesel in engine is very little. As biodiesel from unused mixed biomass of indigenous algae grown in Punjab, India, has not been used as a feedstock for biodiesel production, the emission characteristics of that fuel, obviously, has not been examined.
- v. Combustion analysis and engine testing too, have not been reported for this biodiesel. Hence, the types and measures of effects of this algal biodiesel and their blends have not been investigated.

- vi. Very few statistical experiments have been conducted on algal biodiesel. Their resemblance with the practical study is, hence, unknown.

1.12. OBJECTIVES OF THIS STUDY

The proposed research study will be conducted with the following objectives-

- (i) To optimize production parameters for indigenous algal biomass.
- (ii) To optimize transesterification process for production of algal biodiesel.
- (iii) To characterize algal biodiesel.

CHAPTER 2

REVIEW OF LITERATURE

This chapter encompasses relevant literatures which were gone through before starting this experiment. It enlists the brief design of the apposite literatures of recent and past researches on biodiesel, explicitly, algal biodiesel production, algae culture and so on.

2.1. DIFFERENT SEGMENTS OF RESEARCH DONE IN THIS FIELD

As because rapid use of fossil fuels is now identified as unsustainable for the continuous and irreversible depletion of these fuels and their contribution in generation of greenhouse gases in the atmosphere, huge number of research projects are being involved in search of new resource and sources of eco- friendly renewable fuels.

Usage of 5% bioethanol has become mandatory in India. In U. S. too, a mandatory Renewable Fuel Standard has been established to produce renewable fuel of 36 billion gallons as transport fuel by 2022 (Biofuels Technology Roadmap USA, 2010.). EU intends to replace 10% of transport fuel by biofuels by 2020 (Rosch and Sharka 2009). Due to few inferior characteristics and poor economy, biodiesel is blended with petro- diesel to use in most engines (Montefri et al. 2010).

First generation biofuels including biodiesel from inedible sources like Jatropha, karanj, mahua (Gadge and Raheman 2005) etc. have become very famous in last few years but they are not economically compatible with petroleum diesel and enough crop field is unavailable for their cultivation in required quantity. At the same time, production of biodiesel from the edible oils cannot be entertained in third world countries like India as they import about 43% of edible oil in a year (Kumar et al. 2013). Apart from that, it has become evident that biofuels from different vegetable oils, waste cooking oils along with fats together too are unable to meet the demand of transport fuels all over the world (Smith et al. 2010). In a city like Louisiana, USA, 87.4 million barrels of algal biofuel is required to replace current transportation fuels (Dassey et al. 2014).

Although the future of biofuels derived from algae is uncertain, algae and its fuels have so many advantageous properties to be established as the alternative of petro- diesel (Reijnders 2008). It has been seen that 10% more oil can be derived from low productivity microalgae than that can be derived from soybean plant (Pienkos and Darzins 2009). But

among 30000 studied species (Richmond 2004), morphological variation may take place even for the same strain and each race (Metzger and Largeau 2005). For each of this variations oil content and composition of fatty acids can change. Photosynthetic efficiency is also more in microalgae (exceeds 10%) than that of terrestrial plants (less than 0.5%) (Li et al. 2008). In their exponential phase of growth, algal biomass doubles in 3.5 to 24 hours (Chisti 2007). Farmers do not have to get worried about its culture because neither any external water resource nor any technical tool like tractor is used here. Therefore its cultivation is safe and hopeful for the farmers.

Research on Algal biodiesel production can be into (i) algae culture, (ii) oil content of algae, (iii) biodiesel production (iv) study of engine with the fuel, (v) environmental impact study and (vi) economic and statistical studies. Research on most of these parts is taking place in first world countries (Chisti and Yan, 2011).

2.2. ALGAE CULTURE

Light intensity (Damiani et al. 2010) and supply of CO₂, at the time of algae culture, exert good effect on the lipid concentration of algal species like *Haematococcus pluvialis*, *Dunaliella tertiolecta*, *Nannochloropsis oculata*, *Chlorella* sp. etc. (Chiu et al. 2009). Higher content of lipid can be produced in *Nannochloropsis* sp. with the supply of air with higher CO₂ percentage (0.28%) (Hsueh et al. 2009).

Other than carbon dioxide, algal growth can be enriched with optimized level of nutrients providing nitrogen, potassium and phosphorus (Chisti 2013; Cordell et al. 2009). Nitrogen and phosphorus have been observed to provide 4.47 MJ of energy to a ton of algal mass (Chisti 2013). Some artificial media can be good for algal growth. In a study, medium containing 12% glucose for 12 days culture showed enough suppression of polyunsaturated fatty acids (Matsuura et al. 2012) which can solve the problem of poor oxygen stability of algal oil. Oil content of the algae can be increased by inducing microalgal species to accumulate substantial lipid (Rodolfi et al. 2007). Some scientists have shown that limiting nitrogen in the medium triggers the increase of lipid content of algae up to four times than the lipid content of nitrogen efficient algae (Yamaberi et al. 1998).

Multi-tier cropping (algae- paddy) has been seen as a good alternative which provide food and water security too. A very few mathematical studies have been done on this research

topic. In one of them a mathematical model has been developed and reported (Sudhakar et al. 2012) to show the feasibility of algal biodiesel in India. According to it, yearly algal biomass production would be within 159 and 345 metric tons per hectare. At the same time, 57,000 to 1, 62,000 litres of algal lipids, from a hectare of field, can be produced based on the intensity of solar radiation in that place.

As most of the research works on culture of algae are confined to monoculture, preference, for this purpose, is given to photobioreactors (Fig 2.1). As these are closed glass chambers, monoculture can be done in photo-bioreactors because contaminants from outside cannot affect it.

Both artificial and Sun light can be used for the growth of algae. Different types of PRBs like Helical PBR, Tubular PBR, Flat plate PBR are available with their own speciality and productivity. The drawback of PBRs is their price. Installation and operation costs are very high for these which lead to the increase in oil price. Though algae culture is done largely by means of photobioreactors (Podevin et al. 2017) to reed off any sort of contamination and escalate the lipid content of algae, the installation and operation costs are very high for it (Nosker et al. 2011) and this is one of the yardsticks behind increase in algae- oil price (Mata et al. 2009).

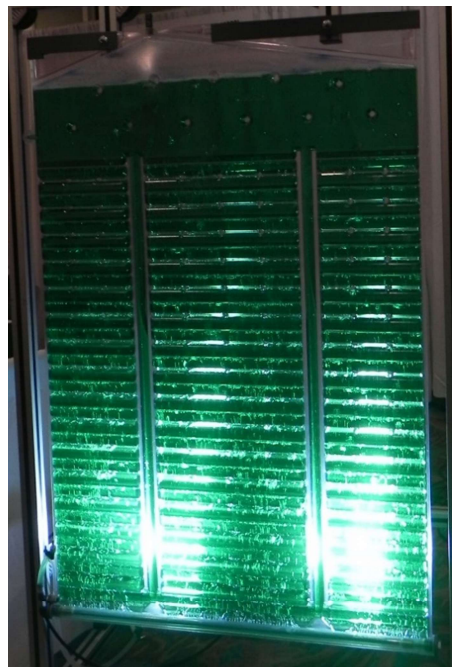


Fig 2.1: Photobioreactor

Recently, algae culture is also preferred to be done in raceway ponds (Fig 2.2). The raceway ponds are also open ponds but these are made of closed loop recirculation channels. They are associated with a paddle wheel which helps in continuous mixing.



Fig 2.2: Raceway pond (source- Google images)

2.3. OIL CONTENT OF ALGAE

Oil content of algae varies from 15% to 77% (Chisti 2007). In most oceanic algae, the oil is more than that of other algae. Whereas lipid content of fresh water algae is average (<20%) (Rodolfi et al. 2007).

Pre-treatment of algal biomass for the destruction of cell with ultra-sonication and other methods have been seen to produce better yield of oil in studies by Suganya et al. (2013) with the algae *Enteromorpha compressa* and *Ulva lactuca*. Conventional pure and quick supercritical fluid extraction or subcritical water extraction has been reported to extract up to 100% of the oil content (Khoo et al. 2011).

The fatty acid content of algal oil has been found to mostly include oleic, linoleic, palmitic. In the study of Seo et al. (2014), algal waste after biofuel production is used to feed *Cryptococcus curvatus* yeast to produce oil. This kind of oil producing microbes, which grows very fast and accumulate very high amount of lipid, can be used for biodiesel production in alternative pathway purpose (Miao and Wu 2006). This will also solve the high amount of residue disposal (Li 2012) of used algal mass.

A study on a specific species of algae *Nannochloropsis salina* has been done by Feinberg (1984) which reports that the concerned algal species contains 34% fatty acid in its total lipid content. Feinberg reported that conversion of 53% of energy content of that alga into liquid fuels is possible where the energy density would be 8.1 MJ. Apart from innovative conventional techniques (Vian et al. 2013), oil extraction by hydro distillation is also getting famous these days (Sardar and Alamgir 2017).

2.4. BIODIESEL PRODUCTION

Biodiesel, generally, are produced by transesterification method which is the reaction of a specific vegetable oil and a short- chain alcohol (Karmakar et al. 2017). This is done in a closed chamber where temperature can be remained constant. The reaction is carried out by stirring the reactants at constant stirring intensity. If the FFA of the biodiesel seems to be low enough, alkali catalysed reaction, for this endeavour, is preferred (Kumar et al. 2017). Catalysts are used to speed up the reaction.

Spirulina platensis alga was used in an experiment as a feedstock for production of biodiesel where extraction and transesterification was done together in a single stage with the aim to study several parameters for production of biodiesel. The optimum conditions for highest yield of biodiesel (75 ± 0.40%) were (i) 60% concentration of catalyst, (ii) 1: 4 algae biomass to methanol ratio, (iii) intensity of stirring of 450 rpm and (iv) reaction temperature of 55°C. The experimental data was found to be a fitted good with the first order reaction kinetics (Szybist et al. 2007).

In a semi- continuous mode of biodiesel production with *Microcystis aeruginosa* 21.3% lipid was achieved followed by a 90.1% biodiesel production (Ashokkumar et al. 2014). The biodiesel had a calorific value of 38.8 MJ/ kg. *Nannochloropsis salina* alga's oil has been used in Wang's research to convert oil to fatty acid methyl ester (FAME) enzymatically. NOVOZYME 435 along with t- butanol and methanol has been used at 25°C for 4 hrs. to produce biodiesel with 99.1% conversion efficiency. Conversion efficiency can be calculated from the GC- MS results using the equation given hereafter (Phan and Phan 2008)-

$$\text{Conversion (\%)} = \frac{(\text{weight of methyl ester collected} \times 100)}{\left(3 \times \frac{(\text{weight of the sample oil})}{(\text{average molecular weight of algal oil})} \times \text{average molecular weight of fatty acid ester}\right)}$$

According to Ponnusamy et al. (2014), 5.6 kg of *Nannochloropsis salina* biomass is needed to prepare 1 kg of biodiesel. In a techno-economic assessment carbon negative production procedure by gasification followed by transesterification to produce biofuels was found to be very economic (Taylor et al. 2013). Although according to this literature larger biodiesel production plants are required to bring a cut down to biodiesel price. They have shown biodiesel price to be reduced to recent price of petrodiesel (\$114/ barrel crude oil) by assuming a 0.8 scaling factor with a rate of production of 100 Mt/ year. According to Wang et al. (2014) if algal biodiesel's use comes in practice, the net revenue that can be achieved is \$24.17 million under base scenario. In a study with algal species *Nannochloropsis oceanica* IMET1, more than 80% of biodiesel was produced using immobilized lipase extracted from *Candida Antarctica* (Wang et al. 2016). In recent days, biodiesel production using heterogeneous catalysts like activated carbon produced by flamboyant pods (Dhawane et al. 2015), calcium diglyceroxide (Gupta et al.) etc. and use of microwave assisted biodiesel production (Babel et al. 2017) are becoming area of interest in many researches.

2.5. ENGINE TEST

Engine test for biodiesel is done to find out the performance of the engine for the use of different biodiesel in them. Examination of engine performance has been done by researchers mostly with algal biodiesel- petrodiesel blends (Pandian et al. 2018). 100% biodiesel has been used in very few researchers.

Very little number of experiments has been done on engine testing and emission characteristics of algal biodiesel throughout the world. Literature from Indian research on engine testing of algal biodiesel is very rare. It has been seen that the algal biodiesel have similar properties of conventional diesel and its use has been successful in operating the diesel engine (Tuccar 2013).

In another experiment, microalgal biodiesel was blended with petroleum diesel in a ratio of 5, 10, 20 and 50 (Haik and Selim 2011). Properties of pure biodiesel and its blends were inspected along with examination of performance of the engine and emissions exhausted from it. The results manifested that that microalgae derived biodiesel is associated with a slight reduction in torque and brake power values of CI engine.

2.6. ECO-FRIENDLINESS OF ALGAE AND ALGAL FUEL

Biodiesel produced from different vegetable oil are evidently eco- friendly because they are produced from renewable natural resources like plants which use carbon dioxide for their photosynthesis. Zivkovik and Veljkovik, in the year 2017, have reported that biodiesel has many benefits including economical, and agricultural and environmental (Zivkovik and Veljkovik 2017).

In a research study fixation of 1.83 kg of CO₂ by 1 kg of algae is seen (Chisti 2007). The research of Dassey et al. indicates that 1.9 g of carbon as CO₂ is required to produce 1 gm of algae (Dassey et al. 2014). Hence, CO₂ emitted from the Power stations can be used for the growth of algae (Chisti 2013). It has been found that recycle of water after harvesting of algal biomass can reduce water usage up to 60% (Yang et al. 2011).

Biodiesel produced from *Monoraphidium* sp. has been shown to produce less greenhouse gases compared to petro- diesel (Holbrook et al. 2014). Harto et al. (2010), suggests that 216 L of water is required for a litre of algal biodiesel production from open ponds. Small scale decentralized on-site treatment of waste water reduces the movement of hazardous waste (Mounz et al. 2006). Management of eutrophication can be done along with algae culture to bring ecological benefits (Dodds et al. 2009). It has been seen that solar energy supply for the entire algal biodiesel production process by means of electricity from Concentrated Solar Power (CSP) is very carbon neutral and economic (Taylor et al. 2013).

2.7. ECONOMIC ASPECTS

In first world countries like United States of America, research on algae based fuels is running in a very good pace. Most of the papers on algal biodiesel have been published by USA (22%) and China (15%) (Chisti and Yan 2011). This indicates that research on this topic is favourable to flexible economy only. In 2009, in USA, it was proposed to invest US\$800 Million on algal biofuel research and its commercialization (Lee 2013).

It has been investigated that developed countries can adopt clean energy easily because of the flexibility in economy (Lee 2011). Algal biodiesel provides a mass balance per unit operation approach to check carbon neutrality and economic feasibility (Himmelblau 1967). The price of crude oil of algae is very high all over the world. In India it costs more than 1000

Rupees/ litre. To be economically competitive algal oil must cost less than \$0.25/ kg as a barrel of petroleum fuel costs around \$100 at current situation (Chisti and Yan 2011).

Biomass production with photobioreactors is also very costly. NNFCC of UK has made calculators to assess the economic feasibility of biofuels for the upcoming investors of biofuel (Gutterson and Zhang 2009). The operation cost of tubular multiple 3 m³ photobioreactor is €69 which produces 1 kg of algal biomass (Fernandez et al. 2001). On the other hand raceway ponds cost only €4.95 for the same (Nosker et al. 2011). Again, high feed stock price contributes 60- 70% of the high product cost (Mata et al. 2009).

According to Pfromm et al. (2011), sustainable production of algal biodiesel is possible by optimistic yield assumptions. If its use comes in practice, the net revenue that can be achieved according to Amano- Boadu (2014) is \$24.17 million under base scenario. The concept of algal biorefinery can make algal biofuel economically viable (Gouveia 2011). It was proposed by Lynd et al (1999). that the systematic design biorefineries can bring advancement in production of bio-based products. An outline of the biorefineries of 21st century has been described by Ragauskas et al. (2006) According to him, from a conventional refinery, a total of 5% of the petroleum goes to chemical products. The remaining is used for fuels for transportation.

Therefore, most of the research works on algae have been done on their cultivation procedure to improve their growth and lipid content. Though biodiesel production from different feed stock has been done, production of the fuel from algae by transesterification procedure is very rare. No work was found to be there on biodiesel production from unused biomass of algae in India. Research works on engine test and emission characteristics of algae based biodiesel is, thus, very few in number.

CHAPTER 3

MATERIALS AND METHOD

This first part of this chapter enlists all the raw materials, including the premier grist, the algae, used in this experiment. The succeeding part of this chapter chronicles the procedure of collection of those materials and the detailed methodology of their use to produce biodiesel including the optimization of growth parameters of those algae, their processing followed by oil extraction and finally the optimization of biodiesel production procedure. It also recounts the physicochemical properties of the algal biodiesel succeeded by emission characteristics of the fuel produced at different load conditions (0%- 110%) of an IC engine.

3.1. SELECTION OF MIXED INDIGENOUS ALGAE- CULTURE FOR THE EXPERIMENT

Algae- culture was gathered from pisciculture- ponds of Guru Angad Dev Veterinary and Animal Sciences University, India. Aquaculture, pisciculture together with experiments on relevant subjects take place in these ponds throughout the year. In winter, required flora and fauna from these ponds are taken away and preserved in laboratories to re-prepare those ponds. Eutrophication, and thereby algal bloom, take place in those ponds during these times. Algae, grown in these ponds, are taken away and discarded in dumping sites. Another algae- culture were hoarded from a canal of Ludhiana city called Simlapuri Nahar. When the water current of this canal becomes very slow or becomes stagnant, bloom of algae takes place due to immobilization of nutrient. These algae causes pollution to the waterbody and generates bad odour after being degraded naturally. Algae, harvested from these two waterbodies, were chosen for this research experiment because of the following reasons-

- (i) They are not important economically
- (ii) They are available very abundantly
- (iii) They are not used as food in India
- (iv) They are trashed in dumping sites without any use.

Harvesting of algae, in this procedure, was done by algae- net or simply by hand (Fig 3.1). Use of any electrical or chemical flocculation was avoided to pursue this job eco- friendly.



(a)

(b)

Fig 3.1: Harvesting of algae from (a) pisciculture- pond of GADVASU (b) Simlapuri Nahar

3.2. CULTIVATION AND OPTMIZATION OF GROWTH PARAMETERS OF ALGAE

Algae collected from their sites were brought to the laboratory of CSIR CMERI CoEFM, Ludhiana and their culture was done in the concrete ponds (5m x 1m x 1.5m) with proper inlet and outlet (Fig 3.2). Identification of the species present in the mixed indigenous algal biomass were done by Department of Biotechnology, TIET, Patiala and The College of Fisheries of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Initially 5 kg of algae were added in every pond. Oil content of these algae at this stage was 8.9%. Several growth conditions were applied on the algae grown in these ponds to find out the optimized conditions for best algal growth in terms of increase of their biomass as well as their oil content. The level of five essential parameters, namely nutrient, carbon dioxide, depth of pond water, temperature and intensity of mixing, were optimized in this process.



Fig 3.2: Algae culture in concrete ponds of CSIR CMERI CoEFM

Growth of these algae was measured in terms of increase of biomass over the initial amount (5 kg) and increase in oil content over the initial oil content (8.9%) of these algae.

3.2.1. DESIGN OF EXPERIMENT

It was decided that five levels of all five parameters would be considered and optimized consecutively in this experiment. While one parameter was getting optimized, every other parameter was being kept constant. When one level of a parameter got optimized, the proceeding experiment with another parameter's optimization was done with that optimized level of the former parameter. It was also planned that opting for experiment with more levels of any growth parameter would be done in required conditions when any good result would not come out of the experiment or search for better result would be expected from any other level of a parameter. The names of the ponds were given according to the levels of parameters used in them.

A statistical analysis of optimization of growth parameters, using Taguchi's approach, was also planned to be carried out after getting positive results from the above said non-statistical study. As this kind of experiment on algae- culture in open pond is very rare and on top of that research on this particular mixed culture of algae which grow naturally in ponds and canals in India and remain unused has never got researchers' attention, statistical analysis was found very hard to design. Therefore, statistical analysis was aimed to be done with the best results received from non- statistical analysis.

3.2.2. OPTIMIZATION OF DIFFERENT GROWTH PARAMETERS OF ALGAE

3.2.2.1. NUTRIENT

As nutrient plays an important role in algal growth, experiment with optimization of nutrient was done at the outset. Nitrogen along with phosphorus are considered as very prime factor for algal growth (Zhang et al. 2017; Havens and Paerl 2015; Fan et al. 2018). A comparative study between fertilizer NPK (40:20:40) and cow dung slurry was carried out in search of the better nutrient. While chemical fertilizer NPK (40: 20: 40) is found to be little costly, cow dung slurry, which contains essential elements for plant growth (Hoekstra et al. 2002; Sorensen and Jensen 1995), has no price value as they are the by- product coming out of the biogas plant.

Five levels of NPK fertilizer, which are 0.5kg/pond, 1kg/pond, 1.5kg/pond, 2kg/pond, 2.5kg/pond were used at first. There were only five ponds available for this optimization of

algal growth parameter experiment. Therefore, experiment with five levels of NPK fertilizer were carried out at first. Corresponding quantities of solid NPK were dissolved in a bucket of water taken from the corresponding pond and the solution was poured back into the pond. The water of the pond was stirred very well for homogenous mixing.

The cow dung slurry were collected from the outlet of cow dung- gas plant installed at CSIR CMERI CoEFM, Ludhiana (Fig 3.3). The liquid part of the slurry was separated by using a muslin cloth and the solid part was kept in the sun for drying and preparing bio- fertilizer or organic fertilizer (Mittal et al. 2018). Optimization of cow dung slurry was carried out with 2l/pond, 3l/pond, 4l/pond, 5l/pond and 6l/pond. Required quantity of this liquid was added in all five relevant ponds and mixed properly. As algae, at this stage, were sitting at the bottom of the pond, no problem was faced to mix the cow dung slurry up with the pond water.

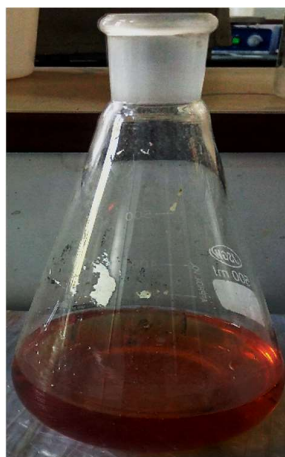


Fig 3.3: Cow dung slurry

3.2.2.2. CARBON DIOXIDE

The next experiment of optimization of growth parameters was performed with carbon dioxide. Five levels of CO₂, which were 10ml/l, 20ml/l, 30ml/l, 40ml/l and 50ml/l, were chosen for this experiment. Supply of carbon dioxide was done from a CO₂ cylinder (Fig 3.4). A pipe attached to the cylinder was kept at its open end on the bottom surface of the pond. Higher pressure causes bubbling of carbon dioxide in the pond and the gas gets released from the pond. To avoid this misuse and make all the carbon dioxide available for algae, the

outlet pressure of the CO₂ cylinder was maintained at 50 psi using a pressure gauge. The flow of the gas was measured with a gas- flow- meter.



Fig 3.4: Supply of CO₂ in the algae- pond

The supply of the gas was done only during daytime when photosynthesis by algae takes place. Injecting CO₂ in ponds during night was avoided because that reduces the pH of the water to a very low level which reduces algal growth.

3.2.2.3. DEPTH OF WATER

Depth of water in algae pond plays an important role on aquatic life (Park et al. 2011) by controlling the penetration of sunlight in it. Thence, optimization of depth of pond was very necessary for carrying out an experiment on algal growth.

Pond water depths of 0.25 m, 0.5 m, 0.75 m, 1 m, 1.25 m were considered to be optimized to find out the best depth of pond water for algal growth. Different levels of pond water were set in five algae- ponds. Then, to initiate the experiment with this parameter, optimized levels of nutrient and carbon dioxide were applied in these ponds and 5 kg of algae were transferred in each of them. Effect of this parameter on algal growth was observed after 10 days.

3.2.2.4. TEMPERATURE

In the state of Punjab, India, four distinct temperature conditions are observed in a year. In extreme winter time, the atmospheric temperature fluctuates from 5°C to 10°C whereas in extreme summer it fluctuates from 41°C to 50°C. In the end of summer and beginning of

winter, the atmospheric temperature is found to fluctuate from 31°C to 40°C. The variation of temperature between 11°C and 20°C is seen in the end of winter and beginning of summer.

Hence, the experiment of temperature on algal growth was planned to be done throughout a year and three trials of experiment were done for each range of temperature. In this way, algae- culture was executed for one year in five different ranges of temperature to find out the best atmospheric temperature for the growth of the selected indigenous mixed culture of algae in terms of increase of its biomass and oil content at the same time. The five ranges of temperature, selected for this optimization process, were 5- 10°C, 11°C- 20°C, 21°C- 30°C, 31°C- 40°C and 41°C- 50°C.

3.2.2.5. MIXING

To avoid usage of electricity and carry out the experiment as eco- friendly as possible electronic stirrer or electronic paddle wheel were not used throughout the optimization procedure and mixing was done manually. As it was not possible to mix the water all the day or in every short time span, optimization of intensity of mixing was performed. Five intensities of mixing, which were mixing in every 2h, 3h, 4h, 5h and 6h, were used in this experiment of optimization of algal growth. This time another pond became available for this experiment. This pond was used as the control- pond and no mixing was done in this pond. Mixing in pond was done from 6 am in the morning to 9 pm at night. Mixing was done using a plastic paddle with long handle.

Table 3.1 displays the levels of five parameters used for optimization of algal growth in this experiment.

Table 3.1: Different growth parameters and their levels

Growth parameters	Levels				
Nutrient (CDS) (l/pond)	2	3	4	5	6
CO₂ (ml/l)	10	20	30	40	50
Depth (m)	0.25	0.50	0.75	1.00	1.25
Temperature (°C)	5-10	11-20	21-30	31-40	41-50
Intensity of mixing	In every				
	2h	3h	4h	5h	6h

As maximum growth of algae was found to take place within 7th and 10th day of algae culture (Karmakar et al. 2012), algae from the ponds were harvested on every 10th day and their growth (increase in biomass from initial 5 kg) and oil content (increase in oil content from initial 8.9%) were measured. Mixing of algae, for homogenization, was done every time of harvest of algae with the help of the algae net.

3.2.3. STATISTICAL ANALYSIS OF OPTIMIZATION OF GROWTH PARAMETERS

A statistical analysis, besides the above analysis procedure, was performed using Taguchi's method. Initially, for each parameter, five levels were available. So, five growth parameters, which were nutrient (X1), carbon dioxide (X2), depth of water (X3), temperature (X4) and intensity of mixing (X5), were considered as variables for this analysis. Those variables along with their five levels were used to prepare the matrix (Table 3.2) to observe their effect on increase in algal biomass (Y1) and increase in their oil content (Y2). Statistical analysis, for optimization of algal growth, was decided to be accomplished by using the best results obtained from the non- statistical analysis. It was also decided that incorporation of any or more levels of parameters, which were not considered earlier but later in the non- statistical analysis and yielded better result, would take place and the matrix would be changed accordingly.

Table 3.2: Different parameters for algal growth (X) and their levels used in statistical analysis of algal growth.

Growth parameters	Code	Levels				
Nutrient (CDS) (l/pond)	X1	2	3	4	5	6
CO ₂ (ml/l)	X2	10	20	30	40	50
Depth (m)	X3	0.25	0.50	0.75	1.00	1.25
Temperature (°C)	X4	5-10	11-20	21-30	31-40	41-50
Intensity of mixing	X5	In every				
		2h	3h	4h	5h	6h

Taguchi's method was used to prepare a set of 25 combinations (L25 orthogonal array) of five parameter's levels (Table 3.3).

Table 3.3: 25 combinations (L25 orthogonal array) of parameters (X) used in optimization of growth parameter of algae

Combination number	Levels of Parameters				
	X1	X2	X3	X4*	X5
1	2	10	0.25	10	2
2	2	20	0.5	20	3
3	2	30	0.75	30	4
4	2	40	1	40	5
5	2	50	1.25	50	6
6	3	10	0.5	30	5
7	3	20	0.75	40	6
8	3	30	1	50	2
9	3	40	1.25	10	3
10	3	50	0.25	20	4
11	4	10	0.75	50	3
12	4	20	1	10	4
13	4	30	1.25	20	5
14	4	40	0.25	30	6
15	4	50	0.5	40	2
16	5	10	1	20	6
17	5	20	1.25	30	2
18	5	30	0.25	40	3
19	5	40	0.5	50	4
20	5	50	0.75	10	5
21	6	10	1.25	40	4
22	6	20	0.25	50	5
23	6	30	0.5	10	6
24	6	40	0.75	20	2
25	6	50	1	30	3

*10 represents 5°C- 10°C; 20 represents 11°C- 20°C; 30 represents 21°C- 30°C; 40 represents 31°C- 40°C, 50 represents 41°C- 50°C

Three trial experiments for each combinations were done. Their mean were calculated eventually and optimization of growth of algae in terms of increase of algal biomass (Y1) and increase of their oil content (Y2) was carried out. The statistical analysis was done by using Minitab software. ANOVA was performed accordingly to analyse the variance and find out whether the results derived from this experiment were statistically significant or not. Contribution of each parameter was calculated and their corresponding ranks were assigned by determining the signal to noise ratio of parameters.

3.3. DETERMINATION OF NPK IN POND WATER

Nitrogen, phosphorus and potassium play very crucial role in increase of biomass and oil content of algae. Wherefore, their presence and quantity in the algal pond water, with all optimized conditions, were measured with pertinent methods each time at the beginning and end of cultivation of algae.

3.3.1. NITROGEN

Determination of nitrogen content was measured by Total Kjeldahl nitrogen method (APHA, Standard Methods for the Examination of Water and Wastewater, 22nd edition, Washington (USA), 2012). Kjeldahl apparatus (Fig 3.5) was used for this purpose.



Fig 3.5: Determination of nitrogen of pond water using Kjeldahl apparatus

At first ammonical nitrogen (AN) content of every pond's water was measured. 500ml of pond water samples, collected at the beginning of algae culture and on the day of harvesting of algae, were taken in long-neck round bottom Kjeldahl's flask. 50ml of borate

buffer was added to each of them and their pH were maintained at 9.5 level. Thereafter the samples were digested. The distillate generated in the procedure of the digestion was collected in 50ml boric acid. Digestion was stopped when the volume of the distillate- boric acid solution reaches 250ml. 2- 3 drops of mixed indicator was added to this solution and the colour of the solution turned to green at this stage. It was, then, titrated with 0.02N sulphuric acid solution till the colour of the solution turn light purple that persists, at least, for ten seconds. The formula for calculating ammonical nitrogen (AN) is as follows-

$$\text{Ammonical N content (AN)} = \frac{\text{Burette reading of sample} - \text{burette reading of blank}}{\text{amount of sample used}} \times 280$$

With the leftover received from the ammonical nitrogen experiment, determination of organic nitrogen (ON) content of the algal pond water was started. 50ml digestion reagent (a solution of K_2SO_4 , H_2SO_4 and $HgSO_4$) was added to the leftover solution and it was digested in an open area of rooftop. When the volume of this solution reduced down to 15ml, the digestion was stopped and allowed to cool down to room temperature. In the next step, 250ml of distilled water followed by 50ml of sodium hydroxide (NaOH) - sodium thiosulfate ($Na_2S_2O_7$) was mixed with the solution. This solution was digested in a Kjeldahl apparatus. The distillate was collected in 50ml boric acid solution and 2-3 drops of mixed indicator was added to it. Titration, to determine the organic nitrogen content of the sample, was done with 0.02N H_2SO_4 till the colour of this solution turns pale purple from green and persists for at least 10 seconds. The formula for calculating the organic nitrogen content (ON) is as follows-

$$\text{Organic N content (ON)} = \frac{\text{Burette reading of sample} - \text{burette reading of blank}}{\text{amount of sample used}} \times 280$$

The formula for TKN is given below.

$$\text{TKN} = \text{AN} + \text{ON}$$

7 trials of each sample were conducted and their means were noted.

3.3.2. PHOSPHORUS

Determination of phosphorus content of algal ponds' water was done spectrophotometrically by acid digestion method (APHA, Standard Methods for the Examination of Water and Wastewater, 22nd edition, Washington (USA), 2012). Standard (stock) solution of phosphorus (PO_4) was produced by dissolving 192mg of monopotassium phosphate (KH_2PO_4) solution in 1l of distilled water. The concentration of PO_4 in this

solution is 50mg/l. To prepare PO_4 samples of known concentration of 0.2mg/l, 0.4mg/l, 0.6mg/l, 0.8mg/l, 1mg/l and 1.2mg/l, 4ml 8ml, 12ml, 16ml, 20ml and 24ml stock solutions were taken in Nessler's tubes and each of their volume was made up to 100ml by the addition of distilled water. 4ml of ammonium molybdate solution was added to each Nessler's tube followed by the addition of 0.5ml of stannous chloride (SnCl_2). The colour of samples, apart from the blank, turned blue (Fig 3.6).

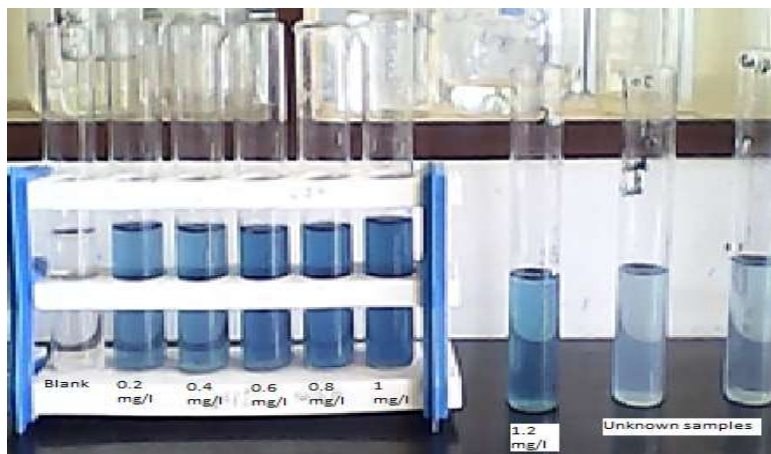


Fig 3.6: Samples with known and unknown concentrations of phosphorus

The OD of these samples with known and unknown concentrations of phosphorus were determined by a spectrophotometer (Fig 3.7) at 690nm. The standard curves and their formula for the samples were prepared by plotting the OD readings generated for known concentration of samples along with those concentrations in the graph. The concentrations of the unknown samples were calculated from the formula generated. 3 trials of each experiment were carried out and their mean were noted.



Fig 3.7: Spectrophotometer

3.3.3. POTASSIUM

Concentration of potassium in algal ponds' water was measured at the beginning of algae culture and on the day of harvesting of algae. It was conducted by Sophisticated Analytical Instrument (SAI) laboratory of TIET by using the method of flame photometry. This measurement was carried out both in summer and winter.

3.4. MEASUREMENT OF DISSOLVED OXYGEN OF POND

Oxygen is the prime element required by all living entities. Although, algae consumes carbon dioxide for their photosynthesis in the day time, their respiration, by which they produce their energy required to perform all required works, goes on throughout the day. During night, they consumes no carbon dioxide but oxygen.

As the algae grow in pond water, a part of the oxygen released by them during day time gets dissolved in the water. This dissolved oxygen is used by algae throughout the day and thus the DO of the pond water dwindles at night. Hence, the measurement of DO in pond water should be analysed while optimizing the growth parameter of algae.

The dissolved oxygen content of each pond was estimated by Winkler's method (APHA, Standard Methods for the Examination of Water and Wastewater, 22nd edition, Washington (USA), 2012; Oudot et al. 1988). 300ml Winkler's bottles were opened beneath the surface of the undisturbed pond- water (quiet before the mixing of pond water) while collecting the samples for DO estimation. Every bottle was capped underneath the pond surface while it was filled up with water. 1ml of manganous sulphate ($MnSO_4$) followed by 1ml of alkali-iodide-azide reagent were added at the middle of each of the bottle with a glass pipette. The bottles were shaken very well and the appearance of brown precipitate was observed (Fig 3.8). As the precipitate settles at the bottom of the bottle, 2ml Concentrated H_2SO_4 was added to each bottle from top. The bottles were shaken once again to dissolve the precipitate completely. 201ml of this dissolved solution was taken inside a conical flask and 3-5 drops of starch indicator was added to it. The colour of the solution turns dark blue. This solution was titrated with 0.025N sodium thiosulfate ($Na_2S_2O_3$) till the solution becomes colourless and the corresponding DO of pond- water was estimated. Dissolved oxygen content of all ponds were estimated from 6am in the morning to 9pm at night both in

summer and winter. Three trials of sample collected at a particular time was done and their mean was calculated.



Fig 3.8: Estimation of DO of pond water

3.5. PREPARATION OF DRY ALGAE POWDER

Wet algae, grown in optimized conditions, were brought to the laboratory and dried thereafter. They were crushed thoroughly for extraction of oil from them.

3.5.1. DRYING OF ALGAE

Drying of algae was done in the sun. After harvesting the algae grown in optimized conditions, they were partially dewatered by squeezing manually. After that chunks of algae were flattened a little and kept in the sun for drying. Use of hot air oven, in this endeavour, was avoided thoroughly to make this procedure as eco- friendly and economically viable as possible. It was observed that the dry weight of algae was only 10% of the wet weight of the algae (Fig 3.9).



Fig 3.9: (a) Wet and (b) dry algae

3.5.2. CRUSHING OF DRIED ALGAE

The dry algae were crushed by using a mixer grinder till their texture turned into almost powder- alike (Fig 3.10). This crushed algal dried algal biomass was stored in a dry glass jar. Some of the dried algae, at this stage, were not crushed but kept for mechanical oil extraction experiment.



Fig 3.10: Crushed algae

3.6. EXTRACTION OF OIL FROM ALGAE

Extraction of oil was planned to be conducted by both mechanical and solvent extraction procedures to carry out a comparative study between these two procedures. While mechanical extraction of oil from oil- seeds is an age old technique and very suitable for seeds with high- oil content, it is being replaced by solvent extraction methods these days (Matthaus 2011). Solvent extraction can also be done for seeds with low oil content and this why the concerned technique is more efficient.

3.6.1. MECHANICAL EXTRACTION

Mechanical extraction of oil (Uquiche et al. 2008) from dried algae by means of a mechanical expeller (3.11 (b)). Some pieces of de- oiled seed-shell of jatropha (Fig 3.11 (a)) were mixed with the dried algal mass to carry out this procedure smoothly because dried algae were found to be very delicate to be used in expeller.



(a)



(b)

Fig 3.11: Mechanical oil extraction (a) jatropha seed- shell (b) mechanical oil- expeller

3.6.2. SOLVENT EXTRACTION

A comparative study between three solvents, namely hexane, acetone and methanol was carried out in the course of solvent extraction (Dutta et al. 2014). Solvent extraction was done by Soxhlet apparatus (Dobush et al. 1985; Hawthorne et al. 2000). Pre- weighed thimbles were filled up with 5 g of crushed algae and placed inside the separator of the apparatus. Continuous flow of cold water was supplied to the condenser of the apparatus. Solvents were taken in the distillation flask placed on the heating mantle (Fig 3.12). The evaporated solvent got condensed in the condenser and dropped inside the thimbles. The oil was extracted hereby from the algae and got deposited in the separator of Soxhlet apparatus until it flows back to the solvent in the distillation flask through the capillary tube. This procedure was repeated for 2- 3 times for each sample. The solvent was evaporated by boiling after extraction of oil of 20 samples and the crude oil was collected in an air tight dry glass jar. The oil content of algae was measured by the following formula-

$$\text{Percentage of algal oil} = \frac{\text{Weight of the oil extracted from dried algae}}{\text{Weight of the dried algae used}} \times 100$$



Fig 3.12: Solvent extraction of algal oil with Soxhlet apparatus

3.7. BIODIESEL PRODUCTION

Biodiesel, from the crude algal oil, was produced by means of transesterification method. Transesterification reaction, for the production of biodiesel, transpires as a result of the interchange of organic R group of a specific oil (triglyceride) with the R' group of a short chain alcohol (Karmakar et al. 2017). The content of free fatty acid (FFA) of the oil was observed before the commencement of transesterification reaction. FFA, of the oil, was found to be 21.3%. As the FFA of oil was very high, a two-step transesterification (Fig 3.13) i.e. acid-catalysed treatment followed by a conventional alkali- catalysed treatment (Sorguven et al. 2010) was decided to be performed for this experiment (Berchmans et al. 2008; Dhawane et al. 2016; Suganya et al. 2013).

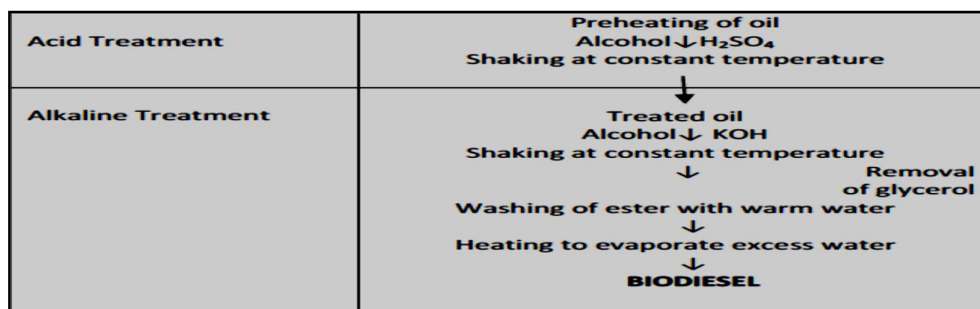


Fig 3.13: Steps of biodiesel production

To carry out this experiment, methanol (alcohol), sulphuric acid- H_2SO_4 (acid- catalyst), potassium hydroxide- KOH (alkali- catalyst) were used. All the chemicals and glass- wares were made available from local shops.

3.7.1 ACID TREATMENT

Acid catalysed esterification was performed to reduce the FFA content of the algal oil below 2% (Kumar et al. 2017) and make the triglyceride suitable for alkali- catalysed reactions because higher level of FFA inhibits the alkali treatment and results in soap formation.

Four parameters were optimized in this course of experiment. They were (i) methanol to oil molar ratio, (ii) acid- catalyst concentration (%), (iii) reaction time (min) and (iv) reaction temperature (°C). Different levels of each parameter used in this experiment were methanol to oil molar ratio of 3: 1; 4: 1; 5: 1; 6: 1, 7: 1 and 8: 1, catalyst amount of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%, reaction time of 30min, 45min, 60min, 75min, 90min, 105min and 120 min, reaction temperature of 40°C, 45°C, 50°C, 55°C, 60°C and 62°C, settling time of 30min, 60min, 90min, 120min, 150min, 180min.

3.7.2. ALKALI TREATMENT

In alkali catalysed reaction also, four parameters, which are (i) methanol to oil molar ratio, (ii) acid- catalyst concentration, (iii) reaction time and (iv) reaction temperature were optimized and when one level of a parameter got optimized, the proceeding experiment with another parameter's optimization was done with that optimized level of the former parameter. The levels of all four parameters used in alkali catalysed reaction were molar ratio of 3: 1, 4: 1, 5: 1, 6: 1, 7: 1 and 8: 1, KOH catalyst concentration of 0.5%, 1%, 1.5%, 2%, 2.5%, 3% and 3.5% reaction temperature of 40°C, 45°C, 50°C, 55°C, 60°C and 62°C, reaction time of 30min, 45min, 60min, 75min and 90min. Use of reaction temperature of 62°C was preferred over 65°C because methanol has a boiling point of 64.7°C. Therefore, keeping the temperature of reaction below methanol's boiling point was given priority for this experiment.

All the acid and alkali catalysed reactions were made inside a waterbath- shaker. The reactants were taken in a screw- capped conical flasks. The flasks were then bolted in the concerned flask- holder of waterbath shaker (Ragit et al. 2011). These flasks were shaken at 250 rpm (Musa 2016) inside the waterbath- shaker at constant temperature to carry out the reaction in the conical flask. When a level of a parameter was selected as optimized level, the proceeding experiment with another parameter's optimization was done with that optimized level of the former parameter. The solution resulted from the transesterification reaction, thereafter, was poured in a separating funnel for separating the glycerol from it. The settling time of glycerol from the FAME was also taken into account. With distilled hot water the FAME was washed. Heating of washed algal FAME was done at 100°C in the hot air oven for the removal of water. The flowchart of the experiment is given in Fig 3.14.

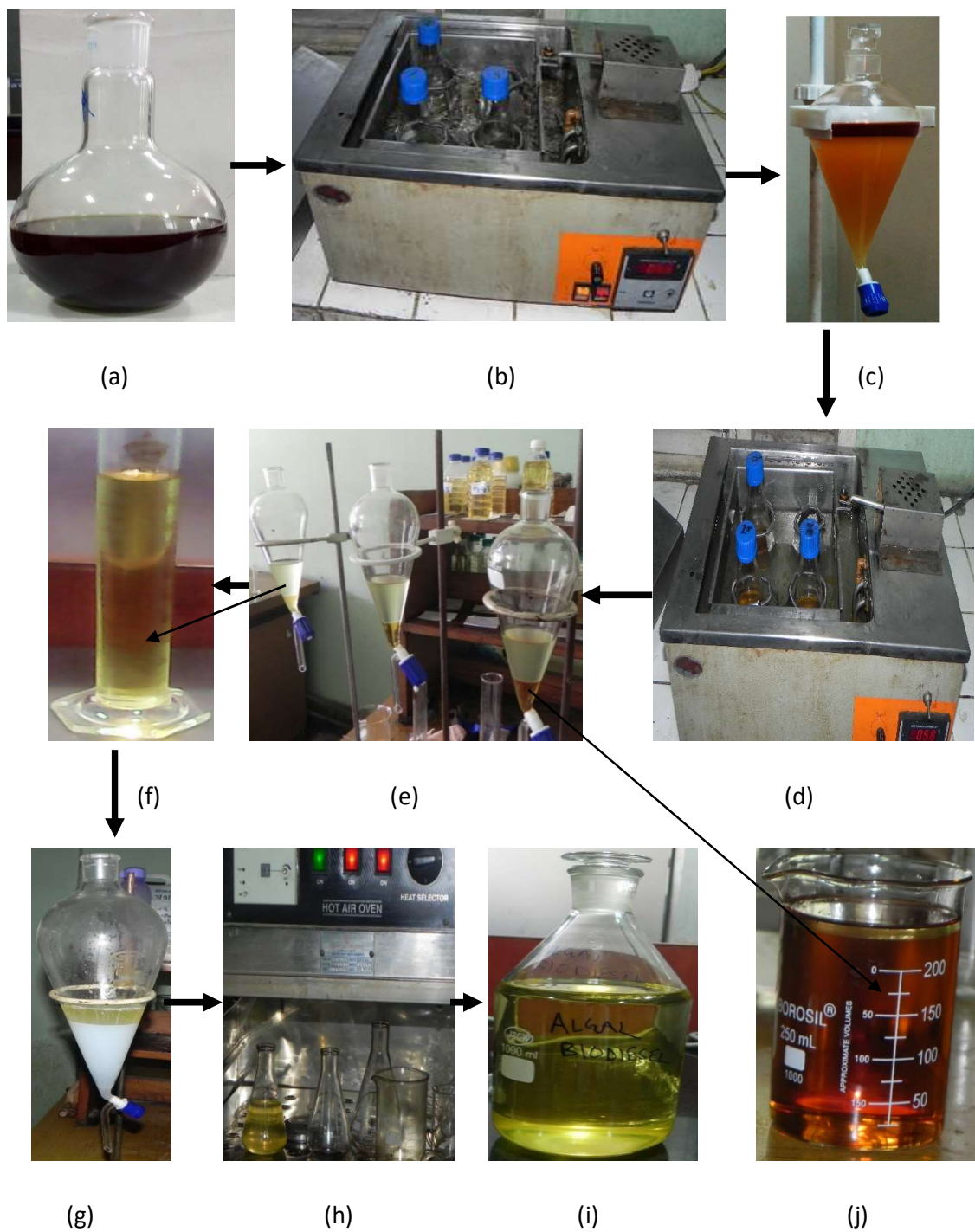


Fig 3.14: Flow chart of transesterification reaction (a) algal oil; (B) acid esterification in waterbath shaker; (c) solution received after acid catalysed reaction; (d) alkali esterification; (e) Separation of FAME and glycerol; (f) algal FAME; (g) washing of FAME with warm water; (h) heating of algal FAME in oven for removal of water; (i) algal biodiesel; (j) glycerol

3.7.3. STATISTICAL ANALYSIS OF BIODIESEL PRODUCTION

Apart from the above explained experiment, a statistically analysed procedure of optimization of transesterification was executed using Taguchi's method (Ross 1989; Buasri et al. 2009). Four parameters, which were, methanol to oil molar ratio (X1), catalyst concentration (%) (X2), reaction- time (min) (X3) and finally reaction- temperature (°C) (X4), were taken into account as variables for this course of statistical analysis (Table 3.4).

Table3.4: Different parameters used in statistical analysis of transesterification procedure

Process parameters	Code
Methanol: oil molar ratio	X1
Catalyst concentration	X2
Reaction time	X3
Reaction temperature	X4

The experimental design of statistical analysis for the optimization of transesterification process was made by Minitab software. It was planned that three levels of each of the four parameters, which would be the optimized value of each level along with the exact upper and lower values of that optimized value, would be chosen from the non-statistical analysis to prepare a set of 9 combinations of parameter's levels (L9 orthogonal array) in the course to observe the effect of four of the parameters to achieve highest yield of biodiesel together with lowest free fatty acid (FFA) content. Practical experiments were performed once again to notice the biodiesel yield and the FFA content of the same resulted from all nine sets of parameters' levels.

Three trials of both yield (%) and FFA (%) of the biodiesel were performed and their results were noted. Corresponding means of the trials were calculated thereafter. The contribution of all four parameters were calculated by determining the signal to noise ratios (S/N ratio) of them and their relevant ranks were assigned to them over yield (Y1) and FFA content (Y2) of biodiesel.

3.8. MEASUREMENT OF BIODIESEL YIELD

To find out the biodiesel with most converted FAME in it and measuring its yield, both thin layer chromatography (TLC) and nuclear magnetic resonance were used. At first three samples, which was found to have highest yield and lowest FFA content, were chosen from statistical and non- statistical methods used for the optimization of transesterification procedure along with algal oil.

The silica gel solution was prepared with hexane and silica gel powder. On a glass plate, a thin layer of silica gel- hexane solution was made subsequently. In an appendrof tube, 1 drop of oil sample was mixed thoroughly with 1ml of hexane. In another three appendrof tubes, the same procedure was done by replacing oil with three biodiesel samples of highest yield and lowest FFA content (S1, S2 and S3). Thereafter, a solution of hexane and ethyl acetate (9:1) was prepared in a glass jar which was followed by incorporation of 1- 2 drops of acetic acid. A tiny drop of each of oil- hexane and algal biodiesel- hexane solutions were placed in a line just above the lower end of glass plate. The glass slide was placed inside the glass jar containing hexane- ethyl acetate solution in such a way so that the drops of oil and biodiesel samples remain above the solution surface. After certain time, when the solution was about to reach the upper end of layer of silica gel, the glass plates were taken outside of the jar and placed in another glass jar containing crystals of iodine. This glass jar was made closed. When the colour was developed on the glass plate, it was brought outside. The presence of FFA, triglyceride and FAME was examined thoroughly from this thin layer chromatography.

The NMR of the algal FAME was done to calculate the exact amount of biodiesel produced from algal oil (Sharma et al. 2013; Satyarthi et al. 2009). From the graph generated from the nuclear magnetic resonance experiment of algal biodiesel, the A_{ME} and $A_{\alpha-CH_2}$ were identified and the percentage of conversion of biodiesel was derived by means of the following formula (Gelbard et al. 1995; Sharma et al. 2013)-

$$\text{Percentage of conversion (C)} = \frac{2A_{ME}}{3A_{\alpha-CH_2}} \times 100$$

Where, A_{ME} stands for the integration value of proton of methyl ester and $A_{\alpha-CH_2}$ indicates the methylene protons' integration value.

The formula used for deriving the yield of the biodiesel is as follows-

$$\text{Yield of biodiesel (\%)} = \text{yield (recovery) of biodiesel observed from experiment} \times C$$

3.9. DETERMINATION OF ALGAL BIODIESEL PROPERTIES

The properties of biodiesel provides with idea not only about the quality of the same but its effect on the engine and, thus, the environment. Generally, use of biodiesel in higher quantity is not preferred because it causes deposition on engine parts and reduces the longevity of it. In this experiment, the properties of algal biodiesel produced under optimized conditions were examined. The properties of this algal biodiesel, such as kinematic viscosity (mm^2/s), relative density (kg/m^3), flash and fire points ($^{\circ}\text{C}$), cloud and pour points ($^{\circ}\text{C}$), acid number ($\text{mg KOH}/\text{g}$), ash content (%), carbon residue content (%) along with gross heat of combustion or the calorific value (kJ/kg) were examined by means of standard methods and apparatus (Table 3.5) (Ragit et al. 2012; Lin and Li 2009). They were, at the same time, tallied with the standard limits. Three trial tests for all properties were done and their means were calculated.

Table 3.5: Standard methods and apparatus used in fuel characterization

Fuel property	Testing apparatus	Standard
Kinematic viscosity	Redwood viscometer	IS: 1448 [P:25] 1976
Relative density	Pycnometer	IS: 1448 [P:32]: 1992
Flash and fire point	Flash & fire point apparatus	IS: 1448 [P:32]: 1992
Cloud and pour Point	Cloud & pour point apparatus	IS: 1448 [P:10]: 1970
Acid number	Burette and glassware (Titration)	-
Ash content	Muffle furnace	ASTM D482-IP 4 of Institute
Carbon residue	Carbon residue content apparatus	ASTM D189-IP 13 of institute of petroleum
Gross heat of combustion	Digital bomb calorimeter	IS: 1448 [P:6]: 1984

3.9.1. KINEMATIC VISCOSITY

Algal biodiesel was kept inside the heating manifold of Redwood viscometer and, then, heated up to 40°C. To let this heated algal biodiesel drain out of this viscometer, the valve rod was dislodged from its position. The biodiesel got collected inside a measuring cylinder placed just under the hole of the viscometer (Fig 3.15). As the volume of the algal biodiesel, inside the measuring cylinder, became 50ml, the valve rod was replaced on the hole to stop biodiesel's flow. The time consumed for this collection process of 50ml of algal FAME was recorded. The formula used to calculate the kinematic viscosity of this algal biodiesel was as follows-

$$\text{Viscosity of algal biodiesel} = (A \times \text{time}) - \frac{B}{\text{time}}$$

Where, A and B are two constants used for the specific Redwood viscometer used

A= 0.26 and B= 179 (while the time taken is <100 seconds)

Or

A= 0.24 and B= 50 (while time taken is >100 seconds)



Fig 3.15: Redwood viscometer used for measuring the viscosity of the algal biodiesel

3.9.2. RELATIVE DENSITY

Pre- weighed pycnometers (Fig 3.16) with plugged in thermometers were filled up with algal biodiesel and kept inside the refrigerator. The time when the temperature of the algal FAME turned 15°C, the pycnometers were brought outside the refrigerator. The mass and volume of the algal FAME at this temperature were noted and the density (D) of the fuel was calculated using the following formula-

$$D = \frac{\text{Mass of the pycnometer containing algal biodiesel} - \text{mass of the empty pycnometer}}{\text{Volume of the algal biodiesel used}}$$



Fig 3.16: Pycnometer

3.9.3. FLASH AND FIRE POINTS

Algal FAME was kept in the concerned slot of the flash and fire point apparatus (Fig 3.17) and heated using a gas- stove. The bottom part of a cotton thread was submerged into the biodiesel. Another cotton thread, which was ignited, was made dragged on the former thread. When the first spark was found to take place, the temperature was recorded as the flash point of this fuel. After certain time, the former thread got ignited and the temperature observed at thermometer at this time was recorded as the fire point of the algal FAME produced.

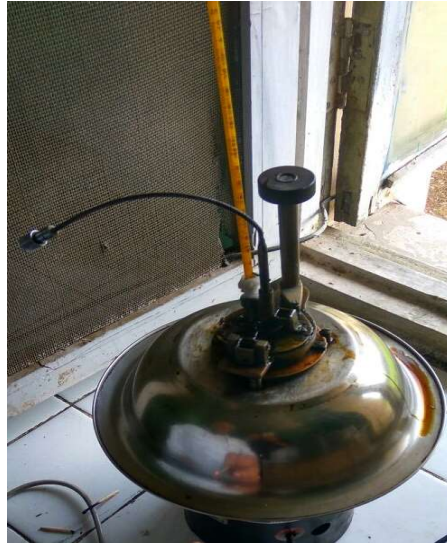


Fig 3.17: Flash and fire point apparatus

3.9.4. CLOUD AND POUR POINT

The cloud and pour point apparatus (Fig 3.18), at first, was filled with crushed dry ice. Glass tubes of the apparatus were filled up with algal biodiesel produced in this experiment and capped with corks. Thermometers were made sieved through all the corks of tubes and submerged into the biodiesel. When the appearance of the algal FAME became cloudy, the temperature reading displayed on the thermometer was recorded as cloud point of the algal biodiesel and when the biodiesel started freezing and become semi solid, the temperature was noted as the pour point of the algal FAME.



Fig 3.18: Cloud and pour point apparatus

3.9.5. ACID NUMBER

Acid number of this algal biodiesel was derived titrimetrically using burette (Fig 3.19). 0.5ml of algal FAME sample was poured into a 250ml conical flask. Solvent mixture of 50ml (equal proportion of diethyl ether and 95% ethanol) was added to it and mixed properly by shaking. 2-3 drops of phenolphthalein indicator got added to it. Titration of the sample was carried out with 0.1N potassium hydroxide (KOH) solution. The burette reading was noted as the colour of the sample turned pale- pink and the colour persists for at least 10 seconds. The formula used for derivation of acid number of the algal FAME is as follows-

$$\text{Acid number of algal biodiesel} = \frac{56.1 \times \text{normality of the KOH solution} \times \text{volume of KOH used}}{\text{weight of the sample used}}$$



Fig 3.19: Titration for the estimation of acid number of algal biodiesel

3.9.6. ASH CONTENT

Ash content of this algal FAME was measured by means of a muffle furnace (Fig 3.20). 5 g of algal biodiesel was taken in a pre- weighed heat- proof quartz crucible. This crucible was put inside the muffle furnace pre-heated at 450°C- 500°C. As half an hour was passed, the muffle furnace was unplugged and the crucible was brought outside of it after an hour. The weight of the crucible was taken when the temperature of the same decreased to room

temperature. Formula which was used to calculate the ash content of the algal biodiesel is as mentioned here-

$$\text{Ash content of algal biodiesel} = \frac{\text{Initial weight of crucible} - \text{Final weight of crucible}}{\text{Weight of algal biodiesel}} \times 100$$



Fig 3.20: Muffle furnace

3.9.7. CARBON RESIDUE CONTENT

For the estimation of carbon residue content of the FAME a digital carbon residue content apparatus (Fig 3.20) was used. 5g of algal biodiesel was taken in a pre- weighed cooking bulb. The bulb was placed in its concerned slot of the apparatus which was preheated at 450°C- 500°C and the apparatus was unplugged when 30 min was passed. After an hour the bulb was brought outside and weight of the same was observed when its temperature reduced to room temperature. The formula which was used to calculate carbon residue content of the algal FAME is as follows-

$$\text{Carbon residue content of algal biodiesel} = \frac{\text{Initial weight of cooking bulb} - \text{Final weight of cooking bulb}}{\text{Weight of algal biodiesel used}} \times 100$$



Fig 3.21: Carbon residue content apparatus

3.9.8. GROSS HEAT OF COMBUSTION

The calorific value (CV) or gross heat of combustion of algal biodiesel was measured by means of a digital bomb calorimeter (Fig 3.22). 0.5 g of the algal biodiesel was taken in the concerned cup of the calorimeter and was placed inside the bomb segment of the same. A cotton thread of 8 cm hanging from a nichrome wire of 8 cm was submerged into algal biodiesel. At 400 psi of pressure, the bomb got filled up with oxygen. Thence, the bomb was put inside the insulated container of the calorimeter which was containing distilled water. Fuse wires of the calorimeter were placed in the concerned slots on the bomb. An 8 cm nichrome wire, inside the bomb, was tied tightly with two sticks which, themselves, were connected with those fuse wires placed on the bomb. Initial temperature inside the insulated jacket of bomb calorimeter was recorded and it was, then, set to zero (0°C). Short circuit on the nichrome wire, inside the bomb, was made by switching on the fire plug to ignite algal biodiesel taken. The temperature increased gradually for some time. The temperature, when stable, was recorded. Gross heat of combustion of this algal biodiesel was derived using the following formula-

$$CV = \frac{(\text{weight of water used} + \text{water equivalent}) \times \text{rise of temperature} \times \text{specific heat of water}}{\text{weight of algal biodiesel}}$$



Fig 3.22: Bomb calorimeter

3.10. TEST OF EMISSION CHARACTERISTICS OF ALGAL BIODIESEL

To examine the emission as a result of combustion of algal FAME a Kirloskar made AV1 engine (Fig 3.23 and Table 3.6) was run by it. The experiment was conducted at Department of Farm Machinery and Power Engineering of G.B. Pant University of Engineering and Technology, Uttarakhand, India.

This time, 100% algal biodiesel (B100) was taken into count. When the gases were emitted from the exhaust of the engine (as a result of combustion of algal biodiesel in the engine, the quantitative presence as well as of carbon monoxide (CO), carbon dioxide (CO₂), oxides of nitrogen (NO_x) and hydro carbon (HC) was observed and recorded. A Testo made AVL 444 di-gas analyser was used for this endeavour.

The probe of this gas analyser was inserted in the engine- exhaust and the quantity of the gases along with the exhaust temperature were displayed on the screen. A comparative study between the emission of algal FAME (B100) and that of petro- diesel was carried out at different engine- loads of 0%, 20%, 40%, 60%, 80%, 100%, 110%.

Table 3.6: Details of the engine used to examine the emission characteristics of algal FAME

Maker	Kirloskar
Model	AV1
Rated speed (rpm)	1500
Rated brake power (bhp/kW)	5/3.73
Number of cylinder	1
Displacement (cc)	552.920
Bore X stroke (mm)	80 x 110
Lubrication system	Forced Feed
Compression ratio	16.5: 1
Cooling system	Water Cooled
Standard injection timing	27° BTDC

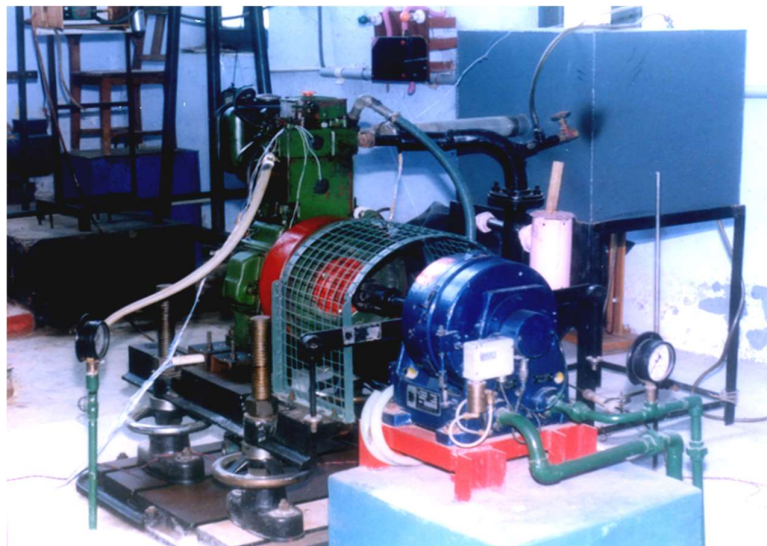


Fig 3.23: Engine used to examine the emission of algal biodiesel (B100)

Besides the above mentioned emission test with B100 algal biodiesel, emission for combustion of three algal FAME- petro- diesel blends, which were B10, B20 and B30, was examined at Department of Mechanical engineering, TIET, Patiala, India. A Kirloskar made 4 stroke engine (Table 3.7) was used to carry out this part of the experiment.

This time also, a Testo made AVL 444 digas analyser (Fig 3.24) was used for the examination of emission of gases like carbon monoxide (CO), carbon dioxide (CO₂), oxides of nitrogen (NO_x) and hydrocarbon (HC) which were generated for the combustion of the algal biodiesel fuel. A comparative study between the emission of petro- diesel and algal biodiesel- petro- diesel blends was done at varied engine loads of %, 20%, 40%, 60%, 80%, 100% and 110% (overload).

Table 3.7: Details of engine used to examine the emission characteristics of algal biodiesel- petro- diesel blends

Maker	Kirloskar
Engine type	Four stroke VCR engine
No. of cylinder	Single cylinder
Engine capacity	661 cm ³
Compression ratio	17.5:1
Dynamometer type	Eddy current, unit loading
Power	3.5 kW at 1500 RPM
Bore	87.5 mm
Stroke	110 mm
Cooling system	Water cooled
Injection timing	23° bTDC



Fig 3.24: Operation of engine and AVL 444 digas analyser to examine the emission of algal biodiesel- petro- diesel blends

3.11. ANALYSIS OF COMPOSITION OF ALGAL BIODIESEL

fatty acid composition of the algal FAME produced from this experiment was carried out by the Department of Biofuel of MERADO institute, Ludhiana, India, by means of gas chromatography (GC) technique (Berchmans and Hitara 2008; Singhasuwan et al. 2015).

CHAPTER 4

RESULT AND DISCUSSION

This chapter documents all the findings derived from the experimental research work carried out. At the outset it provides the optimized conditions for the growth of mixed indigenous culture of algae derived from both non-statistical and statistical analysis. The capacity of different solvents have been enlisted in the next part. The succeeding segment delineates the optimized parameters for the algal biodiesel production procedure emanated from both non statistical and statistical analysis. It also includes the important properties of the fuel. The emission of gases for the combustion of the algal biodiesel produced and its blends with petro-diesel in a CI engine with varied load conditions has also been documented at the end of this chapter.

4.1. ALGAL SPECIES PRESENT IN MIXED INDIGENOUS ALGAL- CULTURE

Ten species were found to be present in the mixed culture of algae collected from GADVASU. The species were- *Scenedesmus* sp., *Chlorella* sp., *Closterium* sp., *Gomphonema* sp., *Spirulina* sp., *Ocillatoria* sp., *Navicula* sp., *Pinullaria* sp., *Zygnema* sp., *Spyrogyra* sp.

In Simlapuri Nahar, *Euglena* sp., *Spirulina* sp., *Spyrogyra* sp., *Chlorella* sp., *Urenoma* sp., *Frustulia* sp., were found to be present.

4.2. OPTIMIZED GROWTH PARAMETERS OF ALGAE

Optimized growth rate of algae in terms of increase in biomass of algae as well as increase of its oil content obtained from non- statistical analysis and statistical analysis were almost similar.

4.2.1. RESULTS OBTAINED FROM NON-STATISTICAL ANALYSIS

4.2.1.1. EFFECT OF NUTRIENT

Although the NPK (40:20:40) was found to exert adverse effect on algal growth cow dung slurry was found to be an important parameter for increase in algal biomass as well as its oil yield. Detailed results obtained from both NPK (40:20:40) and cow dung slurry is given below.

4.2.1.1.1. NPK

NPK fertilizer is used for the cultivation of important plants like wheat (Shaharoon et al. 2008). Among the two nutrients, commercial NPK (40:20:40) was used at first. All the five NPK content was found to exert adverse effect on algal growth (Table 4.1). The final weight of the algae decreased at least to the half of the initial weight (5 kg). With the increase of NPK, the cut down in the quantity of algae took place. The highest reduction was found at 2kg/pond (average growth 0.75 kg from 5 kg) and 2.5kg/pond (average growth 0.47kg) simultaneously (Fig 4.1). The highest average growth (2.23 kg) of the algae was found at 0.5kg/pond level (Fig 4.1). So, another two low levels of NPK (0.25kg/pond and 0.125kg/pond) was used to observe whether lower concentration of NPK causes better growth or not. Although a little better growth was observed (average final weight 2.27 kg) in these two cases, both were much lower than the initial amount of algae used. As the average growth observed in those cases were same, experiment with further reduced quantity of NPK was not done and use of NPK as a nutrient in this experiment was rejected.

Table 4.1: Algal growth with NPK (40:20:40)

		Algal biomass harvested/ pond (kg)					
NPK used 10 th day	0.125	0.25	0.5	1	1.5	2	2.5
	kg/pond	kg/pond	kg/pond	kg/pond	kg/pond	kg/pond	kg/pond
1 st	2.1	2	1.8	2.1	2	0.5	0.2
2 nd	2.3	2.1	1.9	2.2	1.7	0.6	0.3
3 rd	2	2.2	2	2.1	1.9	0.6	0.3
4 th	2	2.3	2.2	2	2	0.7	0.6
5 th	2.5	2.5	2.8	2.5	2.1	0.9	0.5
6 th	2.7	2.5	2.7	2.3	2.3	1.2	0.9

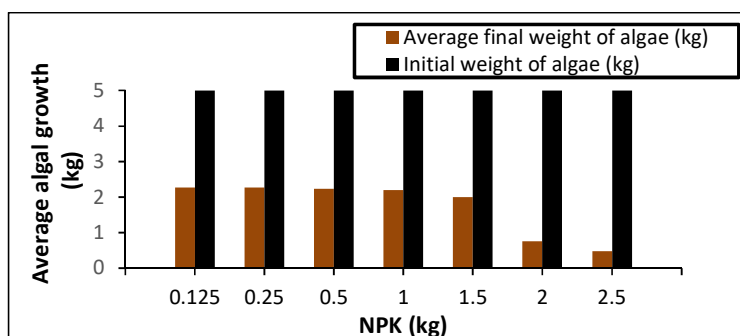


Fig 4.1: Growth of algae with NPK (40:20:40)

4.2.1.1.2. COW DUNG SLURRY (CDS)

When CO₂ and sunlight plays the main role in algal photosynthesis, it cannot be proceeded without the role of essential nutrients. CDS, as nutrient, was used at different concentration in this experiment. From Table 4.2.1 it can be found that very effective growth of algae took place when CDS was added as a nutrient in the algae pond. Algal growth in all five ponds was found to decrease linearly after 90 days when the atmospheric temperature, at the same time, dropped gradually. Highest rate of algal growth was observed in all ponds which were grown between 30th and 40th day. The growth of algae grown in 2l/pond, 3l/pond, 4l/pond, 5l/pond and 6l/pond, during this time, were 11 kg, 11.3 kg, 11.9 kg, 8.6 kg and 6.8 kg from 5 kg respectively. Significant average growth of 9.2 kg of algae was observed in '3l/pond' pond. But the highest growth (average final weight 9.6 kg) was seen in the pond where 4l/pond slurry was added (Fig 4.2(a)). Although further increase in CDS, was found to cause simultaneous cut down in the final growth (average final weight 6.3kg and 5.5 kg accordingly for '5l/pond' and '6l/pond' ponds).

In respect to oil content too, the 4l/pond CDS provided best result (10.8%) (Fig 4.2(b)). Like the growth of algae, the oil content of algae was lowest for algae grown in 5l/pond (9% from 8.9%) and 6l/pond (8.93%) of CDS ponds (Table 4.2.2). No notable increase in oil content of algae was observed in pond receiving lowest quantity of CDS (8.97%). Satisfactory oil content (average final oil content 9.7%) was found in the '3l/pond' pond.

So, 4l/pond CDS was considered as the optimized level for Algal growth. Beyond that level, the decrease in algal growth and its oil content takes place. It was also observed that in first two months of this study, algal growth was at its peak. The growth started decreasing while

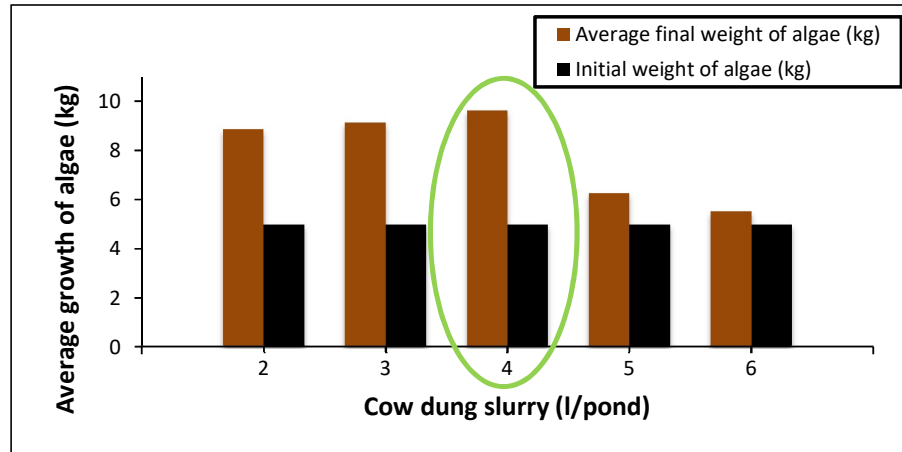
the atmospheric temperature started increasing. Increase of cow dung slurry quantity was found to be associated with significant decrease in DO content of the pond, especially after sunset. This affects the growth of algae and its oil content (Chi et al. 2009) to decrease. Therefore addition of excess of CDS in algal ponds is not recommendable while dealing with the algal growth.

Table 4.2.1: Algal growth with cow dung slurry

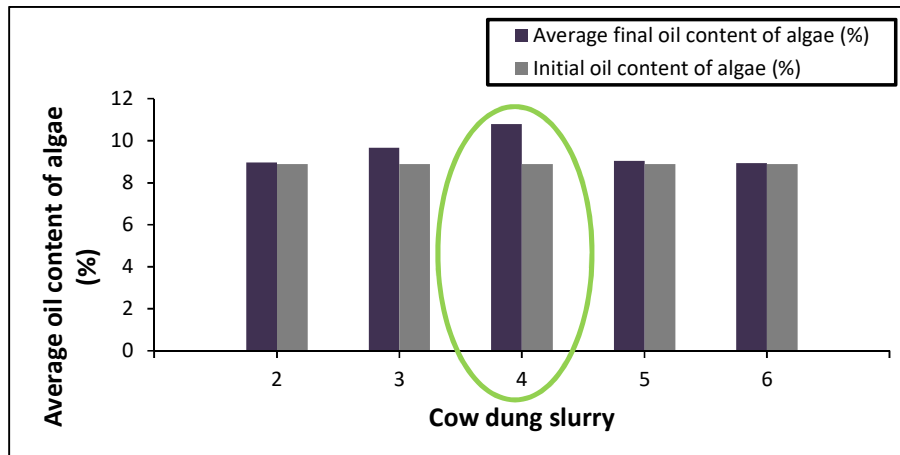
Cow dung slurry used 10 th day	Algal biomass harvested/ pond (kg)				
	2 l/pond	3 l/pond	4 l/pond	5 l/pond	6 l/pond
1 st	10.1	10.5	11.2	8.3	6.9
2 nd	10.8	11	11.5	8.4	7
3 rd	10.9	11	11.9	8.4	6.8
4 th	11	11.3	11.9	8.6	6.8
5 th	10.9	11.3	11.7	8.5	6.5
6 th	10.5	10.7	11.1	7.7	5.7
7 th	10.2	10.4	10.9	6.4	5.6
8 th	9.6	9.9	10.6	5.9	5.6
9 th	9.6	9.9	10.7	5.8	5.5
10 th	8.1	8.3	8.8	5.3	5.3
11 th	8	8.2	8.7	5.3	5.4
12 th	7.8	8	8.6	5.2	5.3
13 th	7.4	7.8	8.1	5.2	5.1
14 th	7.3	7.5	7.8	5.1	5
15 th	7	7.3	7.7	5	4.7
16 th	7	7.3	7.7	4.8	4.6
17 th	6.9	7.1	7.3	4.5	4.1
18 th	6.8	7.2	7.3	4.5	3.8

Table 4.2.2: Change in oil content of algae with cow dung slurry

Cow dung slurry used Trial	Oil content of algae harvested/ pond (%)				
	2 l/pond	3 l/pond	4 l/pond	5 l/pond	6 l/pond
Trial1	9	9.8	11	8.9	8.9
Trial2	8.9	9.6	10.6	9.1	8.9
Trial3	9	9.6	10.8	9.1	9



(a)



(b)

Fig 4.2: (a) Growth of algae (b) change in oil content of algae with cow dung slurry

4.2.1.2. CARBON DIOXIDE (CO₂)

For their photosynthesis and, thus, their growth, algae use dissolved carbon dioxide of the pond water. Algal growth and the oil content of the same increases with the supply of air supplemented with higher proportion of CO₂ (Ramaraja et al. 2015; Hu and Gao 2003; Fabricius et al. 2015; Holbrook et al. 2014). CO₂ was found to be a prime boosting factor for the growth of algae. Initially the growth of algae, in all ponds, specially the 10ml/l and 20ml/l of CO₂ ponds, was less. Increase of algal growth, for the application of carbon dioxide, was found to take place with the decrease in atmospheric temperature. A very

steep growth was observed when CO₂ was applied in the pond water with the algae grown between 151st and 180th day of algae culture (Table 4.3.1). During this period of time, the atmospheric temperature was lowest compared to other times. The growth of the algae was found to increase with the increase of proportion of CO₂ applied in the first three cases (10ml/l, 20ml/l, 30ml/l). From the Fig 4.3(a), it can be observed that the average growth of algae in the 40ml/l pond was very insignificantly lesser (10.02 kg from 5 kg) than 30ml/l pond (10.07 kg from 5 kg). But any further increase of CO₂ (50ml/l) engendered the decline of algae (9.3 kg from 5 kg). From the Table 4.3.1, it can also be concluded that the algae might have taken time to be stabilized with CO₂ supplied from the cylinder at the beginning. As the system got adjusted with the supply of CO₂ from CO₂ cylinder, rate of photosynthesis increased spontaneously and the linear increase of biomass of algae occurred, especially in 30ml/l and 40ml/l CO₂ ponds. Highest growth of algae was observed to take place in all ponds on the last 10 days of the experiment. The growth of all five ponds, which were 10ml/l, 20ml/l, 30ml/l, 40ml/l and 50ml/l of CO₂, at this time were 10.8 kg, 10.9 kg, 11.4 kg, 11.2 kg and 10.3 kg from 5 kg respectively.

No significant increase in oil content of algae was observed in algae grown in 10l/pond and 20l/pond water. Only 0.16% increase of algal oil was observed in 10ml/l pond (9.06% from 8.9%) whereas the oil content of algae grown in ponds enriched with 20ml/l CO₂, increased by only 0.13% (9.03% from 8.9%). The oil content of the algae also reached its peak when 30ml/l CO₂ was applied in the pond (average oil content 11.07%) (Fig 4.3(b)). The increase in content of oil of algae in 40ml/l pond (10.27% from 8.9%) found to be less than that of 30ml/l pond (11.07% from 8.9%). Decrease of oil content of algae occurred linearly for further increase of CO₂ quantity in pond water. The increase in oil content (8.9%) of algae grown in 50ml/l pond water was nil (Table 4.3.2).

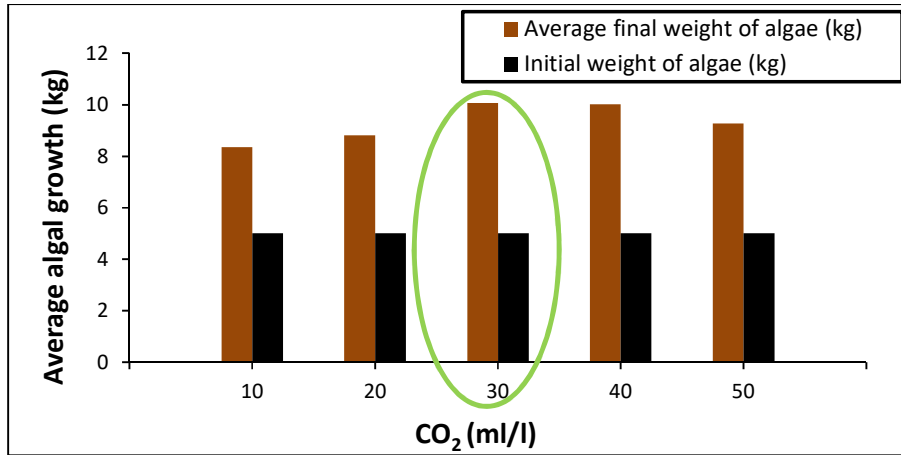
Therefore 30ml/l CO₂ was observed to be the optimum level of CO₂ in the pond for increase in growth as well as increase of oil content of algae. Use of CO₂ beyond this limit lowers the pH of the pond to a noticeable level. This hampers the stability of the algae- pond because, generally, CO₂ applied over the optimized level remains unused and the pH of algal pond decreases thereby and increase in algal biomass works as an add on factor as a result of respiration which takes place simultaneously with photosynthesis. Lower pH of pond starts affecting the growth and algal oil content (Cohen et al. 1988).

Table 4.3.1: Algal growth with CO₂

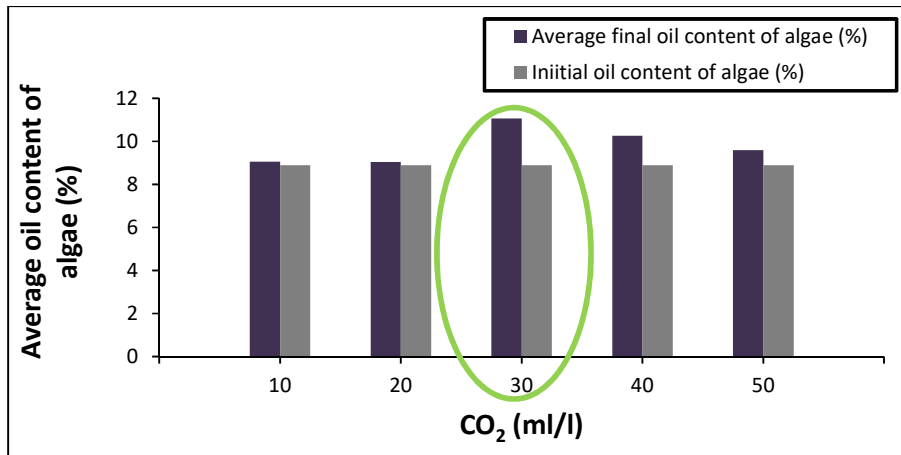
		Algal biomass harvested/ pond (kg)				
10 th day	CO ₂ used	10 ml/l	20 ml/l	30 ml/l	40 ml/l	50 ml/l
	1 st		6.9	7.9	9.8	9.6
2 nd		7	7.8	9.6	9.6	9.2
3 rd		6.8	7.6	9.3	9.5	9.1
4 th		6.6	7.4	9.3	9.3	8.8
5 th		6.5	7.4	9.1	9	8.7
6 th		6.4	7.4	9	9	8.6
7 th		6.9	7.5	9.2	9.2	8.7
8 th		7.3	7.7	9.2	9.1	8.7
9 th		7.9	8	9.6	9.6	9
10 th		8.4	8.5	10	9.9	9.1
11 th		8.8	8.8	10.2	10.3	9.3
12 th		9.2	9.3	10.5	10.6	9.3
13 th		9.6	9.9	10.8	10.8	9.5
14 th		9.7	10.2	10.9	10.7	9.6
15 th		10.3	10.7	10.9	10.9	9.8
16 th		10.6	10.7	11.1	11	9.9
17 th		10.7	10.9	11.3	11	9.9
18 th		10.8	10.9	11.4	11.2	10.3

Table 4.3.2: Change in oil content of algae with CO₂

		Oil content of algae harvested/ pond (%)				
Trial	CO ₂ used	10 ml/l	20 ml/l	30 ml/l	40 ml/l	50 ml/l
	Trial1		9	8.9	10.9	10
Trial2		9	9	11	10	9.6
Trial3		9.2	9.2	11.3	10.8	9.6



(a)



(b)

Fig 4.3: (a) Growth of algae (b) change in oil content of algae with CO₂

4.2.1.3. DEPTH OF WATER

It was found that highest algal growth (10 kg from 5 kg) takes place at 0.5m pond depth (Fig 4.6(a)) and effective decrease of growth rate takes place when the depth of pond water goes beyond 1m (Table 4.4.1). The growth of algae grown in 0.25m pond (9.48 kg from 5 kg) was little less than that of 0.5m pond. The growth of 0.75m pond (9.75 kg from 5 kg) was also as good as the 0.5m pond. The peak growth was observed to take place in algae harvested from all the ponds on 4th, 5th and 6th '10th' day. This time, again, the atmospheric temperature was less among 180 days. Significant decrease of algae was found to take place after 130 days when the atmospheric temperature was at its peak. Least growth, among the whole period, took place in all ponds between 171st day and 180th day. The growth of algae, grown between 41st day and 50th day, in 0.5m and 0.75m ponds was exactly the same (11.6 kg from 5 kg). Although, the difference of this growth in 0.5m pond and 0.75m pond was very marginal, algal growth in other two ponds with more depth, especially 1.25m pond, was significantly less throughout the experiment.

Oil content of the algae of 0.5m pond was highest (11.2% from 8.9%) among others (Fig 4.6(b)) which was found to decrease with further increased depth of pond water. In all three trials of oil content 0.25m pond's algae, the outcome was almost same (10.8%, 10.7% and 10.7%). The difference between the algal oil content of 0.5m pond (11.2%, 11.3% and 11%) and 0.75m pond (11%, 10.8% and 10.7%) throughout all three trials was nominal. The growth of algae was less in 1m pond than 0.75m pond. On contrary, difference in oil content between the algae of 0.75m pond and 1m pond (10.7%, 10.9% and 10.7%) was minimal throughout all three trials. Although, there was a little increase (5.97 kg from 5 kg) in growth of algae in the pond with most depth (1.25m), no increase of oil content was observed in these algae.

This happens because lower depth improves the penetration of light into the pond water and helps to boost photosynthesis (Chisti 2016). As the depth of algae pond increases, penetration of sunlight in the pond decreases. Now, because algae grow at the bottom of the pond at its initial stage, penetration of sunlight to the bottom of the pond is very important to carry on algal photosynthesis. The situation gets worse when algae start growing up and come upwards to commence the formation of an algae- film on the pond surface. This algae- film stops the sunlight from going inside the pond.

Apart from that, change in depth of water in the pond causes change in volume of pond water because the length and width of the pond are unalterable. As a result, in a course of optimization of growth conditions of algae, where parameters like nutrient level, carbon dioxide are being considered, depth of the water plays a prime role because that can change the volume of water and thereby the concentration of nutrient and other essential materials required for algal growth in the pond.

Table 4.4.1: Algal growth with varied depth of pond water

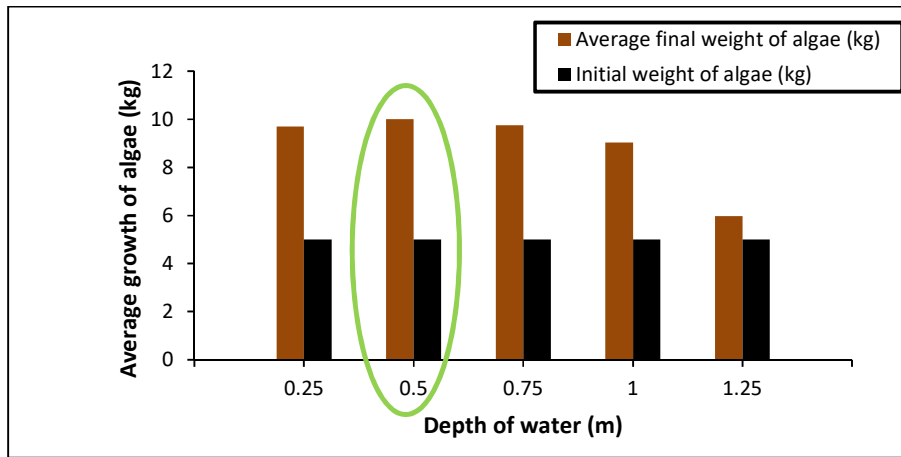
		Algal biomass harvested/ pond (kg)				
10 th day	Depth of water	0.25 m	0.5 m	0.75 m	1 m	1.25 m
	1 st		10.5	11	10.7	9.9
2 nd		10.9	11.1	11	10.1	7.1
3 rd		11	11.9	11	10.2	7.1
4 th		11.4	11.8	11.5	10.6	7.2
5 th		11.5	11.6	11.6	10.8	7.4
6 th		11.3	11.5	11.4	10.7	7.4
7 th		10.8	10.9	10.9	10.2	6.8
8 th		10.1	10.3	10.2	9.8	6.4
9 th		10	10.5	10.1	9.6	6.1
10 th		9.8	10	9.7	9.2	6.1
11 th		9.6	9.8	9.5	9.1	5.9
12 th		9.2	9.3	9.2	8.3	5.6
13 th		8.9	9.1	8.9	8	5.2
14 th		8.2	8.7	8.2	7.7	5
15 th		8.1	8.4	8.1	7.5	4.8
16 th		8	8.2	8	7.3	4.6
17 th		7.9	8.2	8	6.9	4
18 th		7.4	7.9	7.5	6.6	3.7

Algal growth as well as its oil content (4.4.2) were also found to attenuate slightly when depth of water of algae pond decreased further. The reason behind such pattern of growth of algae took place as the ponds were flourished with optimized levels of cow dung slurry (4l/pond) and carbon dioxide (30ml/l). As the depth and width of the pond were constant, decrease in pond water depth caused decrease in volume of the water. This is how the

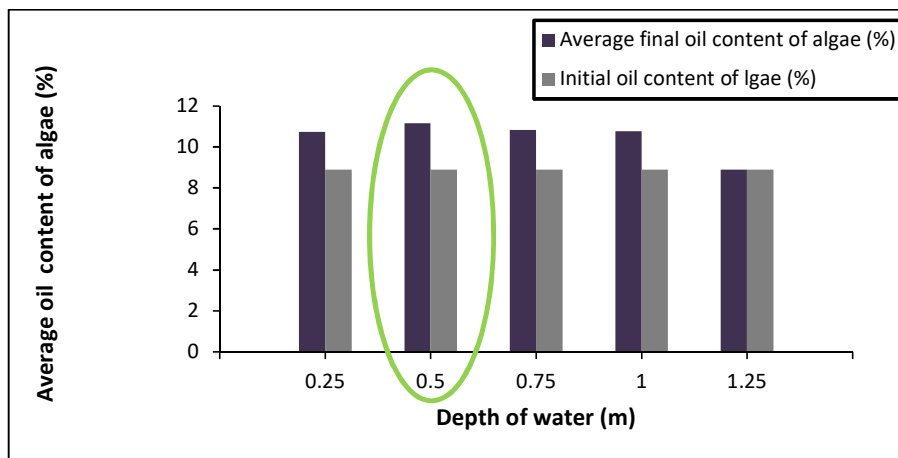
concentration of both the optimized parameters increases in ponds which hampers the stable condition of the waterbody. Therefore depth of 0.5m was found to be optimum for algal growth with 4l/pond cow dung slurry as nutrient and 30ml/l CO₂.

Table 4.4.2: Change in oil content of algae with varied depth of pond water

Depth of water	Oil content of algae harvested/ pond (%)				
	0.25 m	0.5 m	0.75 m	1 m	1.25 m
Trial1	10.8	11.2	11	10.7	8.9
Trial2	10.7	11.3	10.8	10.9	8.9
Trial3	10.7	11	10.7	10.7	9.1



(a)



(b)

Fig 4.4: (a) Growth of algae (b) change in oil content of algae with varied depth of pond water

4.2.1.4. TEMPERATURE

Temperature has a prime impact on the growth and metabolism of microorganisms (Hagerty et al. 2014). Temperature affects not only the growth (Singh and Singh 2015) of algae but change of its oil content also (Schnurr and Allen, 2015).

Temperature was found to exert distinct effect on algal growth (Table 4.5.1) and its oil content (4.5.2). In previous experiment also, the growth of algae was found to be influenced by atmospheric temperature conditions. Most growth of algae (11.6 kg from 5 kg) was found in winter when the temperature of local atmosphere varies from 5°C to 10°C (Fig 4.5(a)). Noticeable growth (10.6 kg from 5 kg) of algae took place in 11°C to 20°C temperature. Growth of algae was found to decrease linearly with increase in atmospheric temperature. Growth of algae during post summer time, when the atmospheric temperature varied from 31°C to 40°C, was 8 kg from 5 kg at most. Almost 9 kg of algae were grown in the temperature range of 21°C- 30°C. Lowest growth was found in summer time when the temperature varies from 41°C to 50°C. Only 7 kg of algae grew from 5 kg of algae during this time.

The oil content of the algae with most growth, which grew in winter with atmospheric temperature of 5°C- 10°C, was found to be the maximum (11.8% from 8.9%). Oil content of the algae grown in summer was significantly low (8.97%). Moderate quantity of oil was yielded from algae grown in 21°C to 40°C (Fig 4.5(b)). The oil content of algae in post winter condition, when the temperature of the atmosphere varied between 11°C and 20°C, was 10%.

Growth of different algal species vary in a particular temperature. Different types of algal growth are seen at different geographical location (Yvon- Durocher et al. 2015; Raven and Gider 1988). Algae- culture grown in this particular geographic zone was found to grow maximum in 5°C to 10°C. Therefore, the availability of these algae is very seasonal.

Because algae- culture, for this experiment, was planned to be done in open ponds to make the procedure economically and ecologically viable, controlling the temperature was not possible in this case. Therefore, dependency on atmospheric temperature was obvious for this experiment. Although, in that case, the result favoured one of the temperature range (5°C- 10°C) and production of seasonal algae was the only solution to the problem, storage

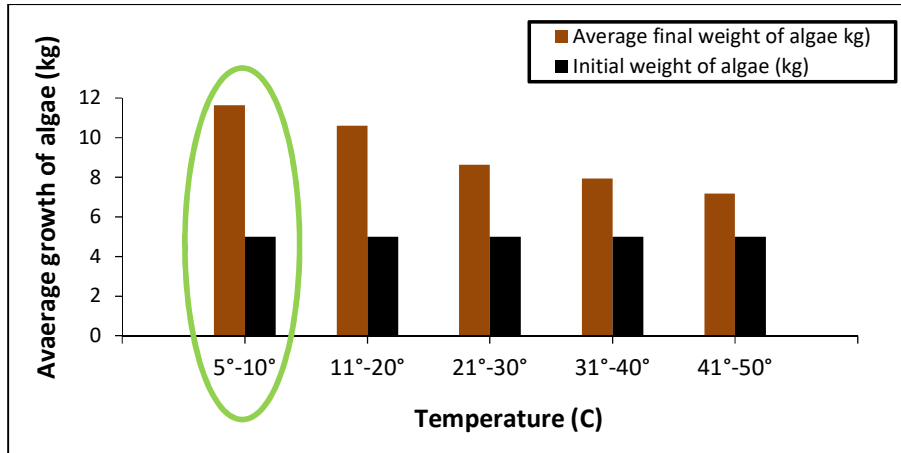
of highly grown algae in optimized temperature range was decided to be the solution of that problem.

Table 4.5.1: Algal growth with change of temperature

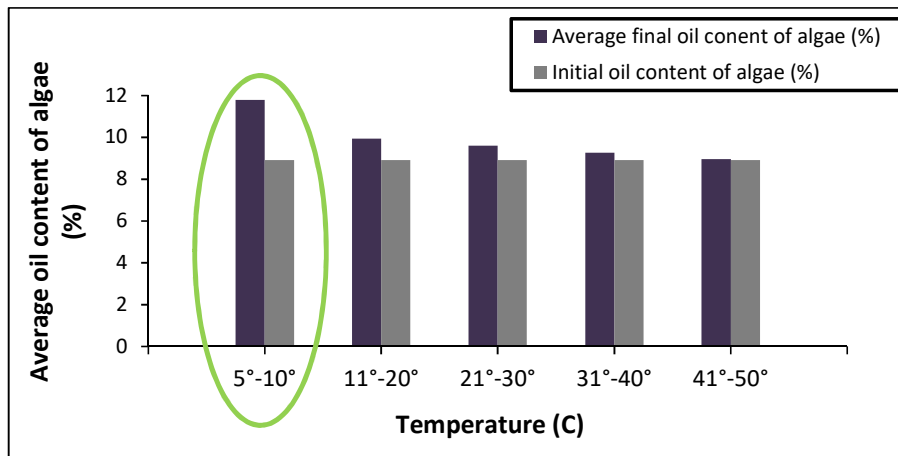
		Algal biomass harvested/ pond (kg)		
Range of Temperature	Trial	Trial 1	Trail 2	Trial 3
	5°C-10°C		11.2	11.8
11°C-20°C		10.2	10.7	10.9
21°C-30°C		8.3	8.6	9
31°C-40°C		7.7	8	8.1
41°C-50°C		6.9	7.2	7.4

Table 4.5.2: Change in oil content of algae with change of temperature

		Oil content of algae harvested/ pond (%)				
Temperature	Trial	5°C-10°C	11°C-20°C	21°C-30°C	31°C-40°C	41°C-50°C
	Trial1		12	9.9	9.6	9.3
Trial2		11.7	10	9.6	9.2	8.9
Trial3		11.7	9.9	9.6	9.3	9.1



(a)



(b)

Fig 4.5: (a) Growth of algae (b) change in oil content of algae with change of temperature

4.2.1.5. INTENSITY OF MIXING

Mixing is a general technique for making the medium of algal growth homogeneous. This is done to make all the required materials available for equally and at its highest level to all the cells of algae. It has been reported by researchers that mixing of algal growth medium, in the process of cultivation of algae, improves the growth of the algae (Reynolds et al. 1984; Rogers et al. 2014).

Higher rate of mixing improves the dissolution of essential materials required for algal growth in pond water and the rate of algal growth, thus, increases thereby (Huang et al. 2014). In this experiment also, highest mixing intensity came out with highest growth of algae.

Algae, in ponds having mixing in every 2 hours were found to have best growth rate (10.1 kg from 5kg) among algae of all the ponds (Table 4.6.1) when the intensity of mixing was varied from 'every 2h' to 'every 6h). The oil content of the algae, during this time, was the most in these algae too (11.4%). The growth rate of algae of pond getting mixing in every 3 hours got slightly lower than that of pond getting mixing in every 2 hours. The growth of algae in 'Every' 1h and 1.5h ponds increased very marginally in first 100 days. Rapid increase of algal growth took place after that in all ponds except the pond with 0 mixing. On the other hand algae grown in the ponds which did not get any mixing had the lowest growth rate as well as lowest oil content (Table 4.6.2).

As the growth rate of algae was found to increase with intensity of mixing, two higher rates of mixing intensity (Every 1.5 hour and every 1 hour) were applied to find out their effect. The growth rate of 'every 1.5h' pond was found to have better growth rate (Fig 4.6(a)) and oil content (11.93%) (Fig 4.6(b)) than algae in 'every 2h' pond. The growth rate of algae grown in 'every 1h' had the same growth (10.20 kg from 5 kg) rate. The oil content of the same was insignificantly higher (11.97%) than the pond getting mixing in every hour. Growth of algae in all ponds, especially in Every 1h, Every 1.5h, Every 2h ponds, were almost constant between 111th and 140th day. Though the growth of algae in pond which did not get mixing any time was 8.28 kg from 5 kg, its oil content was very nominal. Only 0.27% increase in oil content was found to take place in these ponds.

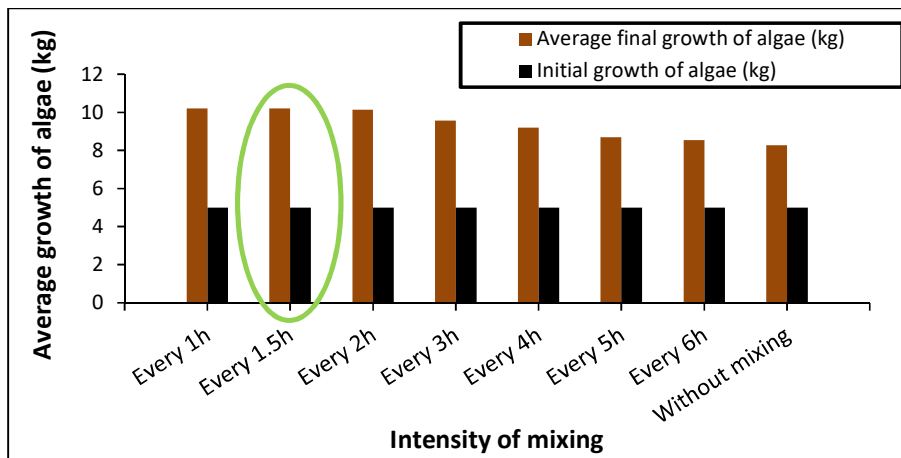
So, mixing in every 1.5 hour was decided to be the optimum level for algal growth because that yielded same amount of biomass and oil as it is yielded by the highest intensity of mixing. So, 4l/pond CDS, 30ml/l CO₂, 0.5m water depth, 5°C- 10°C temperature and 'every 1.5h' mixing intensity are the optimized conditions for the growth of these algae.

Table 4.6.1: Algal growth with varied intensity of mixing

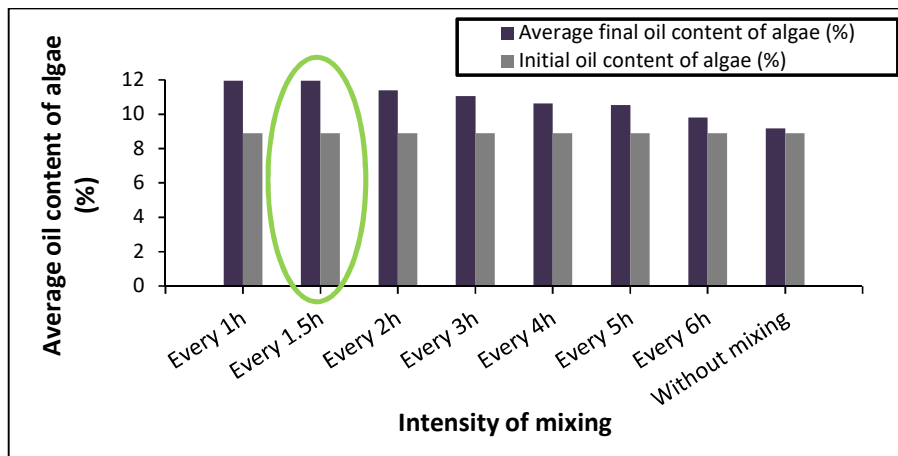
		Algal biomass harvested/ pond (kg)							
10 th Day	Intensity of mixing	Every 1h	Every 1.5h	Every 2h	Every 3h	Every 4h	Every 5h	Every 6h	Without mixing
	1 st		9.8	9.9	9.9	9.3	8.9	8.6	8.4
2 nd		9.8	9.8	9.7	9.3	8.7	8.3	8.3	8
3 rd		9.3	9.3	9.3	8.9	8.6	8.1	8	7.9
4 th		9	9.2	9.2	8.7	8.4	8	8.1	7.7
5 th		8.9	9	9.1	8.6	8.1	7.8	7.8	7.4
6 th		8.8	8.8	8.8	8.4	8.1	7.7	7.5	7.2
7 th		9.5	9.5	9.3	8.9	8.4	8	8	7.8
8 th		9.7	9.8	9.6	9	8.8	8.3	8.1	7.9
9 th		9.8	9.9	9.8	9.1	8.9	8.4	8.3	8.1
10 th		9.9	9.9	9.9	9.2	9.1	8.6	8.4	8.2
11 th		10.7	10.5	10.5	9.6	9.3	8.6	8.6	8.3
12 th		10.6	10.6	10.5	9.7	9.3	8.8	8.6	8.4
13 th		10.8	10.8	10.7	10	9.7	9	8.7	8.6
14 th		10.9	10.9	10.8	10.2	9.9	9.3	8.9	8.7
15 th		11.3	11.2	11	10.5	10.1	9.5	9.1	8.9
16 th		11.4	11.4	11.2	10.8	10.3	9.6	9.3	8.9
17 th		11.6	11.5	11.4	11	10.5	9.9	9.7	9.3
18 th		11.9	11.7	11.8	11.1	10.7	10.1	10	9.6

Table 4.6.2: Change in oil content of algae with varied intensity of mixing

		Oil content of algae grown/ pond (%)							
Trial	Intensity of mixing	Every 1h	Every 1.5h	Every 2h	Every 3h	Every 4h	Every 5h	Every 6h	Without mixing
	Trial1		11.9	12	11.4	11.1	10.7	10.5	9.9
Trial2		11.9	11.9	11.4	11.1	10.7	10.5	9.8	9.3
Trial3		12.1	11.9	11.4	11	10.5	10.6	9.7	9



(a)



(b)

Fig 4.6: (a) Growth of algae (b) change in oil content of algae with varied intensity of mixing

4.2.2. STATISTICAL ANALYSIS OF OPTIMIZATION OF GROWTH PARAMETERS

Five levels of parameters, which were considered for non- statistical analysis of optimization of algal growth, were also used in statistical analysis of the same apart from the parameter ‘intensity of mixing’. As two more levels (‘Every 1h’ and ‘Every 1.5h’) of this parameter apart from five chosen levels (Every 2h, Every 3h, Every 4h, Every 5h, Every 6h) were considered later to be included in the experiment, there were a total of seven levels of this parameter used in the non- statistical experiment. Therefore, for statistical analysis of optimization of algal growth in terms of increase in biomass (Y1) and oil content (Y2), four levels of intensity of mixing best with best results (Every 1h, Every 1.5h, Every 2h, Every 3h) along with 0 intensity of mixing (Table 4.7) were considered to prepare a set of 25 combinations (L25 orthogonal array) of chosen levels of parameters.

Table 4.7: Different parameters (X) with their levels (modified) used for optimization of growth parameters of algae in terms of increase in biomass (Y1) and its oil content (Y2)

Growth parameters	Code	Levels				
Nutrient (CDS) (l/pond)	X1	2	3	4	5	6
CO ₂ (ml/l)	X2	10	20	30	40	50
Depth (m)	X3	0.25	0.50	0.75	1.00	1.25
Temperature (°C)	X4	5-10	11-20	21-30	31-40	41-50
Intensity of mixing (Times/h)	X5	0	1	1.5	2	3

Among 25 combinations of parameters, Combination number 12 was found to come out with best results (Table 4.8). The average growth obtained using 4l/pond cow dung slurry, 20ml/l of CO₂, 1m depth of algal pond, 5°C- 10°C atmospheric temperature and mixing intensity of ‘every 1.5 hours’ was 10.8 kg from 5 kg. The oil content of the same was 11.7% which, again, was found to be the dominating yield among 25 combinations. The optimized conditions obtained from statistical analysis was similar as that obtained from the non- statistical analysis. Only quantity of CO₂ (20ml/l) and depth of pond (1m) obtained from this analysis was different from those used in optimization procedure in non- statistical analysis. The growth rate yielded (10.8 kg) in this analysis, thus, was lower than that resulted from

non- statistical analysis (11.6 kg). Therefore, analysis of contribution of each parameter was clearly an important factor to be calculated.

Table 4.8: Algal growth and its oil yield obtained from 25 combinations of parameters
(where, X= parameters; T= Trials; and Y= Mean of corresponding trials)

Combination number	Levels of Parameters					Growth			Oil content			Average growth	Average oil content
	X1	X2	X3	X4	X5	T1	T2	T3	T1	T2	T3	Y1	Y2
1	2	10	0.25	10	0	9.6	9.6	9.7	10.7	10.7	10.9	9.6	10.8
2	2	20	0.5	20	1	10.2	10.1	10	11.1	11	10.9	10.1	11
3	2	30	0.75	30	1.5	9.3	9.3	9.3	10.7	10.7	10.6	9.3	10.7
4	2	40	1	40	2	8.6	8.6	8.7	10	10	10.1	8.6	10
5	2	50	1.25	50	3	7	7	7.3	9.5	9.5	9.8	7.1	9.6
6	3	10	0.5	30	2	8.2	8.6	8.2	9.7	10.2	9.7	8.3	9.9
7	3	20	0.75	40	3	8	8	8	9.8	9.8	9.8	8	9.8
8	3	30	1	50	0	6	6	5.8	9.2	9.2	9	5.9	9.1
9	3	40	1.25	10	1	10.6	10.4	10.5	11.4	11.2	11.1	10.5	11.2
10	3	50	0.25	20	1.5	10	9.7	9.8	11.1	10.6	10.7	9.8	10.8
11	4	10	0.75	50	1	7.2	7.5	7.6	9.4	9.8	9.8	7.4	9.7
12	4	20	1	10	1.5	11	10.8	10.7	11.9	11.7	11.6	10.8	11.7
13	4	30	1.25	20	2	9.9	9.9	10	11	11	11.1	9.9	11
14	4	40	0.25	30	3	9.2	9.3	9.3	10.6	10.7	10.7	9.3	10.7
15	4	50	0.5	40	0	5.5	5.6	5.8	8.9	8.9	9.2	5.7	9
16	5	10	1	20	3	7	7	6.4	9.8	9.8	9	6.8	9.5
17	5	20	1.25	30	0	5.4	5.4	5.7	8.9	8.9	9	5.5	9
18	5	30	0.25	40	1	6	6	6	9.1	9.1	9	6	9.1
19	5	40	0.5	50	1.5	6.3	6.4	6.4	9.2	9.3	9.4	6.4	9.3
20	5	50	0.75	10	2	8.2	8.2	8	9.8	9.8	9.7	8.1	9.8
21	6	10	1.25	40	1.5	4.7	4.5	4.8	8.9	8.9	8.9	4.7	8.9
22	6	20	0.25	50	2	4.4	4.5	4.7	8.9	8.9	8.8	4.5	8.9
23	6	30	0.5	10	3	6.4	6.6	6.8	9.1	9.3	9.6	6.6	9.3
24	6	40	0.75	20	0	6.2	6.1	6	9.2	9.1	9.1	6.1	9.1
25	6	50	1	30	1	5	4.6	4.5	9	8.9	8.9	4.7	8.9

*10 represents 5°C-10°C; 20 represents 11°C-20°C; 30 represents 21°C- 30°C; 40 represents 31°C- 40°C, 50 represents 41°C- 50°C

4.2.2.1. ANALYSIS OF VARIANCE AND CONTRIBUTION OF EVERY PARAMETER

The signal to noise ratio of different parameters and their corresponding delta (range) were calculated to determine the rank of all five parameters (X1, X2, X3, X4, X5) and their contribution on growth of algae (Y1) and their oil content (Y2). As the target of this experiment was to achieve highest growth of algae and highest oil content of the same, 'larger is better' model was used to find out the rank of the parameters for both Y1 and Y2. Highest rank among all five parameters, for algal growth (Y1), was found to be assigned to nutrient (X1) (Table 4.9) which was followed by temperature (X4), intensity of mixing (X5), CO₂ (X2) and depth of pond (X3) in descending manner. The ranks of all five parameters for oil content of algae (Y2) were exactly same as those for Y1 (Table 4.10).

Table 4.9: Signal to noise ratio of parameters and their rank for growth of algae (Y1)

S/N ratio of parameters					
Level	X1	X2	X3	X4	X5
1	18.96	17.10	17.49	19.06	16.14
2	18.42	17.34	17.22	18.44	17.38
3	18.49	17.33	17.74	17.07	17.89
4	16.26	18.06	16.97	16.17	17.64
5	14.41	16.72	17.12	15.80	17.50
Delta	4.55	1.35	0.76	3.26	1.75
PC (%)	54.04	3.80	0.99	32.78	8.39
Rank	1	4	5	2	3

Table 4.10: Signal to noise ratio of parameters and their ranks for oil content of algae (Y2)

S/N ratio of parameters					
Level	X1	X2	X3	X4	X5
1	20.35	19.77	20.02	20.44	19.44
2	20.11	20.02	19.71	20.21	19.94
3	20.32	19.83	19.83	19.83	20.20
4	19.40	20.02	19.82	19.42	19.91
5	19.10	19.64	19.91	19.38	19.80
Delta	1.24	0.38	0.31	1.06	0.76
PC (%)	47.80	4.41	2.03	34.07	11.64
Rank	1	4	5	2	3

Highest contribution of 54.04%, for the production of highest algal biomass (Y1), was found to be done by nutrient cow dung slurry (X1). Temperature (X4) was found to have second most contribution (32.78%) on algal growth. 8.39% contribution was made by Intensity of mixing (X5). Contribution of both CO₂ (X2) and depth of water (X3), especially the latter, were very low (3.8% and 0.99% respectively).

Once again, highest contribution, for increase in algal oil content (Y2), was found to exert by cow dung slurry (X1) (47.80%). Temperature (X4) of atmosphere was found to have very effective contribution (34.07%) on increase in oil content of algae. Likewise, Intensity of mixing (X5) and carbon dioxide had 11.64% and contribution on oil yield of algae and 4.41% contribution on Y2. The Contribution of depth of pond was found to be very low (2.03%).

Analysis of Variance for this statistical analysis of optimization of growth parameter of algae was carried out by ANOVA. The F value derived for Y1 (increase in biomass of algae), was about 0.005 at 95% confidence level (Table 4.11). This value was found to be much lower than the F_{critical} (3.12) value for the degree of freedom 72 at 95% confidence level. The value of F (0.0002) derived for the experiment on oil content of algae (Y2), also, was much lesser than the F_{critical} value for the degree of freedom 72 at 95% confidence level (Table 4.12). Therefore, the results for both Y1 and Y2 were considered to be significant.

Table 4.11: Analysis of Variance (For Y1)

Source of Variation	SS	df	MS	F	F _{critical}
Between Groups	0.008267	2	0.004133	0.005387	3.123907
Within Groups	55.2472	72	0.767322		
Total	55.25547	74			

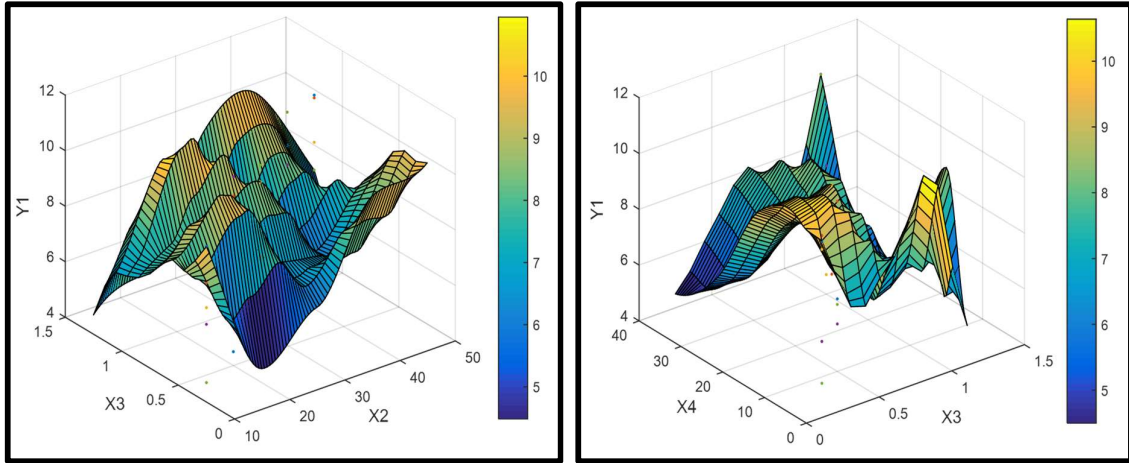
Table 4.12: Analysis of Variance (for Y2)

Source of Variation	SS	df	MS	F	F _{critical}
Between Groups	0.001867	2	0.000933	0.00024	3.123907
Within Groups	280.516	72	3.896056		
Total	280.5179	74			

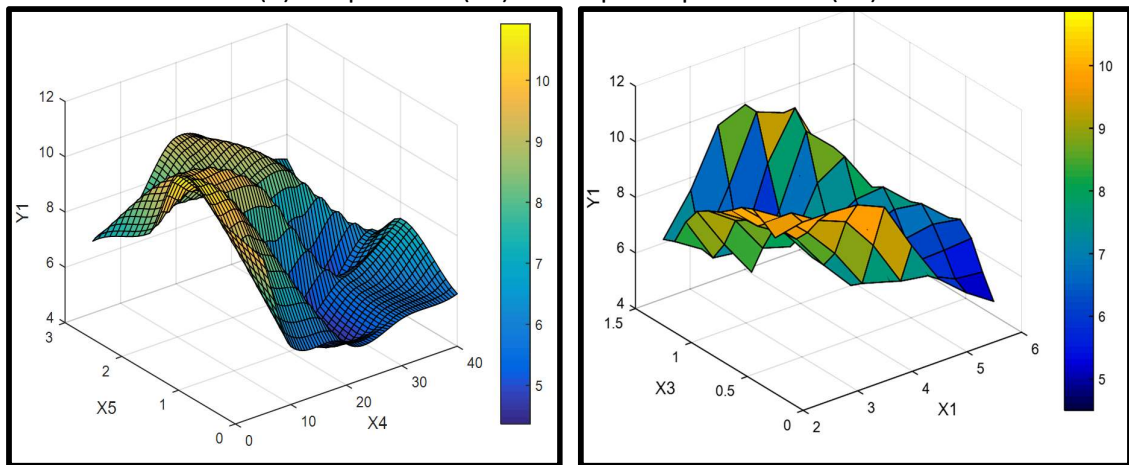
3D plots were drawn to observe optimized conditions for algal growth in terms of increase in biomass (Y1) as well as the oil content (Y2) of the same and distinct effect of parameters (X1, X2, X3, X4, X5) on Y1 and Y2. Higher value of algal growth was marked with yellow colour whereas blue was marked as the colour of lower value of growth. Very distinct effect of cow dung slurry (X1) on algal growth was observed from Fig 4.8(b), 4.9(a) and (b), 4.10(b) and 4.12. Maximum growth of algae was found with 4l/pond cow dung slurry. CO₂ (X2) was found to have very minimal effect on algal growth (Fig 4.7(a), 4.10(a), 4.11(a), 4.12). Although prominent growth was seen at 20ml/l, growth for it was found to be distributed in many parts of the 3D plot. As the depth of pond water (X3) was the insignificant parameter on algal growth, its distribution was not prominent on 3D plots, (Fig 4.7 (a) and (b), 4.8(b), 4.11(b)). Very prominent effect of temperature (X4) was observed in 3D plots of algal growth (4.7(b), 4.8(a), 4.9(a), 4.10(a)), specially for 5°C- 10°C temperature. Region of good algal growth for intensity of mixing (X5) and its optimized level (every 1.5h) was very clearly depicted in Fig 4.8(a), 4.9(b), 4.10(b), 4.11(a) and (b).

Likewise the effect of cow dung slurry (X1) on algal oil content (Y2) was very prominent in 3D plots of Fig 4.14(b), 4.15(a) and (b), 4.16(a) and 4.18. Very clear zone of highest oil content was observed at optimized level (4l/pond) of X1. Optimized level (20ml/l) of CO₂ (X2), also, was found to be expressed prominently in Fig 4.13(a), 4.16(b), 4.17(a), 4.18. Effect of depth of pond water (X3) on oil content of algae (Y2) was expressed more prominently than that on Y1. In Fig 4.13 (a) and (b), 4.15(a), 4.17(b) regions of good algal oil yield for optimized depth of water were found to be clear enough. Effect of temperature (X4) and its optimized level (5°C- 10°C) was found to show prominent zones in Fig 4.13(b), 4.14(a), 4.15(b), 4.16(b). The optimized level of intensity of mixing (X5) was found to be 'every 1.5h'. From Fig 4.14(a) and (b), 4.16(a), 4.17(a) and (b).

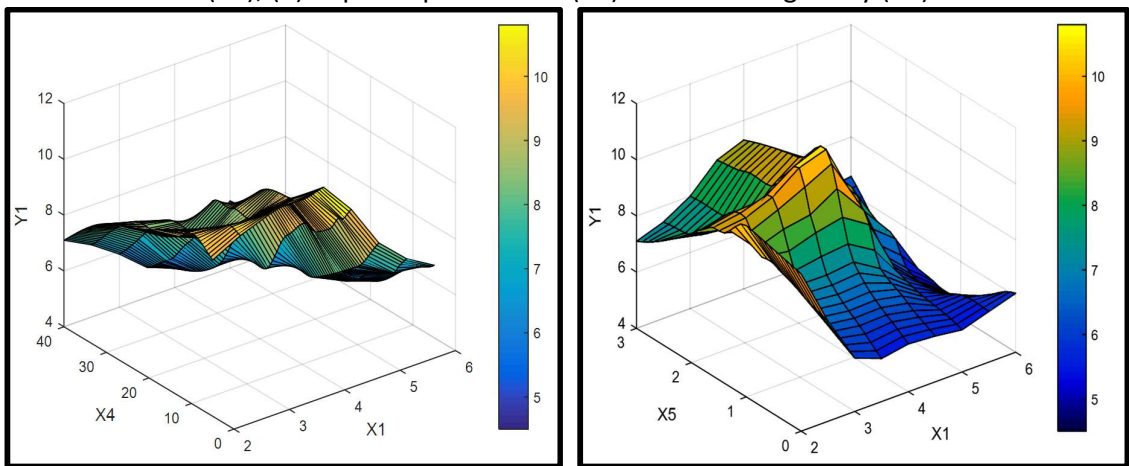
Therefore, from these figures, it was proved again that the optimized conditions for increase in algal growth and their content of oil resulted from 25 combinations of statistical analysis are 4l/pond cow dung slurry, 20ml/l of CO₂, 1m of pond depth, 5°C-10°C reaction temperature and mixing intensity of 'every 1.5h'. These optimum conditions were found to differ from those from non-statistical analysis only for CO₂ level (30ml/l) and depth of pond water (0.5 m) and both of them, already, were found to have least impact on Y1 and Y2.



(a) (b)
 Fig 4.7: Growth of algae (Y1) with respect to (a) depth of pond water(X3) and CO₂ (X2);
 (b) temperature (X4) and depth of pond water (X3)



(a) (b)
 Fig 4.8: Growth of algae (Y1) with respect to (a) intensity of mixing (X5) and temperature (X4);
 (b) depth of pond water (X3) and cow dung slurry (X1)



(a) (b)
 Fig 4.9: Growth of algae (Y1) with respect to (a) temperature (X4) and cow dung slurry (X1);
 (b) intensity of mixing (X5) and cow dung slurry (X1)

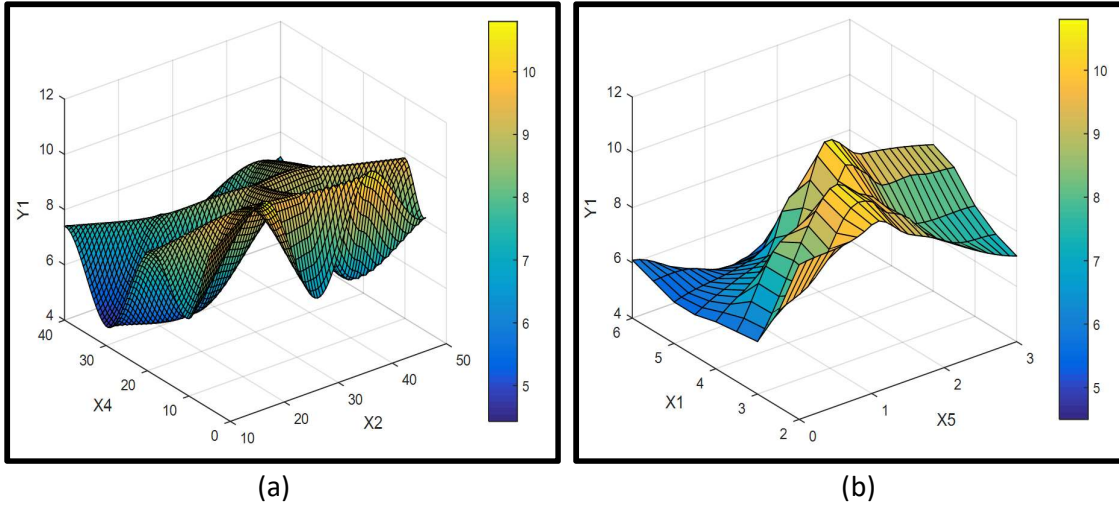


Fig 4.10: Growth of algae (Y1) with respect to (a) temperature (X4) and CO₂(X2); (b) cow dung slurry (X1) and intensity of mixing(X5)

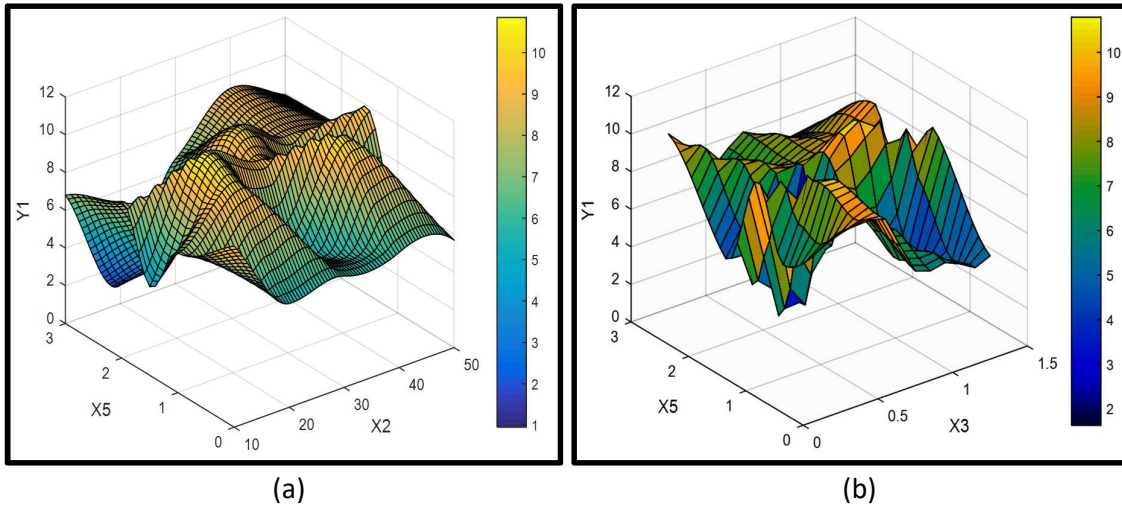


Fig 4.11: Growth of algae (Y1) with respect to (a) intensity of mixing (X5) and CO₂(X2); (b) intensity of mixing (X5) and depth of pond water (X3)

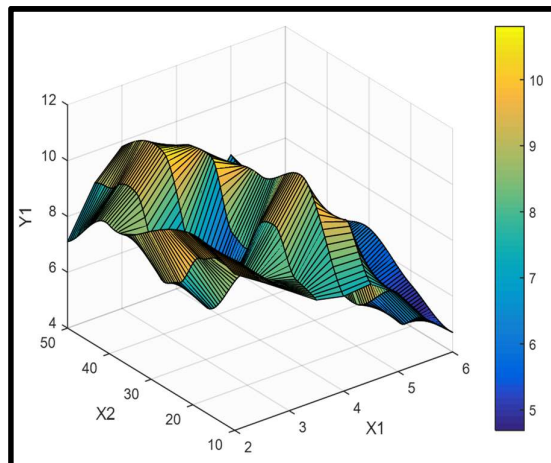
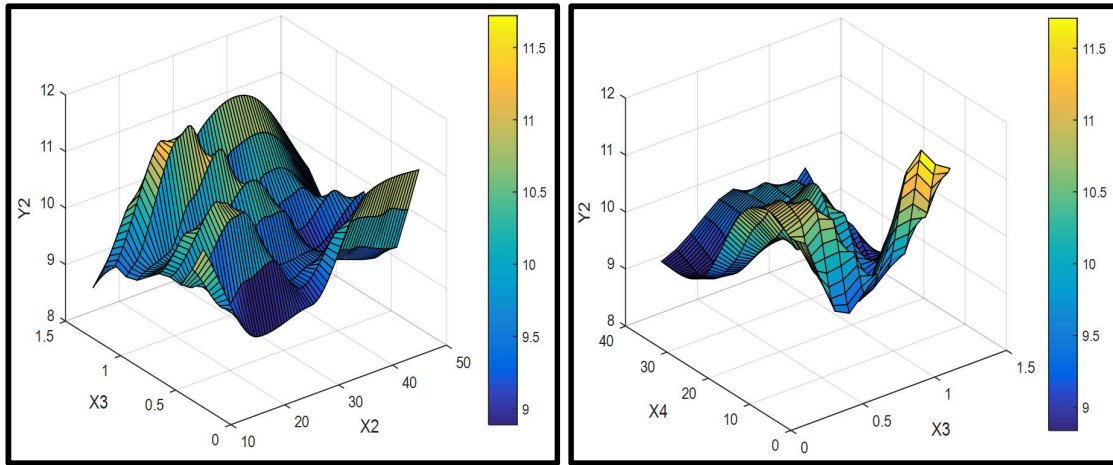


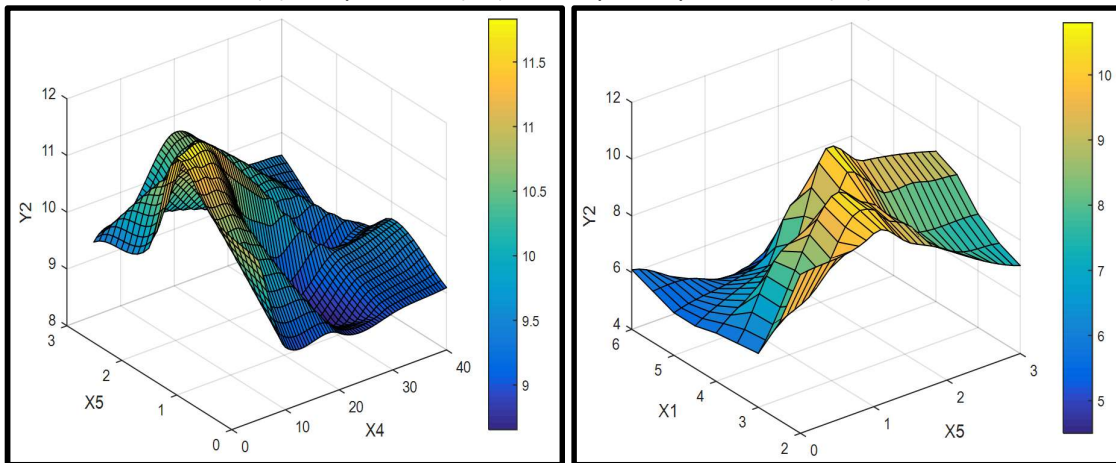
Fig 4.12: Growth of algae (Y1) with respect to CO₂ (X2) and cow dung slurry (X1)



(a)

(b)

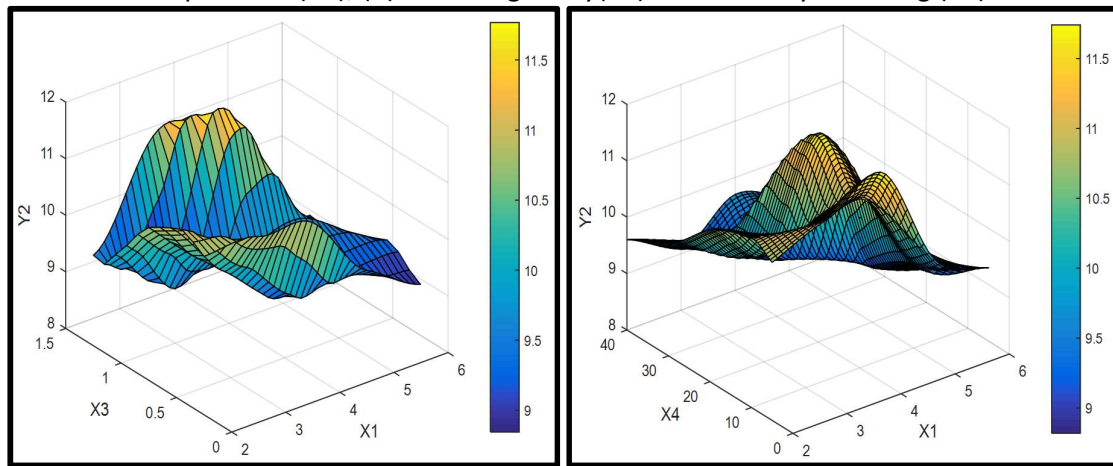
Fig 4.13: Oil content of algae (Y2) with respect to (a) depth of pond water (X3) and CO₂ (X2); (b) temperature (X4) and depth of pond water (X3)



(a)

(b)

Fig 4.14: Oil content of algae (Y2) with respect to (a) intensity of mixing (X5) and temperature (X4); (b) cow dung slurry(X1) and intensity of mixing (X5)



(a)

(b)

Fig 4.15: Oil content of algae (Y2) with respect to (a) depth of pond water (X3) and cow dung slurry (X1); (b) temperature (X4) and cow dung slurry (X1)

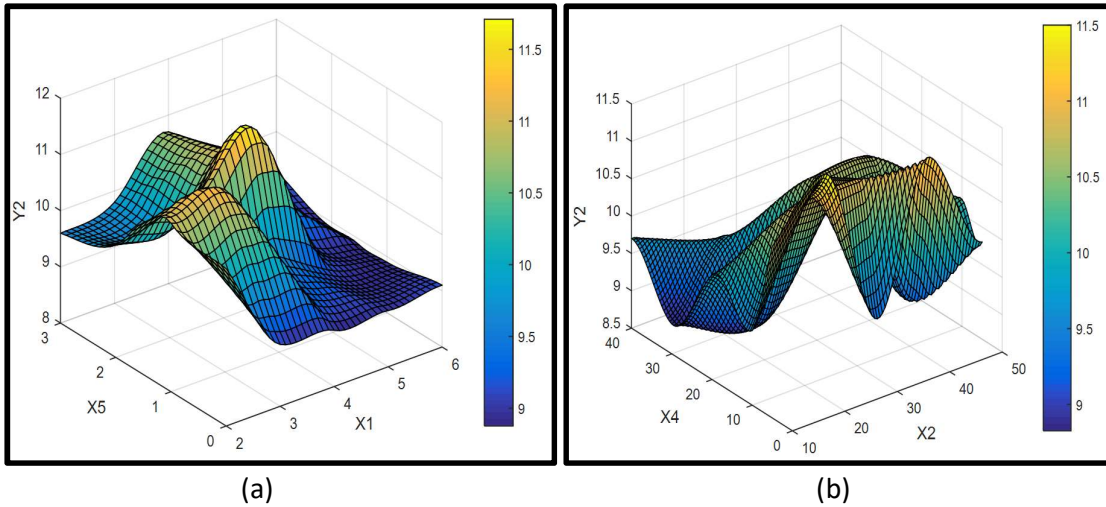


Fig 4.16: Oil content of algae (Y2) with respect to (a) intensity of mixing (X5) and cow dung slurry (X1); (b) temperature (X4) and cow dung slurry (X1)

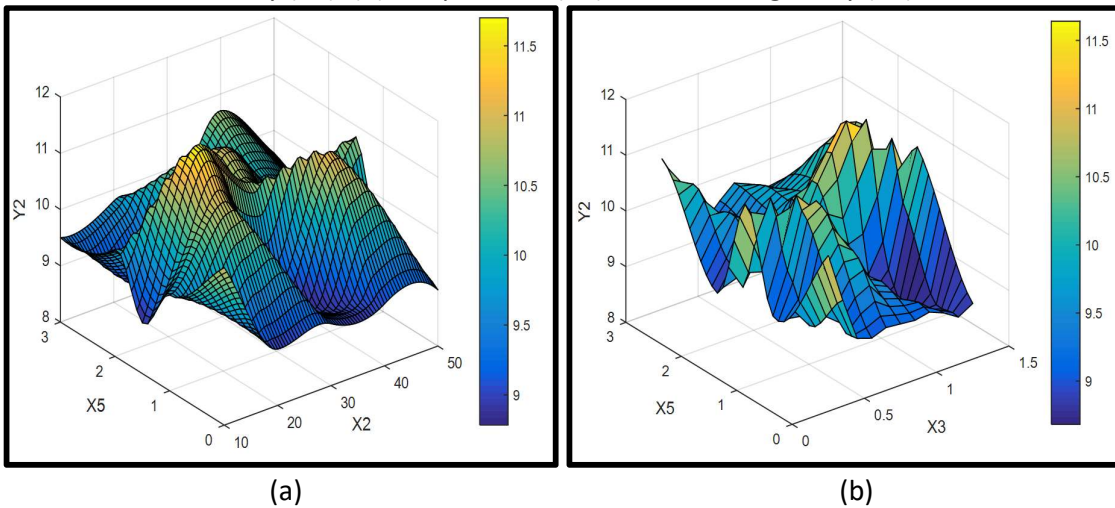


Fig 4.17: Oil content of algae (Y2) with respect to (a) intensity of mixing (X5) and CO₂ (X2); (b) intensity of mixing (X5) and depth of pond water (X3)

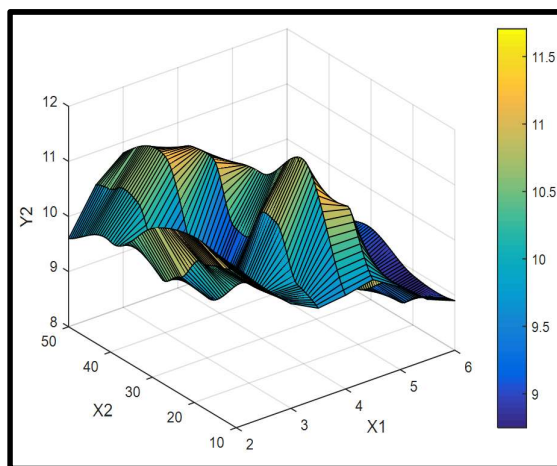


Fig 4.18: Oil content of algae with respect to CO₂ (X2) and cow dung slurry (X1)

4.2.3. NPK CONTENT OF ALGAL PONDS

4.2.3.1. NITROGEN CONTENT

Nitrogen content of algae- ponds with all optimized conditions were found to be very high as the ponds got prepared. N content during this period, before commencing the algae- culture, was about 0.57 mg/l (Table 4.13) in summer. This N content reduced to around 0.36 mg/l just after all algae were harvested (Fig 4.19). In winter, nitrogen content of the pond at initial stage was about 0.6mg/l. The average N content of this pond at the time of harvesting were, again, found to be around 0.36mg/l. Therefore, a huge content of nitrogen was found to be consumed by algae which is an essential requirement for algal growth in terms surge of its biomass and oil content (Li et al. 2008; Barros et al. 2017).

Table 4.13: Nitrogen content of ponds before and after algae- culture

Summer				
Trial	Initial N (mg/l)	Mean initial N (mg/l)	Final N (mg/l)	Mean final N (mg/l)
Trial1	0.58	0.57	0.36	0.36
Trial2	0.57		0.36	
Trial3	0.57		0.35	
Trial4	0.57		0.36	
Trial5	0.57		0.34	
Trial6	0.57		0.36	
Trial7	0.58		0.37	
Winter				
Trial	Initial N (mg/l)	Mean initial N (mg/l)	Final N (mg/l)	Mean final N (mg/l)
Trial1	0.59	0.60	0.36	0.36
Trial2	0.60		0.36	
Trial3	0.60		0.36	
Trial4	0.60		0.36	
Trial5	0.60		0.35	
Trial6	0.59		0.36	
Trial7	0.59		0.35	

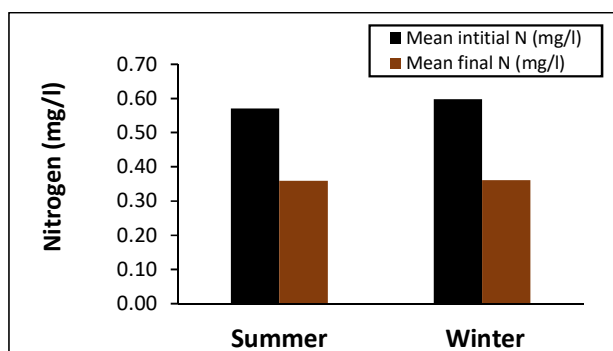


Fig 4.19: Average initial and final nitrogen content of algae- pond in summer and winter

4.2.3.2. PHOSPHORUS CONTENT

Phosphorus is one of the consequential component for the growth of terrestrial and aquatic plants. Standard curve for P was obtained from known concentration of samples (Fig 4.20). OD of the unknown samples were placed in the graph and their concentrations were calculated accordingly. The equations generated from the standard curves were as follows-

- (i) $y=0.7664x-0.0027$; $R^2 = 0.9736$ (For samples used in summer)
- (ii) $y=0.7506x-0.0007$; $R^2 = 0.9806$ (For samples used in winter)

The algal ponds were found to be rich in P. Phosphorus content of pond just at the point of starting of algae- culture was about 0.36mg/l which reduced to 0.07mg/l at the time of harvesting of algae (Table 4.14). During winter, P content of the pond at the time of commencement of algae culture was about 0.42mg/l. The concentration of the same abated to about 0.08mg/l after the harvesting of algae (Fig 4.21). This was found to be very beneficial (Rezvani et al. 2017; Dogaris et al. 2016) for algal growth as increase in both of biomass and oil content of algae was found after algae culture under optimized conditions.

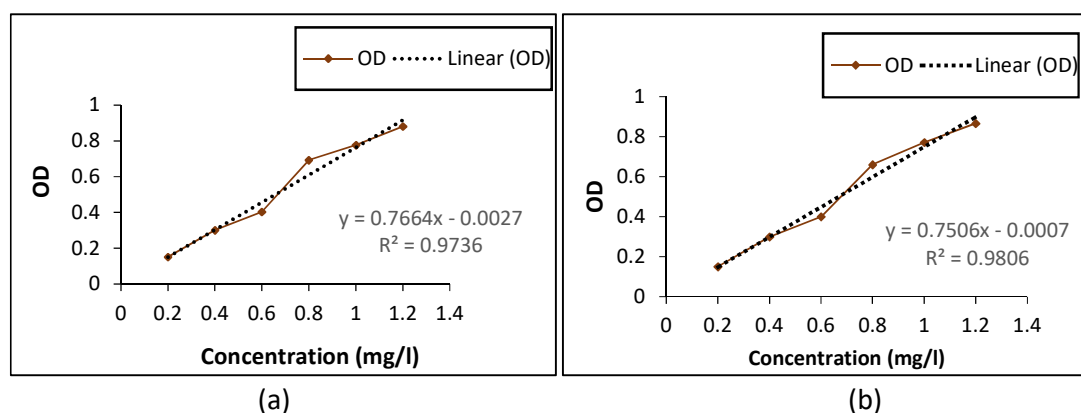


Fig 4.20: Standard curve for (a) samples used in summer; (b) samples used in winter

Table 4.14: Phosphorus content of pond water before and after algae culture

Summer				
	Initial P (mg/l)	Mean Initial P (mg/l)	Final P (mg/l)	Mean Final P (mg/l)
Trial1	0.37	0.36	0.09	0.07
Trial2	0.36		0.07	
Trial3	0.36		0.06	
Winter				
	Initial P (mg/l)	Mean Initial P (mg/l)	Final P (mg/l)	Mean Final P (mg/l)
Trial1	0.47	0.42	0.12	0.08
Trial2	0.40		0.07	
Trial3	0.39		0.07	

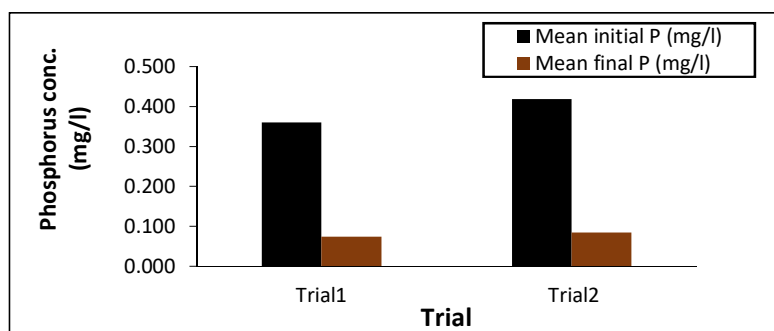


Fig 4.21: Average initial and final Phosphorus content of algae- pond in summer and winter

4.2.3.3. POTASSIUM CONTENT

The result derived from flame photometry method showed that Potassium content of algal pond in summer was 0.33mg/l at the beginning and it was found to reduce to 0.20mg/l in summer (Fig 4.22). In winter, again, the K level of the pond before algae culture was 0.33mg/l and that at the time of harvesting of algae was 0.20mg/l.

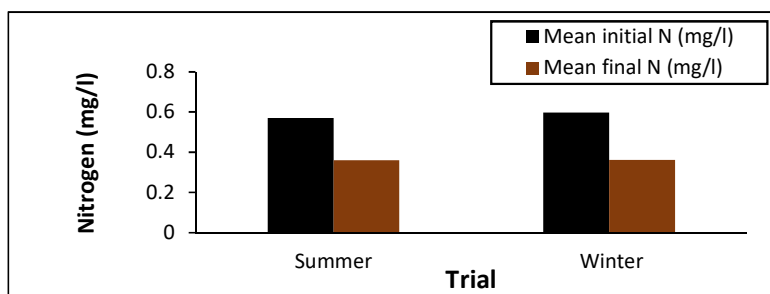


Fig 4.22: Average initial and final Potassium content of algae- pond in summer and winter

4.2.3.4. DISSOLVED OXYGEN CONTENT OF ALGAL POND

Analysis of DO of algal pond is very essential because algae release O₂ very rapidly as a result of photosynthesis by them. Dissolved oxygen of the algae- pond with optimized levels of parameters was found to be very low during early morning (Table 4.15). However DO of the pond during early morning in summer was little lower (5.75mg/l) than that (6.03mg/l) of the pond in winter. Thereafter, the DO of the pond was found to increase with time and reached its peak at noon time (Fig 4.23). DO during this time in winter was much higher (14.53mg/l) than that in summer (12.15mg/l). The reason behind it is the higher growth rate of algae in winter and thereby more oxygen production by photosynthesis. DO level, then, was found to decrease with time as the rate of photosynthesis diminishes to very low level. The DO of algae pond reached its lowest point at night (below 5mg/l).

Table 4.15: Dissolved oxygen content of algae- ponds

Summer				
Time (hrs)	DO trial1 (mg/l)	DO trial2 (mg/l)	DO trial3 (mg/l)	Mean DO (summer) (mg/l)
6	5.71	5.76	5.79	5.75
9	9.2	9.19	9.17	9.18
12	12.11	12.23	12.11	12.15
15	11.33	10.99	10.23	10.85
18	6.98	7.12	6.88	6.99
21	4.29	4.32	4.29	4.3
Winter				
	DO trial1 (mg/l)	DO trial2 (mg/l)	DO trial3 (mg/l)	Mean DO (winter) (mg/l)
6	5.88	6.12	6.09	6.03
9	8.44	8.46	8.37	8.42
12	14.46	14.59	14.54	14.53
15	12.88	12.98	12.93	12.93
18	7.12	7.11	7.11	7.11
21	4.23	4.26	4.21	4.23

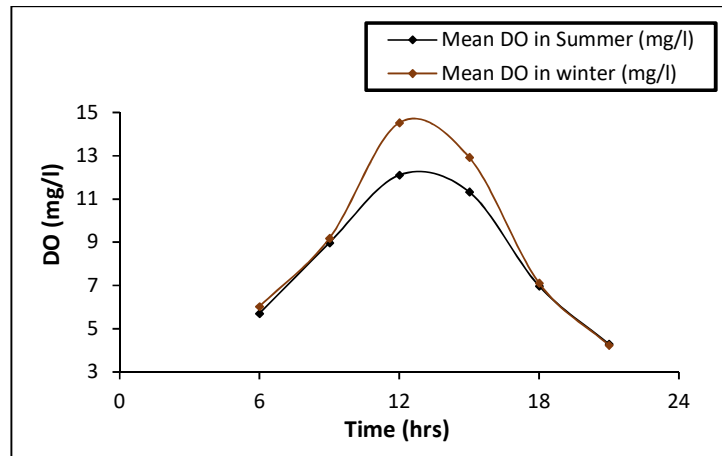


Fig 4.23: Change of DO of pond water with time in summer and winter

4.3. YIELD OF ALGAL OIL

Both mechanical and solvent extraction methods were used for extraction of oil from dried algae. Mechanical extraction procedure was found to fail to extract oil from dried algal biomass (Table 4.16) and thus next experiments were proceeded with solvent extraction by using different organic solvents. Very lower quantity oil was yielded from dry algae by using methanol (2.9%) (Fig 4.24(a)). Hexane (Fig 4.24(b)) and acetone (Fig 4.24(c)) were found to aggregate better quantity of oil (5.7% and 6% respectively) from dried algae. As hexane and acetone were found to extract almost equal quantity of oil, the next experiment was performed with the mixture hexane and acetone (1:1). This time, a huge leap in oil content was found to take place (7.8%).



(a) (b) (c)
Fig 4.24: Oil extraction with (a) methanol; (b) hexane; (c) acetone

As because the Soxhlet came out as a less effective device for oil extraction, new experiment with it was planned to be performed to find out a better way to extract more oil from algae (Fig 4.25). Small bunches of algae packed in muslin clothes, this time, was submerged directly in the solvent. Oil from the dried algae can be drawn out very straightforwardly as the solvent boils. This time, increase in oil yield was found to be done by all solvents. 3.4% of oil was extracted by methanol. But this was very less compared to other solvents. Almost 7% of oil was extracted by hexane. Extraction by acetone, also, increased by more than 1% while the mixture of hexane and acetone (H+A) was able to extract 8.9% of oil by this adapted procedure. Consequently, direct extraction using hexane-acetone mixture (1:1) was used as the ideal procedure all through the experiment.



Fig 4.25: Oil extraction by submerging the algae directly in solvents

Table 4.16: Yield of oil of algae by mechanical and solvent extraction procedures

Trial	Mechanical extraction	Solvent extraction							
		Methanol	Methanol (direct)	Hexane	Hexane (direct)	Acetone	Acetone (direct)	Hexane+ Acetone	H+A (direct)
Trial1	0	30	36	56	69	61	72	77	88
Trial2	0	29	32	57	70	60	74	79	89
Trial3	0	29	35	57	71	60	73	77	89
Average oil extracted	0	29.33	34.33	56.66	70	60.33	73	77.66	88.66
Percentage	0.0%	2.9%	3.4%	5.7%	7.0%	6.0%	7.3%	7.8%	8.9%

4.4. OPTIMIZED PARAMETERS OF BIODIESEL PRODUCTION PROCEDURE

Optimization of transesterification reaction is required to find out the lowest level of parameters to yield maximum quantity of FAME with lowest free fatty acid content. In this way the misuse of reactants can be followed. On the other hand, for every reaction, there are exact levels of parameters which include exact levels of reactants and other conditions of the reaction like temperature, pH etc. Any increase or cut down of that quantity results in imbalance of equilibrium of the reaction and, often, no reaction or reverse reaction takes place.

Hence, optimization of parameters, which includes the reactants too, is very much important to conduct all chemical reactions.

4.4.1 OPTIMIZATION OF ACID- ESTERIFICATION

Presence of FFA in higher quantity in oil (if that is directly involved in alkali catalysed esterification) influences the free fatty acid (FFA) to react with alkali catalyst and produce soap. This causes inhibition of the transesterification reaction.

Treatment with acid catalyst was done only to cut down the FFA content of the sample to the required level (<2%) from where alkali- catalysed transesterification could be actuated. Besides that, this step helped in removal of the impurities of algal oil.

4.4.1.1. MOLAR RATIO

Six molar ratios of methanol and algal triglyceride (3:1, 4:1, 5:1, 6:1, 7:1, 8:1) were juxtaposed to find their effects on transesterification. Other parameters, during this time, were kept constant (1% catalyst concentration; 50°C reaction temperature of; 45 minutes of reaction time).

Molar ratio, for this optimization reaction, was found to exert very important effect. At 3:1 molar ratio of methanol and oil only 3% drop of FFA of algal oil occurred. Huge change of FFA (18.32% to 4.32%) took place when molar ratio of methanol and oil surged from 3:1 to 6:1. For the use of 7:1 molar ratio, FFA of this algal triglyceride was cut down to 3.12%. No more noteworthy change in FFA content was found to take place by raising the molar ratio any further to 8:1 (Fig 4.26). In next experiments with optimization in acid- catalysed esterification, molar ratio of 7:1 was used.

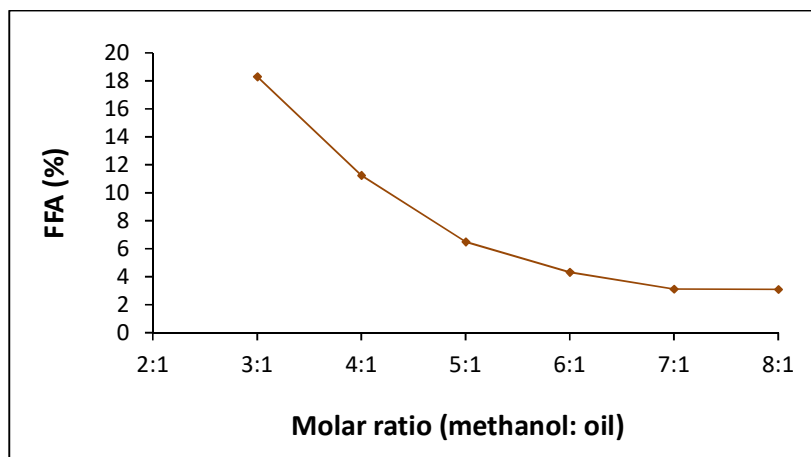


Fig 4.26: Change of FFA with different molar ratio (methanol: oil)

4.4.1.2. CATALYST CONCENTRATION

Seven catalyst concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, 3% and 3.5%) were taken into account for optimizing acid catalysed treatment. FFA of algal triglyceride reduced by almost 17% for the use of 0.5% catalyst concentration (21.3% to 4.76%). FFA content of the sample reduced to 2.16% when 1.5% of catalyst was used. No further decrease of free fatty acid content was found by using more H_2SO_4 catalyst of 2% (Fig 4.27). No separation of layers or formation of product was observed for the use of 2.5%, 3% and 3.5% catalyst concentrations. In the next experiments of acid esterification 7:1 molar ratio and 1.5% of catalyst concentration were taken.

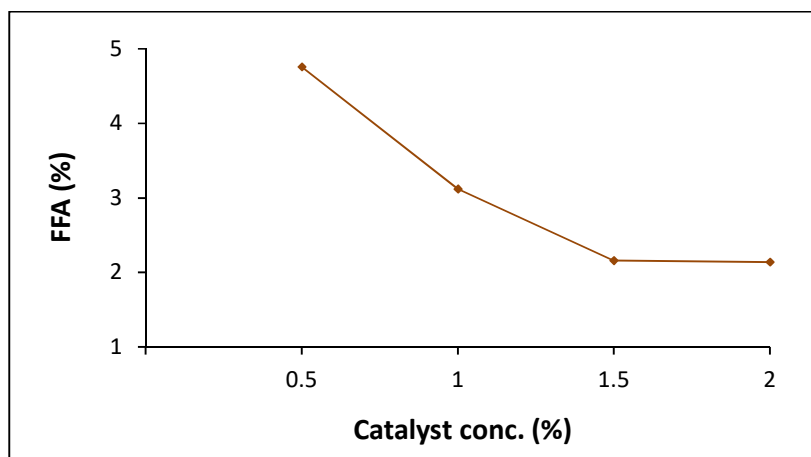


Fig 4.27: Change of FFA with different catalyst concentration

4.4.1.3. REACTION TEMPERATURE

FFA of algal oil was 4.98% when 40°C reaction temperature was applied. It dropped linearly with increase in reaction temperature up to 55°C reaction temperature. Amongst the six reaction temperatures, lowest FFA of algal oil took place at 55°C. But exactly same FFA of algal oil (1.72%) was found while the acid catalysed reaction was performed at 60°C. There was no decrease of FFA for further increase of reaction temperature to 62°C. So, for the optimization of reaction time, both of these reaction temperatures (55°C and 60°C) were tested once again with all of the reaction times together with two of the previously optimized parameters (molar ratio as well as concentration of catalyst) (Fig 4.28).

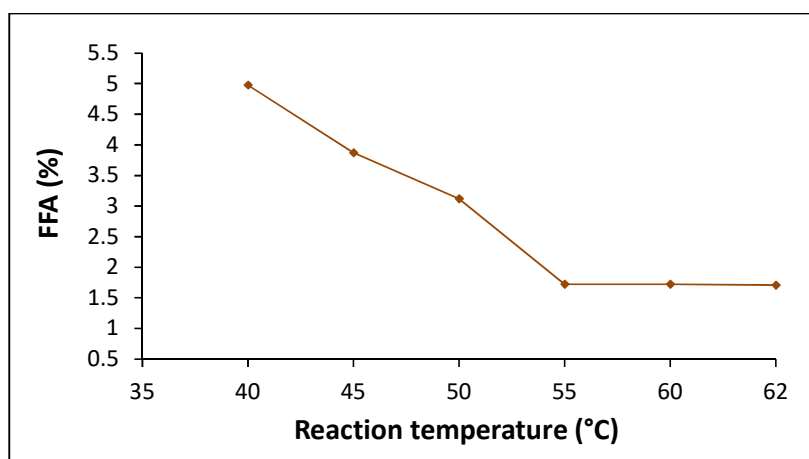


Fig 4.28: Change of FFA with different reaction temperature

4.4.1.4. REACTION TIME

With all previously optimized parameter levels, the reactants reacted for 30 minutes yielded with algae- triglycerides of FFA of 5.55% and 5.69% respectively for 55°C and 60°C reaction temperature. It was found that, for both the reaction temperature, the FFA curtailed linearly up to the use of 75 minutes of reaction time. The FFA, this time, was 1.2% and 1.23% respectively for 55°C and 60°C reaction temperature. Reaction time of 90 minutes was found enough to complete acid catalysed reaction with lowest FFA of oil. The FFA of algae-oil lessened to 1.06% and 1.09% for 55°C and 60°C reaction temperature. For any more increase of reaction time, noteworthy decrease of FFA was not found. Although, a very minute difference was there between values of FFA of FAME derived at 55°C and 60°C reaction temperature, 55°C was considered as the optimized reaction temperature along

with 90 minutes of reaction time for acid catalysed reactions from the point of view of energy saving (Fig 4.29).

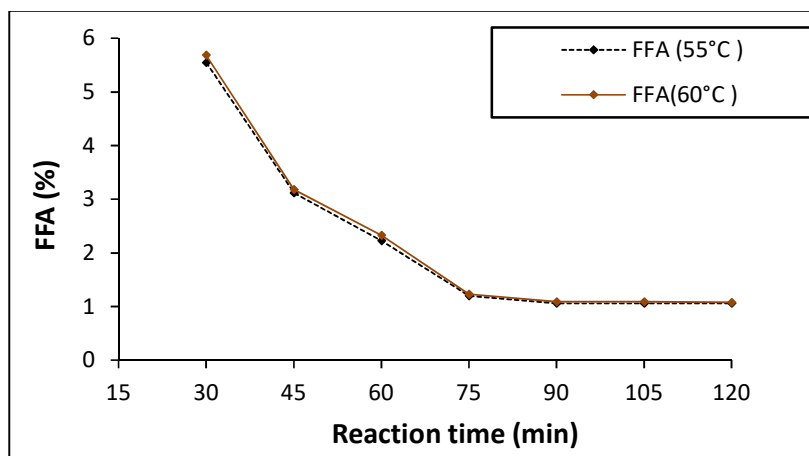


Fig 4.29: Change of FFA with different reaction time

4.4.1.5. SETTLING TIME

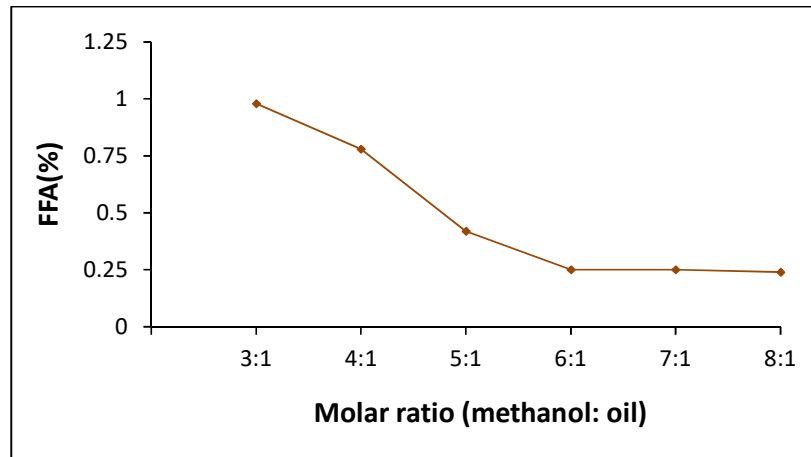
Ester, which was produced in acid- catalysed esterification, settled completely in 80 minutes. Two distinct phases were found to appear. The ester was collected and used in optimization of alkali catalysed reaction.

4.4.2. OPTIMIZATION OF ALKALI- ESTERIFICATION

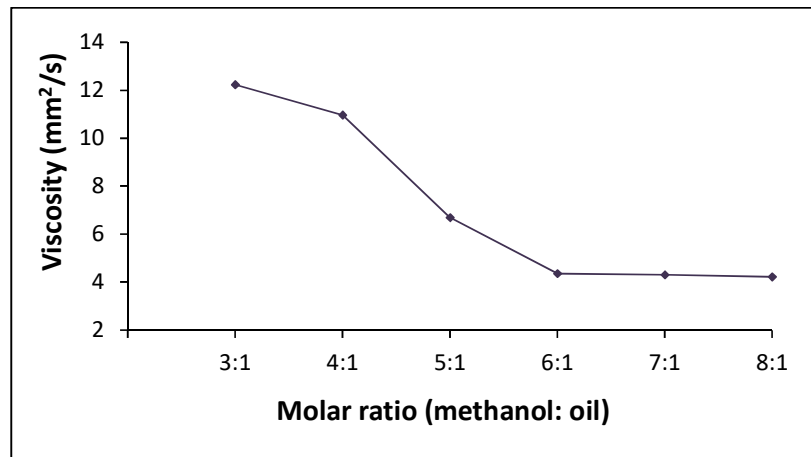
In the alkali catalysed esterification experiment, free fatty acid content together with the viscosity of algal FAME were considered to be set as the yardsticks for optimization of the process because viscosity plays a vital role to maintain the performance of CI engines.

4.4.2.1. MOLAR RATIO

Here, once again, all of six molar ratios of methanol and algal oil-ester (3:1– 8:1) were considered. Free fatty acid content of the sample decreased for the application of molar ratio of 3:1. The free fatty acid (FFA) content of the FAME produced at this stage was 0.98% and the viscosity of the same was 12.23 mm²/s. The FFA and viscosity, as well, of the FAME reduced steeply up to the use of 6:1 molar ratio (methanol: oil). The FFA of the sample for the application of 6:1 molar ratio was 0.25% which was almost the lowest FFA value of FAME resulted from all the alkali catalysed experiments with molar ratio. Viscosity of the FAME also reached almost to its lowest value of 4.35 mm²/s this time. Any further surge in methanol to oil molar ratio did not cause effective change of either FFA content or viscosity of biodiesel (Fig 4.30).



(a)



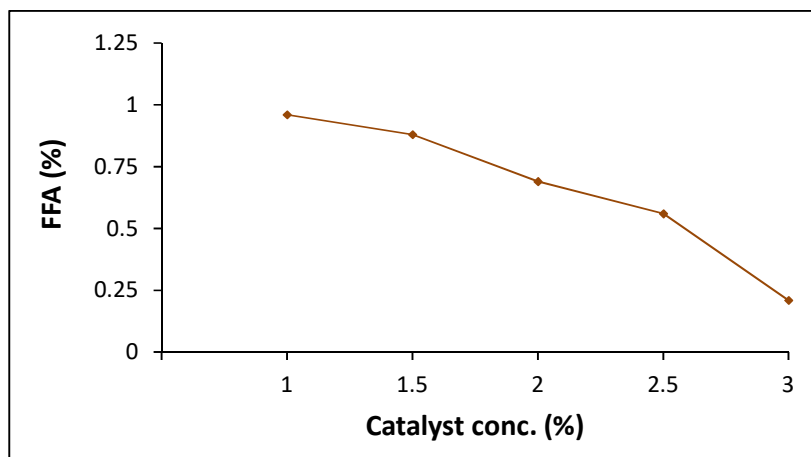
(b)

Fig 4.30: Change of (a) FFA and (b) viscosity with molar ratio (methanol: oil)

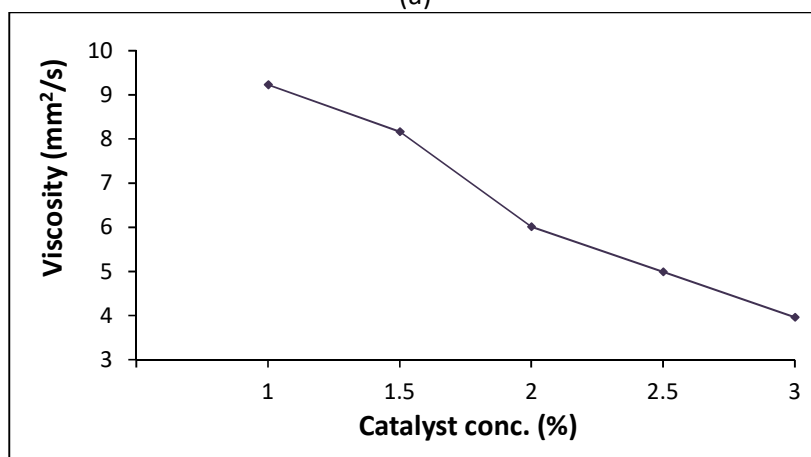
4.4.2.2. CATALYST CONCENTRATION

Catalyst (KOH) concentration was altered, for this experiment, from 0.5% to 3.5%. There was no separation of layer or production of algal FAME for the use of 0.5% KOH catalyst. The free fatty acid (FFA) content of the FAME was 0.96% and the viscosity of the same was 9.23 mm²/s FFA for the use of 1% catalyst concentration. FFA of the FAME reduced rapidly after this time and reached to 0.21% when 3% KOH was applied together with 6:1 molar ratio. The viscosity of the FAME was 3.96 mm²/s this time. Usage of 3.5% KOH resulted in no reduction in viscosity or FFA content but soap formation (Freedman et al. 1984). Same result was found to take place when the repetition of the experiment was done. Therefore, 3.5% KOH was not taken into count for this experiment and the 3% catalyst concentration was reckoned as the optimum KOH concentration of catalyst for this transesterification reaction

(Fig 4.31). In next experiments of alkali catalysed reaction with reaction time and reaction temperature, 6:1 molar ratio and 3% catalyst concentration were considered as constant.



(a)

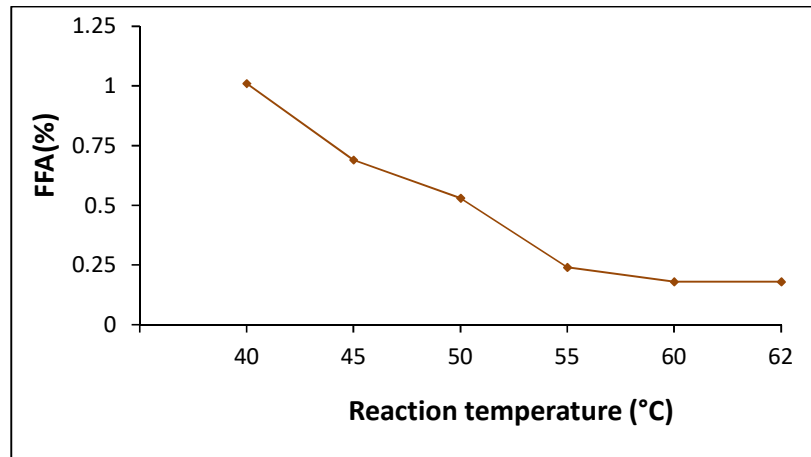


(b)

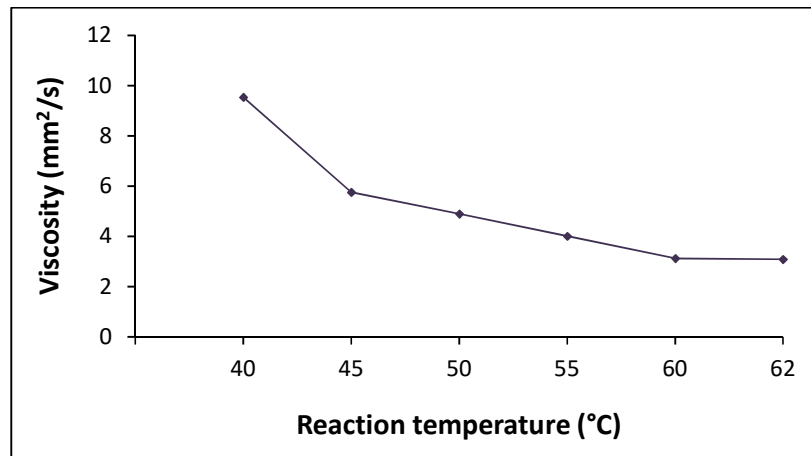
Fig 4.31: Change of (a) FFA and (b) viscosity with different catalyst concentration

4.4.2.3. REACTION TEMPERATURE

FFA of the biodiesel for the use of 40°C as reaction time was 1.01%. Viscosity of the same was 9.54 mm/s. A gradual fall of free fatty acid and viscosity of algal biodiesel took place when the temperature of the reaction surged from 40°C to 60°C. A notable change of free fatty acid content of 0.18% and viscosity of 3.12 mm²/s took place for the increase of temperature to 60°C. There was no change of FFA content of algal FAME but change of viscosity for further increase of reaction temperature to 62°C (Fig 4.32). But the decrease of viscosity (3.09 mm²/s) for this increase in reaction temperature was very much negligible. Hence, reaction temperature of 60°C was recorded as the optimum one and this optimum value of reaction temperature was used in optimization of the reaction time together with molar ratio of 6:1 and catalyst (KOH) concentration of 3%.



(a)



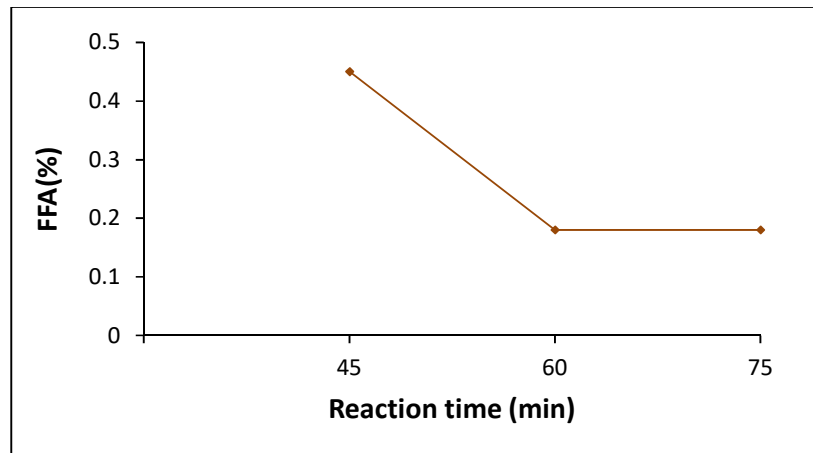
(b)

Fig 4.32: Change of (a) FFA and (b) viscosity with different reaction temperature

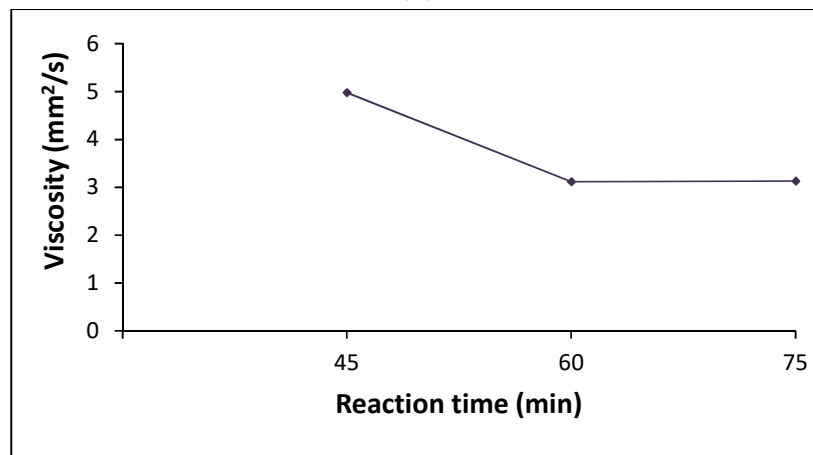
4.4.2.4. REACTION TIME

Genesis of Soap (Deng et al. 2010) took place while the alkali catalysed transesterification reaction was performed for 30 minutes. Therefore, this reaction was performed once again to confirm the result. While the outcome was found to be same, reaction with time 30 minutes of reaction time was not considered further for this experiment. FFA and viscosity of the biodiesel were 0.45% and 4.98 mm²/s respectively with the use of 45 min of reaction time. Best result for the use of reaction time parameter was found to be yielded when with the reaction time of 60 minutes. FFA of FAME, this time, was 0.18%. The viscosity of this FAME was 3.12 mm²/s (Fig 4.33).

As because the free fatty acid (FFA) content and viscosity of the FAME did not change for further increase of reaction time to 75 minutes and no FAME was found to generate for 90 minutes of reaction time, 60 minutes of reaction time was considered to be the optimum for this reaction (Table 4.17).



(a)



(b)

Fig 4.33: Change of (a) FFA and (b) viscosity with different reaction time

4.4.2.5. SETTLING TIME

It took almost 50 minutes to settle down all the glycerol at the lower part of the separating funnel. Thereafter, glycerol, from the funnel, got separated and algal fatty acid methyl ester produced in this experiment was washed with warm distilled water for the removal of excess KOH. 96.4% of algal biodiesel was found to be obtained from this transesterification procedure.

Table 4.17: Optimized conditions for biodiesel production from algal oil

Acid treatment	
Molar ratio (Methanol : oil)	7:1
Catalyst (H ₂ SO ₄) concentration	1.5%
Reaction temperature	55°C
Reaction time	90 minutes
Alkali treatment	
Molar ratio (Methanol : oil)	6:1
Catalyst (KOH) concentration	3%
Reaction temperature	60°C
Reaction time	60 minutes

4.4.3. RESULTS OBTAINED FROM STATISTICAL ANALYSIS

For statistical analysis of optimization of transesterification procedure of this algal oil, the exact upper and the exact lower values of the optimized values of parameters derived from non-statistical process along with the optimized values of parameters themselves were planned to be taken into account. Hence, 7: 1 and 5: 1 of methanol to oil molar ratio, 3.5% and 2.5% of catalyst (KOH) concentration, reaction time of 75 min and 45 min, reaction temperature of 62°C and 55°C were taken into count along with the optimized values of 6:1 molar ratio, 3% catalyst (KOH) concentration, 60 min reaction time and 60°C reaction temperature to prepare the matrix for the evaluation of statistical optimization procedure along with the contribution factors of all the parameters (Table 4.18).

Table 4.18: Different parameters (X) and their levels used to optimize the biodiesel production procedure in terms of biodiesel yield (Y1) and its FFA content (Y2)

Process Parameters	Code	Levels		
Molar ratio	X1	5	6	7
Catalyst Concentration (%)	X2	2.5	3.0	3.5
Reaction time (min)	X3	45	60	75
Reaction temperature (°C)	X4	55	60	62

A L9 orthogonal array was prepared to design the experiment to observe the impact of all of the four parameters in the course to attain highest yield of algal biodiesel together with lowest free fatty acid content of the same (Table 4.19). Experiments were carried out to discern the yield as well as the FFA content of algal FAME come out of all of the nine sets of parameters arrange by Taguchi's approach. Three trials of each of the experiment were performed for yield and FFA content of the FAME and their means were recorded.

Table 4.19: Yield and FFA of the algal biodiesel resulted using orthogonal L9 array and their means (where, X= parameters; T= Trials; and Y= Mean of corresponding trials)

S. No.	Levels of the Parameters				Yield of biodiesel (%)			FFA of biodiesel (%)			Mean Yield (%)	Mean FFA (%)
	X1	X2	X3	X4	T1	T2	T3	T1	T2	T3	Y1	Y2
1	5	2.5	45	55	70.23	73.88	71.35	0.37	0.36	0.36	71.82	0.36
2	5	3.0	60	60	20.71	18.59	19.87	0.78	0.80	0.83	19.72	0.80
3	5	3.5	75	62	10.12	9.58	9.99	0.97	1.09	1.04	9.90	1.03
4	6	2.5	60	62	92.01	88.97	91.45	0.25	0.23	0.25	90.81	0.24
5	6	3.0	75	55	94.65	93.98	92.53	0.20	0.21	0.19	93.72	0.20
6	6	3.5	45	60	23.86	22.80	24.00	0.59	0.57	0.56	23.55	0.57
7	7	2.5	75	60	82.40	82.02	83.12	0.82	0.81	0.83	82.51	0.82
8	7	3.0	45	62	86.73	88.32	87.86	0.79	0.77	0.80	87.64	0.79
9	7	3.5	60	55	5.20	7.33	5.70	1.11	1.15	1.13	6.08	1.13

By using the Minitab software, the signal to noise (S/N) ratios of all four parameters and their corresponding deltas (ranges) were calculated which were used to calculate the contribution of all parameters. The ranks of these parameters (X1, X2, X3, X4) were generated accordingly for the yield (Y1) (Table 4.20) and the FFA (Y2) of the FAME (Table 4.21).

Paramount contribution (67.7%) for the yield of FAME was found to be exerted by concentration of catalyst (X2). Molar ratio (X1) was found to be the parameter with second most contribution (17.3%) for biodiesel yield and which was followed by reaction time (X3) and reaction temperature (X4) which came out with contribution of 8.9% and 6.1% accordingly.

For lowest free fatty acid content, molar ratio was found to exert highest contribution of 58.3%. Amongst the other parameters, contribution of catalyst concentration was best (33.7%) and it was followed by the contribution of reaction temperature (4.5%) and reaction time (3.4%) sequentially.

Table 4.20: Signal to noise ratio along with the contribution and rank of parameters for yield of biodiesel (Y1)

Level	S/N ratio corresponding to Y1			
	X1	X2	X3	X4
1	27.65	38.21	34.47	30.75
2	35.35	34.73	26.91	30.56
3	30.95	21.01	32.56	32.64
Delta	7.70	17.20	7.56	2.09
PC (%)	17.31	67.71	8.91	6.06
Rank	2	1	3	4

Table 4.21: Signal to noise ratio along with the contribution and rank of parameters for the FFA content of biodiesel (Y2)

Level	S/N ratio corresponding to Y2			
	X1	X2	X3	X4
1	3.52	7.67	5.27	7.26
2	10.42	5.99	4.42	2.85
3	0.90	1.19	5.15	4.73
Delta	9.52	6.48	0.84	4.42
PC (%)	58.43	33.71	3.37	4.49
Rank	1	2	4	3

ANOVA was used to calculate the variance of the experiment of optimization of transesterification. The F value was 4.56×10^{-6} for the results derived for Y1 at 95% confidence level which was much lesser (Table 4.22) than the $F_{critical}$ value (3.40) for the degree of freedom 24 at 95% confidence level. The F value derived for the results of Y2 (0.004) was, again, lesser than the $F_{critical}$ value for the degree of freedom 24 (Table 4.23). So, results derived for both Y1 and Y2 from the statistical analysis were considered as significant.

Table 4.22: Result derived from ANOVA for biodiesel yield (Y1)

Source of Variation	SS	df	MS	F	$F_{critical}$
Between Groups	0.013156	2	0.006578	4.55773E-06	3.402826
Within Groups	34637.13	24	1443.214		
Total	34637.14	26			

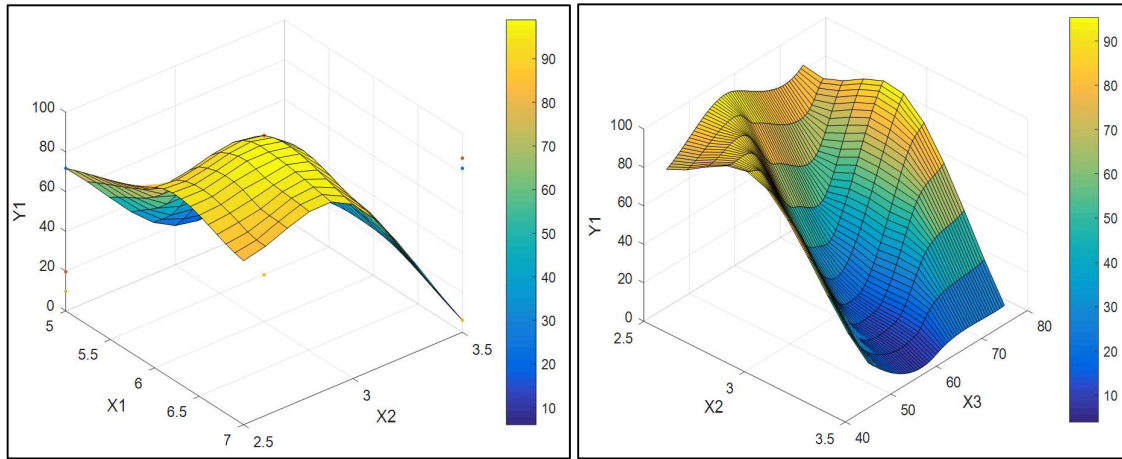
Table 4.23: Result derived from ANOVA for FFA content of biodiesel (Y2)

Source of Variation	SS	df	MS	F	F _{critical}
Between Groups	0.000896	2	0.000448	0.003955	3.402826
Within Groups	2.719644	24	0.113319		
Total	2.720541	26			

To visualise the effect of all four parameters (X) on the yield (Y1) and free fatty acid content (Y2), six three dimensional graph plots were made using three prime contributing parameters (catalyst concentration, molar ratio (methanol to oil) and reaction time for highest yield of FAME (Y1); molar ratio (methanol to oil), catalyst concentration together with reaction temperature for least free fatty acid content of FAME (Y2)) derived from Table 4.20 and 4.21.

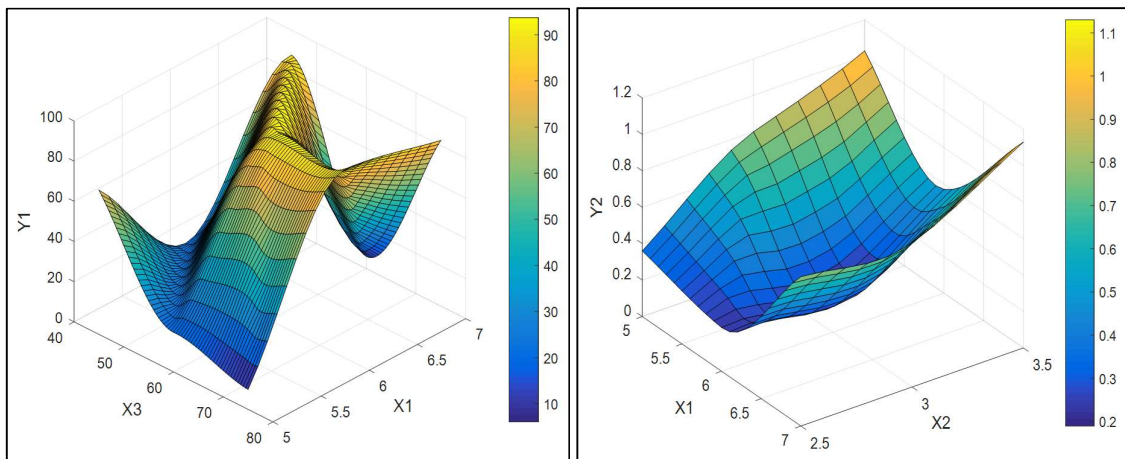
The paramount yield of algal FAME was found when the molar ratio increased from 5:1 to 6:1 and the catalyst concentration along with reaction time increased consecutively from 2.5% and 60 minutes to 3% and 75 minutes. From the previous tables, it has been proven that for yield of highest quantity of biodiesel, catalyst concentration (X2) was most dominant contributing parameter. Same result was found from the 3D graph this time. Foremost yield of algal FAME was found to be plotted at catalyst concentration of 3%. Increase in amount of catalyst concentration was found to cause significant reduction of biodiesel yield.

When 3D plots for free fatty acid were prepared by means of three of most contributing parameters (molar ratio (methanol: oil), catalyst concentration, reaction temperature consecutively), the lowest FFA was found to take place for the molar ratio of 6:1, catalyst concentration of 3% and reaction temperature of 55°C.



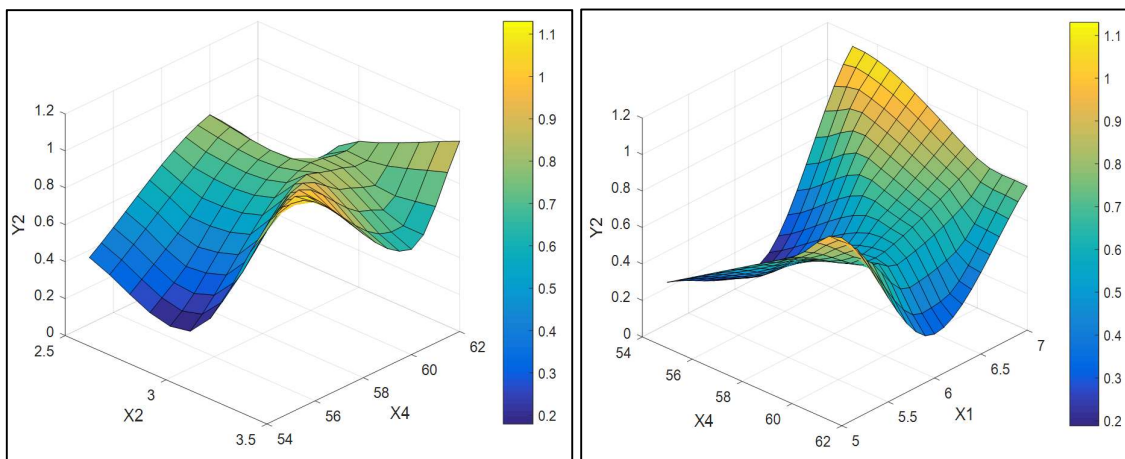
(a)

(b)



(c)

(d)



(e)

(f)

Fig 4.34: 3D plots of algal biodiesel yield with respect to (a) molar ratio (methanol: oil) and catalyst concentration, (b) catalyst concentration and reaction time, (c) reaction time and molar ratio (methanol: oil); and free fatty acid content with respect to (d) molar ratio (methanol: oil) and catalyst concentration, (e) catalyst concentration and reaction temperature, (f) reaction temperature and molar ratio (methanol: oil)

4.4.4. PERCENTAGE OF BIODIESEL CONVERSION

Three biodiesel samples were planned to be chosen from both non- statistical and statistical experiments. Therefore, the biodiesel yielded from the non- statistical analysis, which was derived by applying optimum levels of parameters, was chosen to be used here. The yield of the biodiesel was 96.4% and the FFA of the same was 0.18%. Other two biodiesel samples were chosen from the statistical analysis. The biodiesel produced from combination 4 and combination 5 had the yield of 90% and 93% respectively. The FFA of the same was 0.24% and 0.20% respectively. So, these three biodiesels were the best three algal FAME samples chosen for TLC experiment along with algal oil as the reference. The codes assigned for these biodiesels are given in Table 4.24.

Table 4.24: Codes assigned to algal oil and algal biodiesel samples for TLC

Sample	Code
Algal oil	Oil
Algal biodiesel derived from non-statistical analysis	S1
Algal biodiesel derived from combination 4 of statistical analysis	S2
Algal biodiesel derived from combination 5 of statistical analysis	S3

From thin layer chromatography, it was observed that S1 has completely converted to FAME (Fig 4.35). Whereas the conversion of S2 sample was very less and good quantity of oil was found to be as it is in it. Noticeable conversion of S3 sample was observed. But little quantity of oil in it was found to remain unconverted in this sample too.

Therefore, biodiesel produced with optimized conditions in statistical analysis (S3) was not taken in count and only sample S1, which was the biodiesel produced with optimized conditions in non- statistical analysis was chosen to be proceeded with NMR test to observe percentage of conversion of algal oil to algal FAME and calculate the yield of the biodiesel thereby.

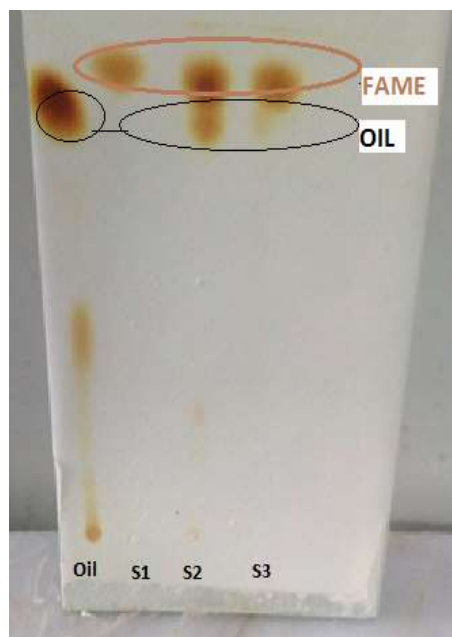


Fig 4.35: TLC of oil and biodiesel samples

From Fig 4.36, percentage of conversion of biodiesel was calculated.

$$\text{Percentage of conversion (C)} = \frac{2A_{\text{ME}}}{3A_{\alpha\text{-CH}_2}} \times 100$$

$$\text{Percentage of conversion (C)} = \frac{2 \times 1.27}{3 \times 0.88} \times 100 = 96.2\%$$

4.4.5. PERCENTAGE OF BIODIESEL YIELD

Therefore, the yield of the biodiesel was calculated by means of the recovery (yield observed from the experiment) and the percentage of conversion of the biodiesel derived from NMR report.

$$\text{Yield of biodiesel (\%)} = \text{yield (recovery) of biodiesel observed from experiment} \times C$$

$$\text{Yield of biodiesel (\%)} = 96.4 \times 96.2\% = 92.74 \approx 93\%$$

As the conversion of sample was high enough (>96%), S1 sample was considered as the full-proof algal oil FAME and conversion of 93% was proved as its exact yield. At the same time, this also manifested the optimized conditions (6:1 methanol to oil molar ratio, 3% catalyst concentration, 60°C reaction temperature together with 60 minutes of reaction time) for biodiesel production from oil of unused algae.

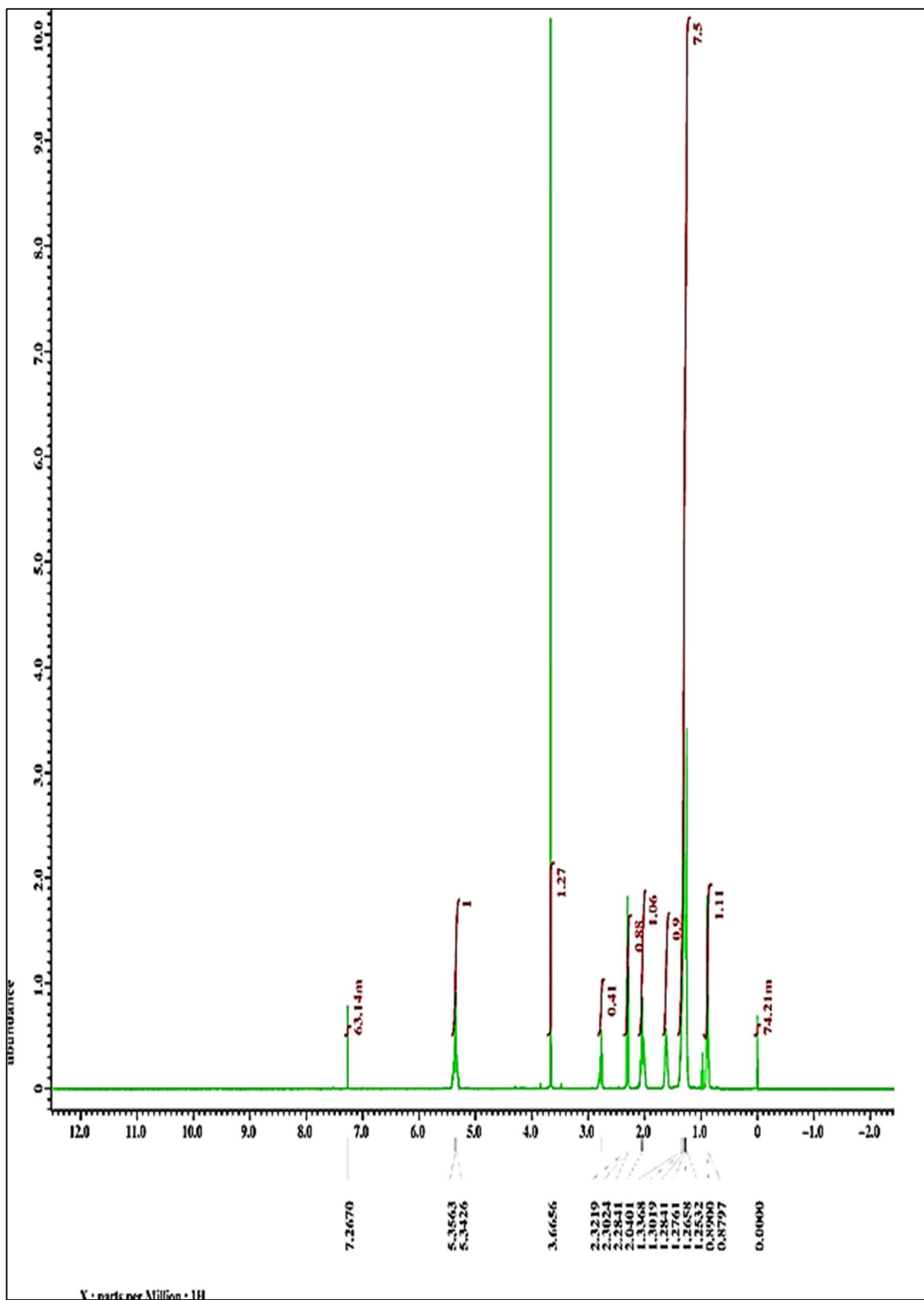


Fig 4.36: NMR report of biodiesel sample S1

4.5. FUEL CHARACTERISTICS

All of the properties of algal biodiesel produced under optimized conditions were found to be within the limits of ASTM standard (Table 4.25) (McCurdy et al. 2014; Diesel Net Technology Guide 2009).

The acid number of algal biodiesel was 0.38mg KOH/g. This was almost similar to the acid value of petroleum derived diesel (0.35) (Singh et al. 2009) and therefore it can do very least harm to the parts of CI engine. Calorific value (CV) of all fuels play a dominant important role because higher calorific value or gross heat of combustion, in other words, of them indicates higher rate of generation of power to run a CI engine. Though, calorific value of this biodiesel produced was lower (40882 kJ/kg) than the calorific value of petrol or diesel (44800 kJ/kg), it was found to be more than the calorific values of coal or well-known biodiesels derived from Jatropha and palm oil (Fig 4.37). Both kinematic viscosity and relative density of the algal FAME produced (3.12 mm²/s and 872.9 kg/m³ respectively) were found to be approximately same to those of petro-diesel (1.9– 4.1 mm²/s (Knothe et al. 2005) and 832 kg/m³ consecutively)). This gives an indication that atomization and combustion of this biodiesel, inside CI engine, would be very good and engine would have a healthy life. The flash point (153°C) and fire points (158°C) of this biodiesel were much higher, and therefore better, than those of petro-diesel which has a flash point of 93°C and a fire point of 102°C. Higher values of flash point and fire points lessen the probability of fire hazard (Lee and Ha 2003). On the other hand, the cloud point and pour point of algal FAME were very high (-1°C and -6°C respectively) than diesel. In spite of that, these values of cloud and pour points were lesser than the cloud point and pour points of many other biodiesels. Therefore, these cold flow properties of algal FAME that was produced in this research can be used in low atmospheric temperature conditions without the usage of additives or modifying engine parts (Sarin et al. 2009). Requirement of additives may take place if the atmospheric temperature reduces to its extreme condition. Lower ash and carbon residue content of biodiesel lowers the carbon deposition on parts of an engine and thereby increases the life of engine. Ash content of this algal FAME was 0.01% and the content of carbon residue the same FAME was 0.03%. Both of these last two properties of algal FAME were also found to be very low and the biodiesel, as a result, can be used in unmodified CI engines.

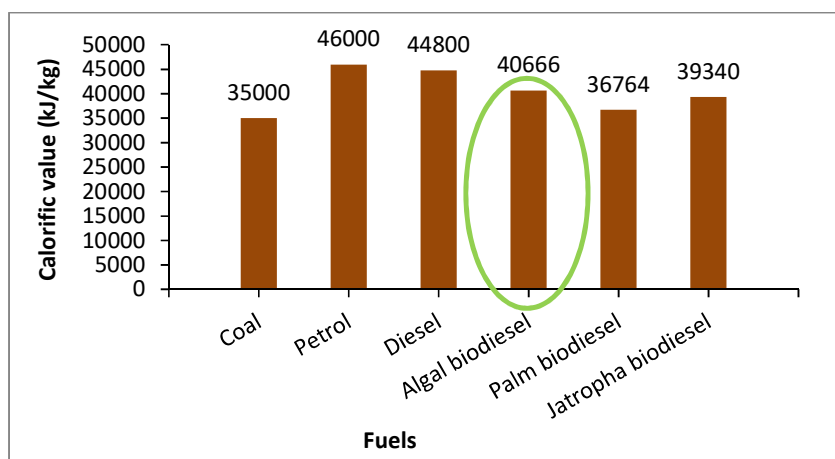


Fig 4.37: Calorific value of different fuels

Table 4.25: Properties of algal FAME and the corresponding limits of ASTM/ BIS standard

Properties	Trial1	Trial2	Trial3	Mean of properties	ASTM / BIS standards
Kinematic viscosity at 40°C (mm ² /s)	3.12	3.12	3.12	3.12	1.9- 6
Density at 15°C (kg/m ³)	873	872.9	872.8	872.9	860- 900
Flash Point (°C)	153	153	153	153	93 (minimum)
Fire Point (°C)	158	158	159	158°C	-
Cloud point (°C)	0	-1	-1	-1°C	3°C(Maximum)
Pour point (°C)	-6	-6	-6	-6°C	-
Acid number (mg KOH/g)	0.38	0.38	0.3	0.36	0.50 (maximum)
Ash content (%)	0.01	0.01	0.02	0.01	0.01 (maximum)
Carbon residue content (%)	0.04	0.03	0.03	0.03	0.050 (maximum)
Calorific value (kJ/kg)	40881	40880	40884	40882	-

4.6. EMISSION CHARACTERISTICS OF ALGAL BIODIESEL

4.6.1. EMISSION OF CARBON MONOXIDE

CO emission of diesel or alike fuels is influenced by incomplete combustion. In accordance to the reports of researchers, increase in load on engine fosters the decline of fuel to air ratio inside CI engines. This comes out with better rate of combustion of that fuel and, thus, reduces the emission of carbon monoxide gas.

As all biodiesels are composed of in-built oxygen, their combustion rate is manifestly more complete than that of petro-diesel because oxygen is the key factor for combustions. Hence, emission of CO for the combustion of biodiesels is lesser than that of diesel.

Initially the CO emission at 0 load condition was 183 ppm for biodiesel and 560 ppm for petro- diesel. In this experimental work, surged load of engine was observed to be associated with the gradual dwindle in emission of CO. CO Emission for algal FAME reduced linearly from 151 ppm to 27 ppm for the relevant increase of load of engine from least load of 20% to 110%. Whereas the CO emission for petro-diesel reduced, for the same range of increase in loads, from 531 ppm to 101 ppm. At all load conditions, emission of CO for algal biodiesel was found to be very lower than CO emission for petro- diesel (Fig 4.38). This kind of carbon monoxide emission was found to be in line with relevant research done with waste cooking oil biodiesel (An et al. 2012) and blend of cashew nut shell biodiesel and hexanol (Pandian et al. 2018).

Carbon monoxide emission for algal biodiesel- diesel blends were found to decrease with increased proportion of biodiesel in them (Fig 4.39). At 0 load condition, the CO emission for blends were 530 ppm, 510 ppm and 492 ppm for B10, B20 and B30 respectively. The CO emission for petro- diesel at this stage was 563 ppm. While B10 of those blends was found to emit most CO, B30 emitted lowest amount of CO at all engine load points. Emission of CO by diesel was found to be more than all algal biodiesel- diesel blends. Emission of carbon monoxide with biodiesel blends were found to decrease linearly (from 498ppm, 450ppm and 423 ppm to 80 ppm, 71 ppm and 63 ppm respectively by B10, B20 and B30) with increase in engine load (20% to 110%). The petro- diesel, here also, found to be decreased linearly with increase in load of engine (534ppm to 102ppm for 20% load to 110% load).

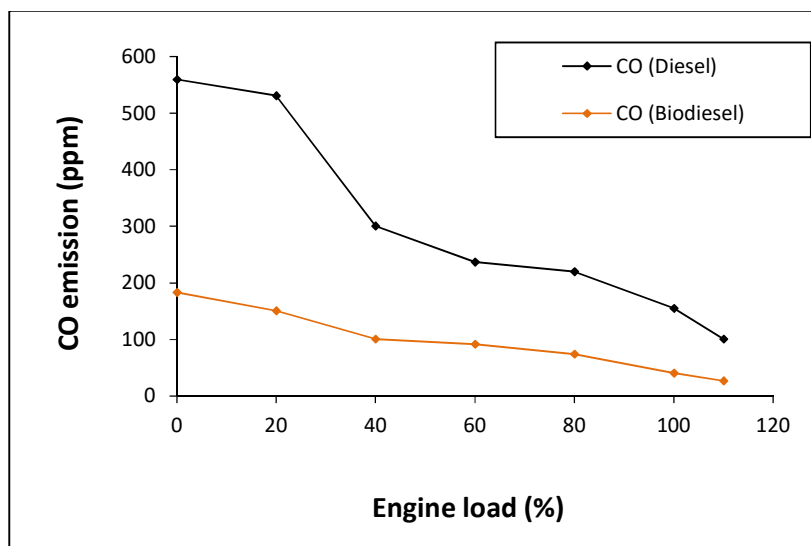


Fig 4.38: Emission of CO with different loads on engine

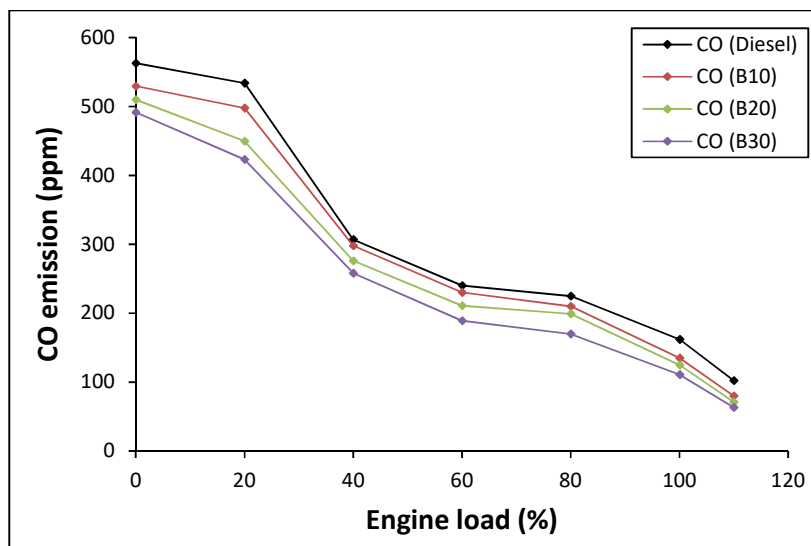


Fig 4.39: CO emission by diesel and algal biodiesel blends with different loads on engine

4.6.2. EMISSION OF CARBON DIOXIDE

More air rich mixture, in the combustion chamber of CI engines, is injected with the surge in load of the engine. So, it is evident that in an engine, the rate of combustion of fuels gets better when the engine load increases. Therefore, the pattern of carbon dioxide emission from a diesel- engine is reverse to the emission pattern of CO because emission of CO₂ escalates with escalation of engine load.

In this current study, a gradual escalation of emission of CO₂ was discerned with the surge in load of engine. Carbon dioxide emission for the combustion of algal FAME was found to be least (2.1%) when there was no load on engine. At overload condition, highest CO₂ emission for algal biodiesel (3.8%) was found to take place. Whereas the carbon dioxide emission for petro- diesel at 0 load condition and overload condition were 2.9% and 4.3% respectively (Fig 4.40). At all engine load conditions, CO₂ emission by algal biodiesel was less than the CO₂ emission of petro- diesel. Both algal biodiesel and petro- diesel increased very steeply with the surge in load of the engine. As because biodiesels have lower ratio of carbon to hydrogen than that of petro- diesel, it is obvious that CO₂ emission, for the combustion of biodiesel would be lesser (Xue et al. 2011).

Likewise carbon dioxide emission for the combustion of algal FAME- diesel blends together with petro- diesel were found to escalate with the increase in engine load (Fig 4.41). When there was 0% load on engine, 2.9% carbon dioxide was emitted by algal biodiesel blends of B10 and B20. The CO₂ emission by B30 was 2.8% during this stage. Emission of CO₂ by all these blends were much less than that of petro-diesel at all engine- loads. Highest CO₂ emission by algal biodiesel- diesel blends was found to take place by blend with lowest proportion of biodiesel (B10) at all loads on engine (2.9%, 3.1%, 3.1%, 3.3%, 3.4%, 3.4%, 3.4% for 0%, 20%, 40%, 60%, 80%, 100% and 110% engine loads respectively). Whereas the lowest CO₂ emission (2.8%, 2.9%, 3%, 3%, 3.1%, 3.2%, 3.3% for 0%, 20%, 40%, 60%, 80%, 100% and 110% engine loads respectively) by blends was done by B30, the blend with highest proportion of algal biodiesel. Higher carbon to hydrogen ratio of diesel results the back bone for pattern of emission.

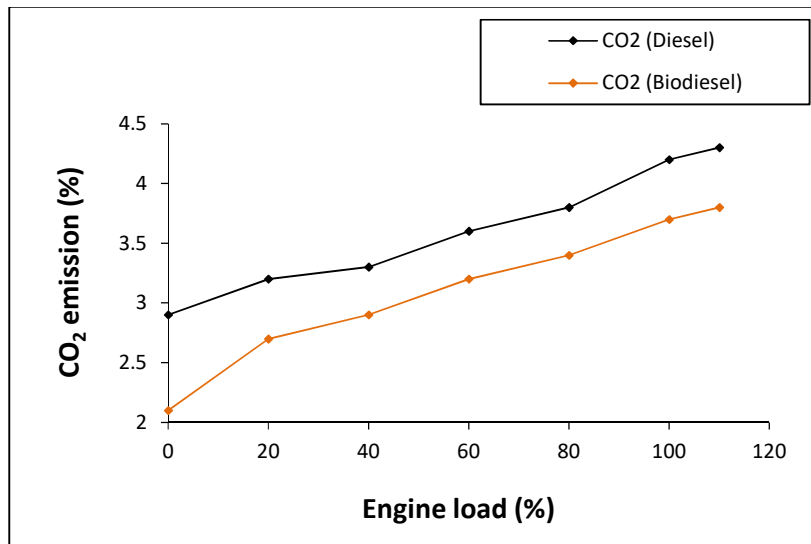


Fig 4.40: Emission of CO₂ with different loads on engine

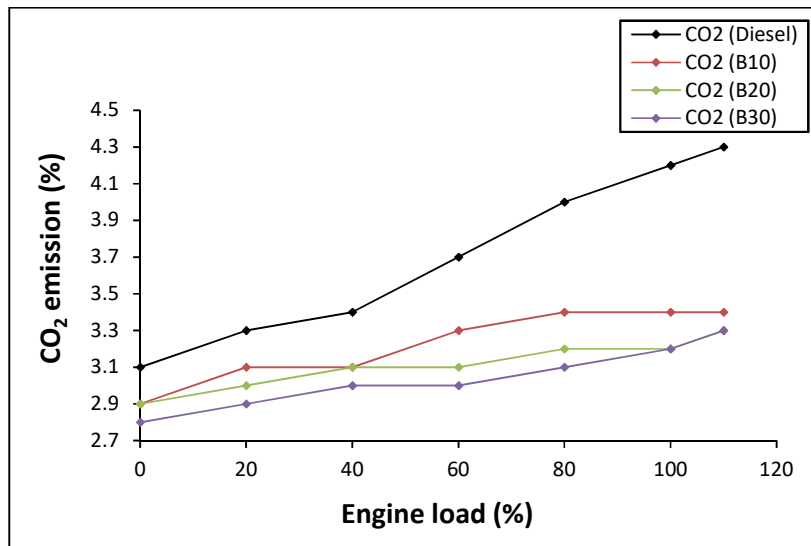


Fig 4.41: CO₂ emission by diesel and algal biodiesel blends with different loads on engine

4.6.3. EMISSION OF NO_x

More heat is engendered in combustion chamber of CI engine when the load of engine increases. This is keystone reason behind rapidly increasing NO_x emission with the increase of engine- load (Raheman and Ghadge 2007; Zhu et al. 2010).

In this experiment, emission of NO_x was found to be increased with the load on engine for the combustion of both petro- diesel and algal FAME (Fig 4.42). At all engine- load conditions, emission of NO_x was more for algal FAME (surged from 42 ppm to 257 ppm for increase from 0% engine load to 110% engine load) than that of petro-diesel (surged linearly from 20 ppm to 154 ppm for increase in engine load from 0% to 110%). The keystone reason behind the increased rate of NO_x emission by algal biodiesel is its in-built oxygen which enhances the chance of formation of oxides of nitrogen. The other reason for such behaviour of emission of NO_x might be the temperature of cylinder of CI engine.

It can be observed from Fig 4.46 and 4.47 that for the combustion of this algal biodiesel, more exhaust temperature, than that was produced for the combustion of petro- diesel, came out. Hence, this can be pretended that temperature of the engine- cylinder would be higher too when the CI engine would be operated by biodiesels. So, more amount of NO_x would be generated for combustion of this algal FAME compared to that of petro-diesel. NO_x emission can be cut down by the addition of biodiesel additives to this algal FAME (Palash et al. 2014) or by attaching a catalytic converter (Zukerman et al. 2009) or a de-NO_x catalyser (Madia et al. 2002) in the exhaust part of CI engine. NO_x emission, by algal biodiesel- petro- diesel blends and petro- diesel itself, escalated linearly with the increase in engine load. NO_x emission by all the blends of algal biodiesel was found to be more than that of petro- diesel at all load conditions (Fig 4.43). As the heat generation by biodiesels is more than that of petro-diesel for the presence of its in-built oxygen and thus its higher cetane number, NO_x emission by algal biodiesel- petro- diesel blends was observed to be least by B10 (23 ppm, 31 ppm, 43 ppm, 80 ppm, 99 ppm, 129 ppm, 170 ppm for 0%, 20%, 40%, 60%, 80%, 100% and 110% engine loads respectively), the blend with lowest proportion of biodiesel. NO_x emission was highest for the combustion of blend with highest fraction of algal biodiesel (B30). For the increase in engine loads from 0% to 110%, its rates of emission were 27 ppm, 38 ppm, 60 ppm, 98 ppm, 109 ppm, 247 ppm, 201 ppm.

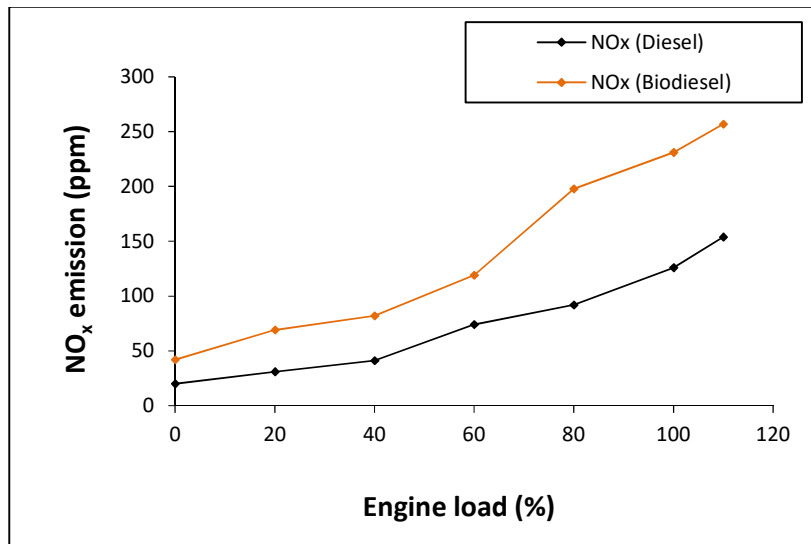


Fig 4.42: Emission of NO_x with different loads on engine

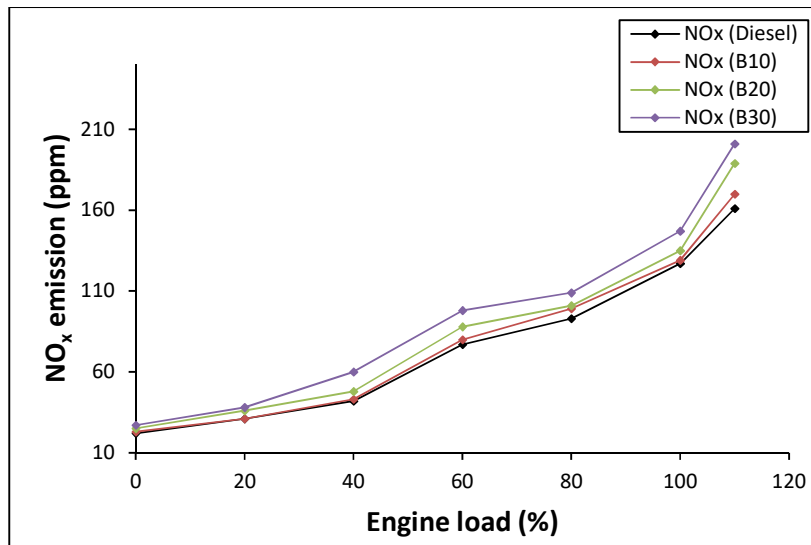


Fig 4.43: NO_x emission by diesel and algal biodiesel blends with different loads on engine

4.6.4. EMISSION OF HYDROCARBON

Emission of partially burned or unburned hydrocarbon (HC) occurs as a result of incomplete or partial combustion of fuels in engine. Therefore, HC emission for the combustion of biodiesel is evidently less than that of petro-diesel because biodiesels are composed of in-built oxygen which boosts better rate of combustion of the former fuel.

Emission of HC, at all loads of engine, was found to be less for the combustion of algal FAME than that for petro- diesel (Fig 4.44). HC emission by petro- diesel was observed to drop (from 38.9 to 25.7 ppm) for the escalation of load on engine. Emission of HC, on contrary, by algal FAME, was found to surge at the beginning of this research experiment from 0 ppm (at engine load of 0%) and reached its highest point of 11 ppm at 40% engine load. Then it declined gradually up to the overload engine condition (110% engine load). From the report of Shirneshan et al. 2012, and Shirneshan 2013, it was found that most emission of HC for combustion of waste frying oil- FAME, takes place at 40% engine load. According to that report, HC emission, thereafter, declined gradually with increasing engine load. This occurred as an outcome of higher value of density of biodiesel than that of petro-diesel. For its higher density, bigger droplets of biodiesel is generated at the time of atomization of it in CI engine. This bigger size of biodiesel droplets does not allow the fuel to burn wholly and, thus, emission HC escalates initially in CI engines. In spite of that fact, when the engine load is escalated, more amount air rich mixture (higher air: fuel) gets injected into the combustion chamber of the CI engine. Injection of more air brings more oxygen in the combustion chamber and at the same time quantity of in-built O₂ surges for the presence of increased quantity of biodiesel. This results in better rate of combustion of biodiesel and this is how the emission of HC declines when the engine load starts escalating from 40%.

But the emission of unburned hydrocarbon for biodiesel blends did not found to increase at first but decrease gradually with increase in engine load from the very beginning (Fig 4.45). This happened as the dominant measure of the blend was petro- diesel in those blends. At all load conditions, this time too, the emission of HC for algal FAME was found to be lesser than HC emission for petro- diesel. As better combustion transpires with FAME, least HC emission was done by B30 (33ppm, 28ppm, 22ppm, 19ppm, 17ppm, 15ppm and 14ppm at respective engine loads) and highest emission, done by blends, was done by B10.

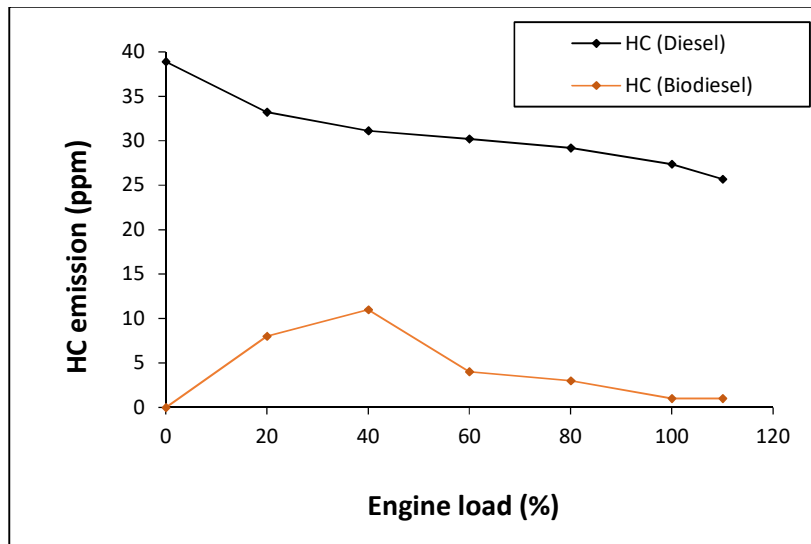


Fig 4.44: Emission of hydro carbon with different loads on engine

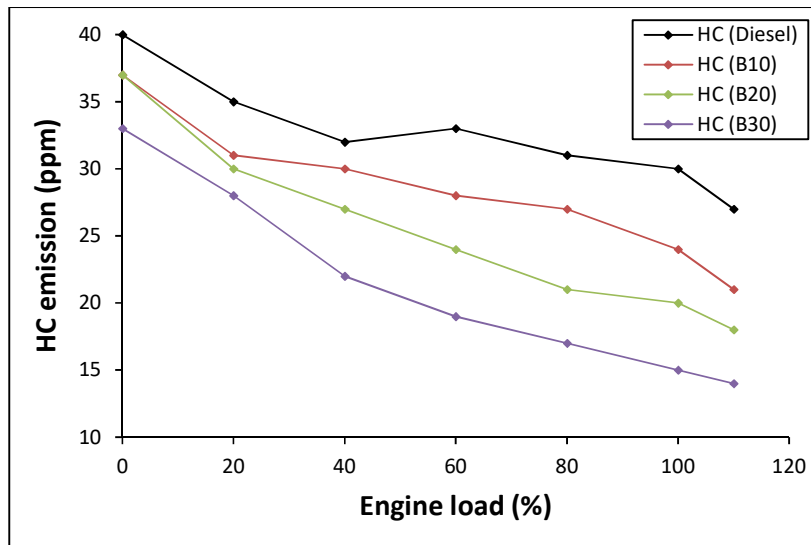


Fig 4.45: HC emission by diesel and algal biodiesel blends with different loads on engine

4.6.5. EXHAUST TEMPERATURE

From the point of environment, exhaust temperature plays an important role because it affects the flora and fauna of the terrestrial and aquatic systems and thereby the ecology of the surrounding environment. Apart from that, the exhaust temperature provides with a brief idea of the increase and decrease of temperature inside the CI engines. The role of engine temperature on emission of different gases is evident and dominant at the same time (Karmakar et al. 2017).

In the present study, exhaust temperature, at all the engine load conditions, was much higher for the combustion of algal FAME than for the combustion of petro- diesel (Fig 4.46). In both of the cases of FAME and petro- diesel, the temperature increased linearly (41.2°C to 59.7°C for diesel and 57°C to 88°C) from 0% load condition to 110% load condition.

With the increase in proportion of algal biodiesel in algal biodiesel- diesel blends, increase in exhaust temperature was found to take place (Fig 4.47). Lowest exhaust temperature was found to take place in CI engines for the combustion of petro- diesel this time too. The exhaust temperature increased from 41.1°C to 59.5°C for petro-diesel.

B10, the biodiesel- diesel blend with least part of algal biodiesel, was found to be associated with the generation of exhaust temperature which is little higher than that for petro-diesel (43.6°C, 50.2°C, 51°C, 55°C, 59.9°C, 61.2°C for 20%, 40%, 60%, 80%, 100% and 110% engine load respectively) apart from 0 load condition when exhaust temperatures for petro- diesel and algal FAME were exactly the same (41.1°C). On the other hand, generation of highest of exhaust temperature (45.1°C, 46°C, 53°C, 55.8°C, 58.3°C, 62.9°C and 66°C for 0%, 20%, 40%, 60%, 80%, 100% and 110% engine loads) was done by the combustion of blend with highest proportion of algal biodiesel (B30).

Therefore, it has been observed that more generation of heat would take place if petro-diesel gets replaced by algal biodiesel produced in this experiment which will help the engine to run with lower quantity of the fuel.

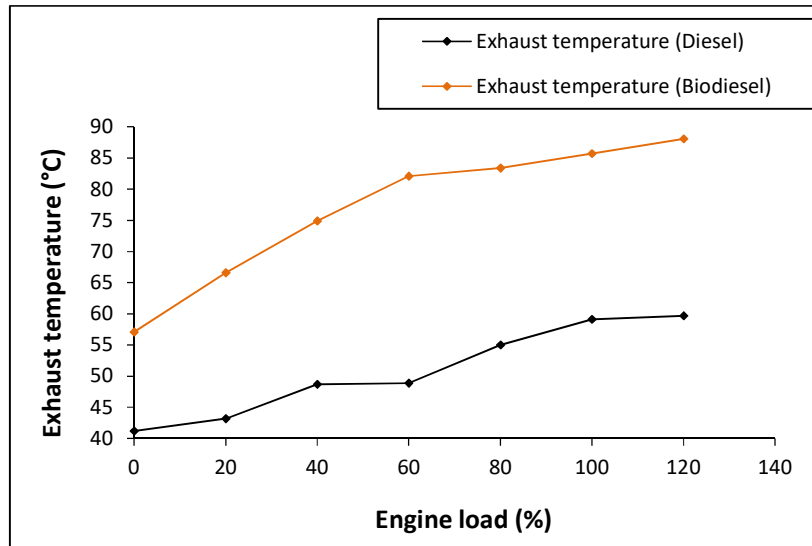


Fig 4.46: Change in exhaust temperature with different loads on engine

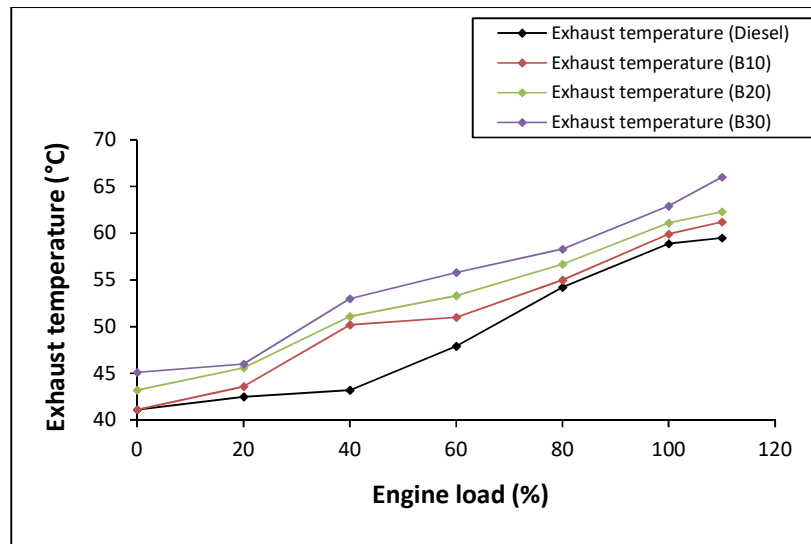


Fig 4.47: Change in exhaust temperature by diesel and algal biodiesel blends with different loads on engine

4.7. COMPOSITION OF ALGAL BIODIESEL

The fatty acid composition of algal biodiesel was determined by gas chromatography technique. It was found that this algal biodiesel was composed of esters of palmitic, stearic, oleic, linoleic and linolenic acids. The algal FAME was found to be predominant in oleic and linoleic acid esters. The composition of the biodiesel is given in Table 4.26.

Table 4.26: Composition of algal FAME

Fatty acid	Percentage (%)
Palmitic	9.15
Stearic	6.20
Oleic	38.35
Linoleic	46.42
Lonolenic	4.77
Others	1.1

Biodiesels with lower quantity of linolenic acid and increase proportion of oleic are considered to be of good quality. With lower quantity of linolenic acid, the oxidative stability, i.e. chance of oxidation, of biodiesel increases (Santos et al. 2013). Though the algal FAME, produced in this process, was low in linolenic acid (4.8%) and thus it can be assumed that it would have high oxidative stability, it was found to be composed of very higher quantity of unsaturated fatty acids like linoleic acid (46%). Therefore the, oxidative stability of this biodiesel is questionable and use of biodiesel additives is recommended if it is planned to be stockpiled for long time.

CHAPTER 5

CONCLUSIONS

This chapter encompasses the conclusions gleaned from all the experimental works brought off step by step. It also includes the drawbacks of this experiment and their possible solutions which can be accomplished in future research endeavours.

5.1. GROWTH PARAMETERS OF ALGAE

- I. 5 parameters, which are nutrient, CO₂, depth of pond water, atmospheric temperature and intensity of mixing, were selected for the optimization of algal growth. It was found that algal biomass as well as its oil content increases to its acme when they are cultured in an open pond of 0.5m depth with 4l/pond cow dung slurry and supply of 30ml/l of carbon dioxide from CO₂ cylinder. The growth of algae gets boosted if the pond water is mixed thoroughly by stirring in every 1.5 h a day.
- II. Although these optimized levels of parameters were found to be yielding abundant growth of algae, these optimized levels are constant for the ponds having length and width of about 5m and 1m respectively. Profound change of the size of pond, which is important to achieve the production of industrial level, may require change in optimized level of growth parameters. Therefore research, in future, may be conducted in larger ponds to accomplish the optimization of growth parameters of algae in them and tally the results with that of this research work.
- III. The feedstock used in these experiment was mixed culture of indigenous algae which is not used in any way in this country. It was found that these algae grow best in winter when the temperature ranges between 5°C and 10°C. Although production of these algae was found to take place throughout the year, the biomass and oil content of algae was found to decrease with the increase in atmospheric temperature. Therefore, production of biodiesel from these algae would be, though, not seasonal but higher in winter. So, to make this biodiesel available equally throughout a year, it can be stored. More research is required to maintain the peak level of growth and oil content of these algae. Genetic engineering (Chisti et al. 2011) to these algae might be a solution to this problem. However extensive research and study id required on the investment cost, legal issues etc.

- IV. Though NPK fertilizer is good for growth of many terrestrial plants, it exerts adverse effect on these growth of these algae. Therefore its use is not recommendable for the cultivation of these algae. The alternate nutrient of it, the CDS which can be achieved from any cow- dung gas plant without paying a penny, comes out with abundant algal growth. So, use of CDS for this purpose is recommended from the point of sustainable development and to make the procedure economically cheap.
- V. It was found from the experiment that nutrient and temperature plays the key role for growth of algae in terms of increase in biomass and oil content of the same. Therefore, use of these parameters, during the culture of these algae, should not be avoided or neglected. Mixing of pond water, as the culture is done in open pond, is also an important parameter when dealing with growth of these algae.
- VI. If culture of these algae is done under the optimized conditions derived from this experiment, almost 12 kg of algae with almost 12% oil content can be achieved from 5 kg of algae with 8.9% oil content.
- VII. As this procedure of biodiesel production dealt with unused and indigenous algae, supply of such feedstock might be a problem when the production would be at industrial scale. But growth of indigenous algae is seen throughout a year in all over India, especially, in unused waterbodies, cattle- ponds and sewage treatment plants. Algae, from those sites can be supplied for production of biodiesel. But research should be conducted for that endeavour because the type or combination of algae might be different from that of the current study.
- VIII. CO₂ from CO₂ cylinder is the only artificial thing that was used in this experiment. The use of it might be avoided by culturing them in a highly CO₂ polluted area. This may decrease the CO₂ level of air in that area and increase the oxygen proportion by means of algal photosynthesis.

5.2. ALGAL OIL EXTRACTION

- I. Least or no energy should be used for the production of some other energy resource. So, from that ethical point of view, algae should be dried in the sun instead of using an oven. It can easily be pursued in hot countries like India.
- II. It was found from the experiment that the mixture of hexane and acetone of equal proportion is best for the extraction of oil from these algae.

- III. Extraction with Soxhlet apparatus was found not to be very promising for extraction of oil from these algae. As the Soxtherm (Silva et al. 2011; Fernández et al. 2010), a better oil extracting apparatus, was not available for this experiment oil extraction, at later stage of this experiment, was done by submerging the dried algae into the solvent. This was found to come out with better result with 8.9% (oil content of algae at initial stage i.e. algae which were not grown under optimized condition) of oil extraction. Therefore, use of this procedure of oil extraction by boiling the algae in solvent directly is recommendable if pursuing the procedure in Soxhlet apparatus. Use of Soxtherm apparatus can also be done for this endeavour. Though, improved technologies are required to extract algal oil when doing it at industrial scale.

5.3. ALGAL BIODIESEL PRODUCTION

- I. For biodiesel production from algal oil with very high FFA content (21.3%), two step procedure (acid- catalysed treatment followed by alkali- catalysed treatment) is always recommendable. Though, acid catalysed reaction should only be carried out to reduce the FFA below 2% to avoid saponification reaction.
- II. The FFA of the algal oil can be reduced to 1.06% by using 7: 1 molar ratio, 1.5% H₂SO₄ catalyst, 90 minutes of reaction time and 55°C of reaction temperature.
- III. For the production of this FAME, especially for reduction of its FFA, both catalyst concentration and molar ratio were found to exert very important contributions. Therefore, while dealing with production of this biodiesel, use of molar ratio and catalyst concentration as production parameters, should not be avoided or neglected.
- IV. Algal FAME with 0.18% FFA and 93% yield (96.4% recovery × 96.2% conversion) can be achieved by using 6: 1 molar ratio, 3% KOH catalyst concentration, 60 minutes of reaction time and 60°C of reaction temperature.
- V. Although, two- step procedure is time consuming and uses more resource, total time of biodiesel production can be reduced by using any heterogeneous catalyst or by some other suitable procedure. There are abundant scopes of researches to produce biodiesel from this algal oil in more conveniently, eco-friendly and economically cheaper procedure.

- VI. Though, it can be assumed that the biodiesel produced from this unused mixed indigenous algal culture, is a budget fuel, research works on economic analysis of the whole procedure for this production is very much essential.

5.4. ALGAL BIODIESEL PROPERTIES

- I. All of the properties of this algal FAME were within the limit of ASTM/BIS standards.
- II. The calorific value of this FAME (40882 kJ/kg) was higher than that of many other famous biodiesels. Therefore, this biodiesel is a more promising one than those biodiesels as the former would generate more power in engine.
- III. The flash and fire points of the algal FAME (153°C and 158°C) were also very high compared to petro- diesel which lessens the chance of fire hazard.
- IV. For its lower cloud and pour points (-1°C and -6°C respectively) it will be able to run an engine in cold atmospheric conditions too. Use of biodiesel additive is preferred if the temperature of the atmosphere gets further reduced.
- V. For its lower ash and carbon residue contents (0.01% and 0.03% respectively) less deposition would occur on engine parts and the life of the engine will increase as a result.
- VI. As the viscosity (3.12 mm²/s) and density (873 kg/m³) along with acid number (0.38 mg of KOH/g) of this FAME were very low and within the limit of ASTM/ BIS standards, least corrosion on engine parts would take place which will increase the life of engine. Although more research on modification of engine parts are required to run it by 100% algal biodiesel (B100).

5.5. EMISSION OF GASES

- I. While CO and HC emission for the combustion of algal FAME and its blends (algal FAME- petro- diesel) were found to decrease with escalating engine loads from 0% to 110%, emission of CO₂ and NO_x decreased with such increase of load of engine.
- II. CO, CO₂ and HC emission would reduce if petro- diesel is replaced by this algal FAME.
- III. Only the NO_x emission was found to be higher for this biodiesel which can be resolved by using biodiesel- additives, catalytic converter or de- NO_x catalyser in the engine.
- IV. Therefore, this algal biodiesel is a very eco- friendly option for generation of energy.

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