**ISLAMIC UNIVERSITY OF TECHNOLOGY (IUT)**

# MASS PRODUCTION OF MICROALGAE USING LOW COST PHOTOBIOREACTOR

**B.Sc. Engineering (Mechanical) THESIS**


**AUTHORS**

**MD. ASIF REZA**

Student ID: 151406

**MOHAIMIN RAHMAN**

Student ID: 151441

**SUPERVISED BY**

**DR. ARAFAT AHMED BHUIYAN**

**DEPARTMENT OF MECHANICAL AND PRODUCTION ENGINEERING**


**November 2019**

# CERTIFICATE OF RESEARCH

The thesis title **"MASS PRODUCTION OF MICROALGAE USING LOW COST PHOTOBIOREACTOR"** submitted by **MD ASIF REZA (151406)** and **MOHAIMIN RAHMAN (151441)** has been accepted as satisfactory in partial fulfillment of the requirement for the Degree of Bachelor of Science in Mechanical Engineering on November 2019.

Supervisor

______________________________

**DR. ARAFAT AHMED BHUIYAN**

Assistant Professor

Department of Mechanical and Production Engineering

Islamic University of Technology

# Declaration

We hereby declare that the work presented in this thesis has not been submitted for any other degree or professional qualification, and that it is the result of our own independent work.

**Signature of the Candidates**

| | |
|---|---|
| **MD ASIF REZA** | **MOHAIMIN RAHMAN** |

**Signature of the Supervisor**


**DR. ARAFAT AHMED BHUIYAN**

**Assistant Professor**

Department of Mechanical & Production Engineering (MPE)

Islamic University of Technology (IUT), OIC

Board Bazar, Gazipur

Dhaka, Bangladesh

# Table of contents

## List of Figures



## List of tables

# Acknowledgements

Foremost, we would like to express our heartfelt gratitude to Dr. Md. Ahiduzzaman sir, Associate Professor , Department of Agro-processing , BSMRAU for his continuous support, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped us during the time of research and writing of this thesis. We could not have imagined having a better advisor and mentor for our thesis.

Our sincere thanks go to our Supervisor, Dr. Arafat Ahmed Bhuiyan, Assistant Professor, Department of Mechanical and Production Engineering, IUT for giving us the opportunity to work under his supervision and guide us in the right direction with his knowledge and support. It has been an honour being part of his enthusiastic vision.

Last but not the least, we would like to thank everyone who has been indirectly involved in our study and helped us throughout the entire span of time

## Abstract

We are using fossil fuels as the main source of energy for a long time. As fossil fuels are harmful for its greater $C_2$ emission and are also a limited source of energy they can't be used as a main source of energy forever . We need to find an alternate source of energy that can be used instead of fossil fuels . That is why people are tending more towards a renewable source of energy which is environment friendly as well as easy to extract . Biofuel can be a perfect alternate in this case . There are various sources of biofuel such as microalgae, corn, biogas, vegetable oil, ethanol etc. Above all the sources of biofuel , the fuel that is produced from microalgae is the most useful one , as it is easy to produce and microalgae has the ability to mitigate $CO_2$ emission. They can be produced in waste water and they can also be produced in large scale in pond .So they have the potential for application in producing biofuels . For that purpose in this study the selection of preferable microalgae species has to be done at first .A low cost photobioreactor setup was created for the culture medium. Then providing appropriate culture medium in the photobioreactor a definite amount of microalgae biomass was produced .The rate of change of growth rate using different microalgae species is shown in this paper . Air and $CO_2$ were separately used to compare the growth rate of the microalgae species in each case .

# Chapter 1: Introduction

## 1.1 Overview of the Biofuel

### 1.1.1 Need for an Alternative Source of Enengy

The world population has experienced continuous growth since the last 50 years, which directly resulted in a large increase in primary energy consumption. In 2010, world primary energy consumption grew by 5.6%, which is the largest percentage growth in almost 40 years [1,2] Hence, the world is currently facing two detrimental challenges, which is energy crisis and environmental pollution. Energy crisis happened in the past decades due to the sub-stantial reduction of unsustainable sources like fossil fuels. Extensive use of fossils fuel for power generation and astrans-portation fuel has caused high carbondioxide (CO2) emissions to the atmosphere and there is an urgent need to reduce its emission to avoid detrimental impact of global warming. [3]. The rapid growth of human population and technological advancements have led to mounting energy demands, which is projected to increase by 50% or more by the year 2030 [4]. The natural petroleum can not compensate the current consumption rate which is already reported to be 105 times faster than nature can provide [5]. Moreover, the use of fossil fuels is devastating to the environment through greenhouse gas emissions and the consequent global warming [6,7]. Rittmann [8] described the danger of depending on fossil fuels from three point of view: depleting fossil-fuel reserves; dwindling resources, leading to geopolitical conflict; and climate change resulting from increasing atmospheric CO2 concentration. Therefore, the search for ‘clean’ energy has become one of most overwhelming challenges [9]. Following this, several alternative sources of energy including solar energy, hydroelectric, geothermal, wind, and biofuels are being studied and implemented. Of these potential sources of energy, biofuels are seen as real means of achieving the goal of replacing fossil fuels in short term [6]. High energy demand in future coupled with the concern of environmental hazards, national security have heightened attention towards production of clean liquid fuel, termed as biofuels, as a suitable alternative source of energy. The increase in fossil oil prices has caused more burden to consumer , businesses and investor as it generates high competi-tion against other countries which utilize biofuel as alternate energy .Therefore , these countries that used biofuels can prolong and maintain low inflation rate .Since fossil fuel is based on global pricing, therefore the country that used such fuel for transporta-tion, production, and all oil related products prices will fluctuate. Biofuel is a renewable energy source which provide an assurance to the economy of the country while promising to achieve a green environment. [3]

### 1.1.2 Sources of Biofuel

Biofuels are fuels that contain energy from geologically recent carbon fixation i.e. living organisms. Biofuels can be produced from starch, vegetable oils, animal fats, waste biomass, or algal biomasses, which are non-toxic, biodegradable and renewable [10]. Based on the feedstock types used and their current/future availability, biofuels are categorized into 1st, 2nd, 3rd and 4th generation biofuels [11]. They provide environmental benefits since their use leads to a decrease in the harmful emissions of CO2, hydrocarbons and particulate matter and to the elimination of SOx emissions, with consequent reduction of greenhouse effects. In fact, burning biofuels adds less carbon to the environment than burning fossil fuels as the carbon released by burning biofuel already existed as part of the modern carbon cycle [12]. Unfortunately, the present biofuel projections are based on feed stocks that are also food commodities [13] and resources suitable for conventional agriculture [14]. Satyanarayana et al. [15] have pointed out that substitution of diesel by biodiesel involves the use of lands used to produce food and the fiscal incentives by governments are decreasing the lands available for food production. To this effect, microalgae have attracted much global attention in recent years for the valuable natural products they generate, their ability to remediate effluents and their potential as energy crops [eg. 5]. Algal fuel (oilgae or third-generation biofuel) is a biofuel which is derived from algae. This is the right move for the production of biofuels as algae possess enormous potential (like low-input, high-yield prospect) for renewable energy applications [15–17]. Thus, this potential may enable to completely displace petroleum-derived transport fuels without the controversial argument “food for fuel”.

## 1.2 Microalgae Production

### 1.2.1 Microalgae

Microalgae are microscopic algae . They are typically found in freshwater and in marine systems . they are unicellular species which means they have only one cell .They can exist individually or in chains or even in groups . Their sizes vary from a few micrometers(μm) to a few hundred micrometers (μm) . They can perform photosynthesis .

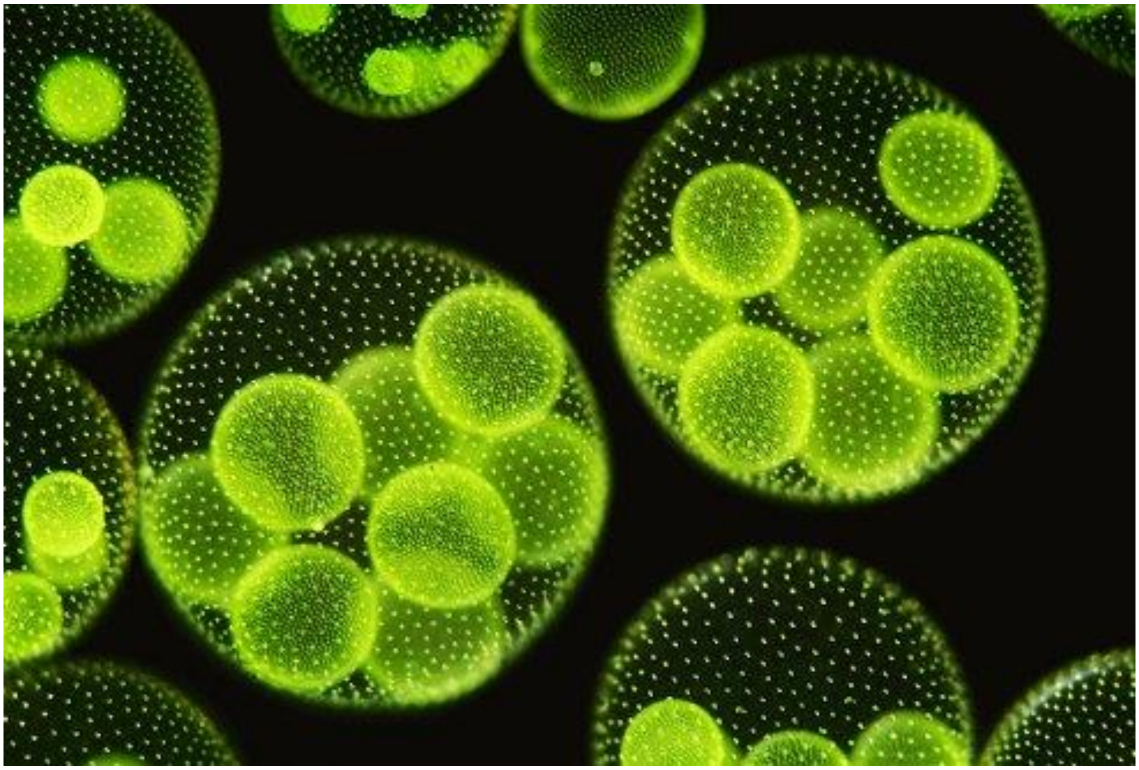

Figure 1. Microscopic structure of a microalgae .

Several features of algal physiology are relevant for evaluating their possible incorporation into renewable biofuel applications. these attributes can be summarized as: i) the solar energy yield with algae could be up to 6–12 times that of terrestrial plants as they are inherently more efficient solar energy converters (3–8% greater than terrestrial); ii) unlike terrestrial, the absence of intractable biopolymers eliminates the need for pretreatments to breakdown cellular products; iii) their metabolic and ecological diversity allows selection of taxa that are adapted for growth in locally available aquifers or have morphological features that allow cost-effective harvesting and iv) manipulating their end-products through the biosynthetic control of chemical composition by nutrient and environmental stresses.[6,16,17] . Based on the above criteria, many microalgal species have been identified as suitable feedstocks for biofuel production. Each species of microalga produces different ratios of lipids, carbohydrates, and proteins. Since the bulk of the natural oil produced by microalgae is in the form of triacylglycerols, which is the right kind of oil for producing biodiesel, microalgae are the exclusive focus in the algae-to-biofuel arena [18] .

### 1.2.2 Photobioreactor

Cultivation of microalgae seems quite easy as only simple nutrients need to be provided. Most research on microalgae cultivation has focused on growing algae in clean but expensive photobioreactors, or in open ponds, which are cheap to maintain but prone to contamination [9]. Therefore, currently two main microalgal cultivation systems are adopted: open ponds and photobioreactors [19].

Figure 2. Tube type photobioreactor .

Photobioreactors are such devices that utilizes light source to cultivate phototrophic microorganisms. Inside a photobioreactor specific conditions are carefully controlled for respective species.  Photobioreactors could be constructed in different shapes: flat-plate, tubular or pyramidal photobioreactor.They are made of glass or plastic tubes with central utilities installation with pumps, sensors, nutrients and $CO_2$. The Pyramid photobioreactor has advantage over the other design in that it is using fully controlled and automatic system that increases the production rate and it enables to grow any microalgae at any climate conditions [20].

## 1.2.3 Production of Microalgal Biomass

Human have been using microalgae, such as blue–green algae, as food sources since past thousands of years. Only in few decade ago, the actual microalgae cultivation has begun [21]. The first ever successfully uni algal cultured was Chlorella vulgaris by Beljerinck in 1980. In the early 1900's, Warburg have developed plant phy-siology study based on such culture [22]. Commercial microalgae cultivation in large scale was started in Japan using microalgae Chlorella species in the early1960's and followed by cultivation and harvesting of Athrospira species in Lake Texcoco by Sosa Tex-coco. There are numerous research studies on microalgae but not always resulted in commercial applications [21]. Microalgae cultivation systems are widely classified in to open or closed systems depending on their design conditions. In open systems, microalgae are cultivated in open area environment such as ponds, lagoons, deep channels, shallow circulating units and others. In closed systems, microalgae are cultivated in vessels with transparent wall and exposed under sunlight or artificial radiation to facilitate photosynthesis [23]. Phototrophic cultivation is the most common way of micro-algae cultivation [24]. The photosynthesis required two sources which are solar radiation and carbon sources. The light is needed for carbon fixation, and to increase the growth rate of microalgae and lastly resulted in high production of microalgae biomass. However, low intensity of light will hamper the photosynthesis

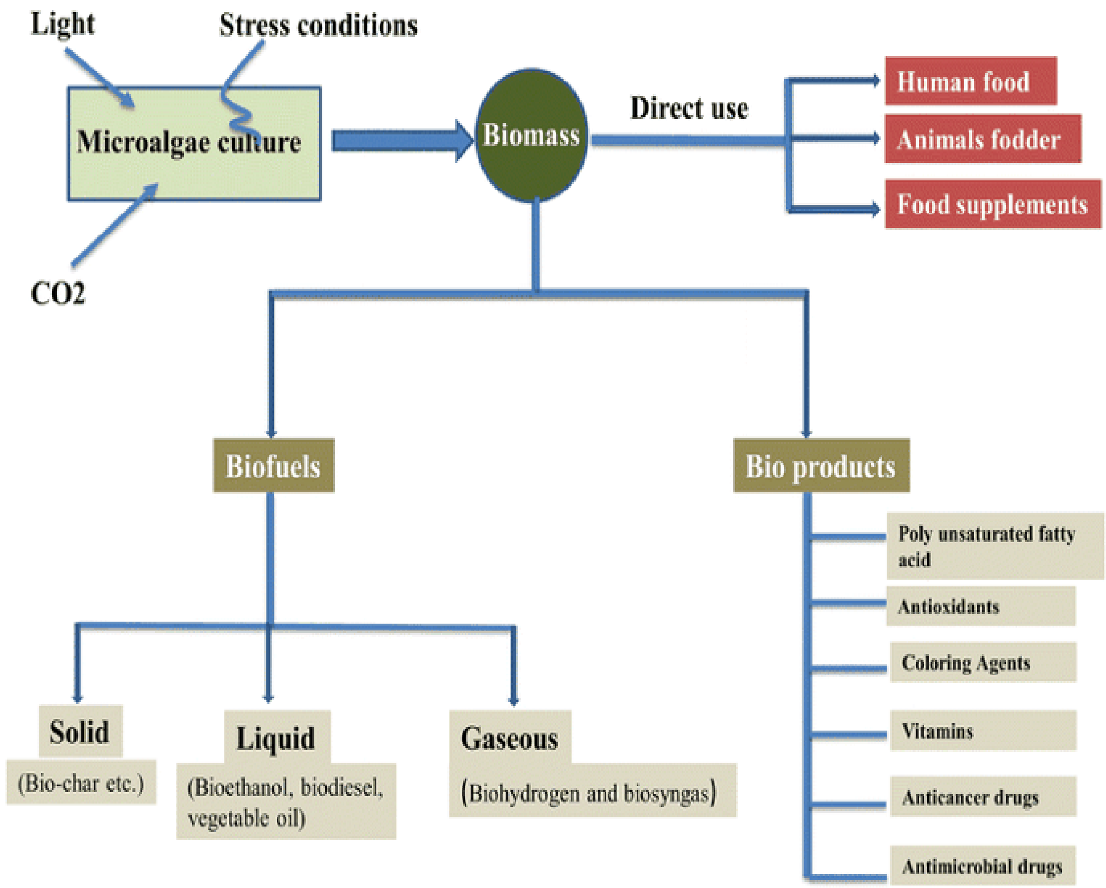


Figure 3. Different usage of microalgae biomass.

process. The carbon source is a fundamental need for the growth of microalgae [25–27]. Microalgal biomass production is usually more costly than growing crops. The cost can be minimized if microalgal biomass productions rely on freely available sunlight although there are daily and seasonal variations in light levels. Moreover, coupling with other uses, targeting high value products and using residual byproducts can boost production economics [28].

# Chapter 2 : Literature Review

Since the atmospheric pollution is rising alarmingly due to increase in atmospheric $CO_2$ concentration, resulting in the depletion of fossil fuel reserves causing hectic situations bringing fossil fuels to the wedge of depletion. Utilization of the algae biomass is not only advantageous for $CO_2$ fixation in the atmosphere but also marks for the production of biodiesel which is environmental friendly as well as also safe for human beings. Hence the biodiesel produced from the algal source plays an important role in maintaining the environment clean, can be used for transportation and will show less impact in the growth of biodiesel crops which have high impact in growing vegetable crops which can be used for human welfare [29] .

In the work of Eyasu Shumbulo Shubaa and Demeke Kifleb it is found that Microalgal biofuels are promising to replace fossil fuels in light of microalgae's intrinsic efficiency to convert solar energy into chemical energy, and their significantly higher potential yield of oils suitable for biofuel production than terrestrial crops. A number of achievements in genetic and metabolic engineering of algal strains to optimize their production costs have been recoded during the last few years. In addition, many technical aspects have been upgraded in the course of algal biomass production and biomass processing techniques to yield biofuels. Owing to the current growing concern over fossil fuels, energy security, greenhouse gas emissions, and the potential of other biofuel feedstocks to compete for limited agricultural resources, there is mounting worldwide interest in microalgal biofuels.  The availability of plenty of sunshine and favorably high temperature in the tropics, may favor the production of hydrogen in a low-cost system in a closed photobioreactor. This would be a big opportunity especially for landlocked countries like Ethiopia, which are yet without petroleum-based oil, where there are “thirteen months of sunshine”, to boost the economy [30] .

Muhammad Rizwan and Ghulam Mujtaba said in their work that large scale applications of microalgae can not be deemed unless improving their biomass yield or coupling with other technologies. Microalgae offer interesting features to qualify them as alternative feedstocks for various bio-refinery applications. Microalgae can be used as feed for animal and aquaculture, fertilizers, medicine, cosmetic products. A consolidated bio-refinery approach may be adopted to improve the utility of microalgae biomass [31] .

In the work of Lin-Lan Zhuanga and Dawei Yu it has been found that , comparing with the suspended microalgae cultivation, attached microalgae cultivation has advantages on easily biomass harvest, less space and water consumption, higher microalgae biomass productivity and better water purification performance, among others, making this culture pattern more potential for scale-up biomass/bioenergy production and wastewater treatment. the accumulation of the attached microalgae biomass showed a linear increase and then reached the plateau. The productivity showed a huge difference among reactors, which could be up to 20.7 $g \cdot m^{-2} d^{-1}$ [32] .

In the paper of Jassinnee Milano and Hwai Chyuan Ong it is showed that biofuels from microalgae biomass have the potential to replace fossil fuel for power generation while mitigating $CO_2$ emission from atmosphere. Chemical compositions of microalgae fuels are similar to petrol and diesel thus can be used to replace fossil fuels for transportation. In addition, microalgae have high growth rates and lipid content which make them a feasible choice for $CO_2$ fixation and biodiesel production. microalgae have high potential to produce biofuels and replace fossil in power generation, challenges to commercialize the production at large scale has been addressed . The challenges are such as : (a) the demand for biofuels is not high in viewing low market price of fossil fuel; (b) the cost for the biofuels production from microalgae is high and (c) the energy ratio of above unity needs to be achieved.Some challenges such as Low cost with high efficient and low contamination harvesting techniques and extracting oil from dried microalgae has been addressed . These problem can be solved by introducing natural flocculants, which is biodegradable with lower cost than mineral flocculants. Even though the microalgae are recognized of high lipid contents, but oil extraction is not as anticipated. Thus, biofuels production from microalgae biomass is considered expensive and the returns of investment are quiet slow and low. However, with the improvement of advanced technologies and possible government incentives it is hoped that microalgae biofuels will soon become economic feasible and comparable to fossil fuels in term of cost of production [33] .

El-Sayed Salama and Mayur B. Kurade showed in their paper that Microalgal biomass as a feedstock is advantageous over terrestrial plant biomass and provides great potential for the sustainable production of biofuels. The challenges to the direct use of wastewaters for microalgae cultivation should be considered because they limit the exploitation of the readily available and cheap wastewater growth medium. Currently, the most relevant approaches consider sterilized wastewater for microalgal cultivation.Isolation or genetic modification of microalgal species with superior performance is essential to maintaining stable biomass production even in the presence of the biotic inhibitory factors in wastewater at large scale. In the last five years, industries have made development in the area of coupling wastewater and microalga cultivation using open and closed systems, providing groundbreaking improvements in the low-cost production of algal biomass. A new approach involves mixing different wastewaters to attain the optimum N:P ratio for better biomass production [34] .

F. Iasimone and A. Panico showed in their research that the light intensity and nutrients content in the growth medium affect the microalgal production efficiency and lipids accumulation in batch cultivation systems fed with urban wastewater. The highest light intensity and the lowest nutrients concentration in the growth medium produced the most performing and promising activities. From an in-depth analysis of experimental activities, it was found that pH in the growth medium is the parameter that drives, most than any others, the microalgal cultivation chain for the production of biomass used as biofuel source. Besides, in batch condition, pH varies progressively during cultivation time due to inorganic carbon absorption, showing an increasing trend during microalgae exponential growth phase. High pH values are positive because they induce the auto flocculation phenomenon in microalgae and consequently promote and favour their settling without using chemical flocculants. On the other hand, high pH values inhibits the photosynthetic process, causing nitrite accumulation and consequently death of microalgae. The use of wastewater to feed batch microalgal cultivation systems is feasible and profitable since wastewater is a zero cost substrate and microalgae can conveniently grow using wastewater as source of nutrients, especially under high light intensities. Auto flocculation process at high pH values makes economically sustainable the biomass harvesting, especially if it is conducted at the time of the exponential growth phase, avoiding cells lysis processes [35] .

From the study of Kit Wayne Chew and Shir Reen Chia we get to know that Microalgae can produce a greater oil yield per hectare of land than compared to existing field crops, which emphasizes the high potential of microal- gae to be used at the industrial scale. It is important to choose the right microalgae strains for optimal lipid accumulation. Microalgae strains like *Chlorella* sp. and *Nannochloropsis* sp. contain high lipid content but cannot produce top-grade biodiesel yet. Fresh water is most commonly used, but nutrients need to be added for microalgae growth that results in higher cost. Salt water and wastewater provide cultivation conditions for certain species of microalgae only, where not all microalgae will survive under these conditions. It can be seen that closed systems can be tuned to operate more efficiently but open systems remain more popular due to the lower cost and high productivity. The main issue with open systems is the systems being harder to control to obtain the optimum productivity. Meanwhile, it is reported that heterotrophic and photoautotrophic cultivations are more commonly used in industry, but both have their own advantages and drawbacks. Heterotrophic cultivation grows microalgae with higher lipid content but the microalgae get contaminated easily especially in open cultivation systems, while photoautotrophic cultivation is environmental friendly but the lipid content produced is low. Research on this is still on-going and trade-offs between cultivation methods need to be considered. Microalgae have great potential to produce a variety of products and can be used in wastewater remediation as well. The possible prospects of microalgae cultivation will en- courage more researches to be done by creating a drive to further enhance this technology into a new era of food and fuels [36] .

M. Motijur, M.G. Rasul , N.M. S Hassan and M. N. Nabi mentioned in their paper that microalgae represent the third generation feedstock for biodiesel, with much higher yields than other crops. In recent year's biodiesel production from microalgae have received much attention worldwide. Finding the most efficient method and optimized conditions for the extraction of lipid are important for reducing the cost of biodiesel production from microalgae. Making biodiesel from microalgae oil is similar to the process of making biodiesel oil from any other oilseed, and thus can quite possibly use the same conversion processes to produce biodiesel. The use of biodiesel in diesel engine reduces the engine power slightly but also reduces the engine emission except the NOx emission. The engine output response varies with the operation condition. Research on microalgae combustion in internal combustion engine should be done to improve the engine performance and lower the emission [37] .

Muhammad Imran Khan, Jin Hyuk Shin and Jong Deog Kim Showed that microalgae can be used in bioenergy, nutraceutical and pharmaceutical industry and the associated challenges and limitations and how it can be overcome to make them feasible and viable for commercialization [38] .

Srikanth Reddy Medipally suggested that Microalgae have the potential to be important and sustainable renewable energy feedstock that could meet the global demand. In spite of the many advantages, microalgae biofuels also have some disadvantages such as low biomass production and small cell size that makes the harvesting process costly. These limitations could be overcome by designing advanced photobioreactors and developing low cost technologies for biomass harvesting, drying and oil extraction. In addition, application of genetic engineering technology in the manipulation of microalgae metabolic pathways is also an efficient strategy to improve biomass and biofuel production. Genetic engineering technology also plays an important role in the production of valuable products with minimal costs. Biotic interaction with bacterial biofilms is also an important aspect in microalgae biomass and biofuel production. However these technologies are still in the early stages and most have not been applied on a commercial scale. Therefore, further research in the development of novel upstream and downstream technologies will benefit the commercial production of biofuels from microalgae [39] .

Despite of various benefits associated with the production of microalgae biodiesel, economic feasibility for large scale-scale production is yet to be realized as there are a number of limitations blocking its real competition with fossil diesel. A substantial improvement and development is urgently needed to ensure economic feasibility for large-scale production of biodiesel derived from microalgae [40] .

# Chapter 3 : Methodology

## 3.1 Species Selection

At first we have to select specific species for the whole production process.The selected species should have some specific characteristics . Such that it has to be easily found in nature. It must have high growth rate. Lipid percentage in it must be high . In this case following the above mentioned characteristics we selected two species . They are *c. vulgaris* and *c. sorokiniana* .

Figure 4. *c. vulgaris* .

Figure 5. *c. sorokiniana*

Both of the species are found in natural pond water. *c. vulgaris* is light green in colour and *c. sorokiniana* is olive in colour . 600 ml of each species is collocted for the production purpose . The wet mass of the species was collected . The collected species were then preserved in a contamination free environment.

## 3.2 Creating Culture Medium

Culture medium is the mix of the tris buffer solution and trace metal solution . It is the medium where the seeds are placed in nutrients for the rapid growth of the microalgae seeds . Tris buffer solution and trace metal solution are the nutrients of the seeds. In this case 10 trace metals were used for the culture medium .At first a beaker/flask is selected for the culture medium. Separate solutions of the 10 trace metals were made in 10 separate flasks at first . The culture medium varies in size . Depending on the production it varies from a small beaker to a large drum . For commercial production large ponds are used as the culture medium . Then from there 2 ml from each of the solutions were taken in a pipet and then those 2ml solutions were poured into the beaker that were selected as the culture medium . After that tris buffer solution were made taking definite amount of each chemicals and mixing it in one jar .

| **Tris Buffer Solution** | | **Trace Metal Solution** | |
|---|---|---|---|
| **Chemicals** | **Amount (mg/L)** | **Chemicals** | **Amount (mg/L)** |
| 1.Tris (hydroxymethyl) aminomethane | 2,420 | **1.** $Na_2EDTA$ | 50 |
| 2. $NH_4Cl$ | 400 | 2.$ZnSO_4 \cdot 7H_2O$ | 22 |
| 3. $K_2HPO_4$ | 108 | 3. $H_3BO_3$ | 11.4 |
| 4. $MgSO_4 \cdot 7H_2O$ | 100 | 4. KOH | 16 |
| 5. $KH_2PO_4$ | 56 | 5.$MnCl_2 . 4H_2O$ | 5.06 |
| 6. $CaCl_2 . 2H_2O$ | 50 | 6.$FeSO_4 \cdot 7H_2O$ | 4.99 |
| 7.Glacial Acetic Acid | 1 ml | 7. $CoCl_2 . 6H_2O$ | 1.61 |
| | | 8.$CuSO_4 \cdot 5H_2O$ | 1.57 |
| | | 9.$(NH_4)6Mo_7O_{24} . 4H_2O$ | 1.10 |
| | | 10. $CaCl_2 . 2H_2O$ | 0.05 |

Table 1. Chemical List of the culture medium .

In the tris buffer solution special care should be taken while mixing the Glacial Acetic Acid . Because it is very harmful for skin and also it smells bad . The mixture then needed to be stirred for a while until all the chemicals mix together . In case of a small beaker stirring can be done by shaking the beaker in hand . When it is mixed properly then it is ready to mix with the trace metal solution in the culture medium .

Figure 6. Culture medium.

Next the tris buffer solution was mixed with the trace metal solution in the culture medium . Now the culture medium is ready with the proper nutrients. Finally the collected seeds were poured into the culture medium at a rate of 1ml/L .

## 3.3 Providing Anti-contaminant

Contamination is one of the basic problems in microalgae production . It happens during the production in the culture medium and ruins the whole batch of production . Bacterial attack is the main source of contamination . It can happen at the day of production or it can happen gradually . If any white or red layer of bacteria can be seen at the top of the culture medium then we have to understand that the culture medium has been compromised . If heavy bacterial attack happens then it will be clearly visible from outside otherwise it might not . In that case there is another way to identify . The culture medium tends  to turn greener from the day of production. If no change is visible within few days then we have to understand it has been attacked by bacteria .

Figure 7. Bactrial attack

As we can see in the figure there are a white layer at the top of the culture medium and another red layer immediate after it . This has been formed because of bacterial attack . To get rid of this problem we have to use anti-contaminant . In this case we used anti bacterial such as penicillin and amoxicillin . Penicillin was used at a rate of 500 mg/L and Amoxicillin was used at a rate of 250 mg/L . These two anti bacterial was mixed in a separate jar and then that was mixed in the culture medium . Now the culture medium is contaminant proof .

## 3.4 Setting up the Lowcost Photobioreactor

After creating the culture medium and mixing the anti bacterial in the medium it has to place inside a photobioreactor setup . Because for the rapid growth of the seeds in the culture medium it needs enough light and air . Natural sunlight can not be a option in this case as we can’t get it whole twenty four hours . So an artificial set up has to be introduced . Because of the high costing of the photobioreactors available in the market we had to go for a photobioreactor of our own . That we have named ‘Lowcost Photobioreactor’ . This setup can be made with a small amount of money . For this setup we need

1. Flood light of 100 W .
2. Air pump .
3. Carbondioxide cylinder .
4. Plastic box .
5. Rubber pipes .

We have to connect one end of the rubber pipes with the air pump . Another end of the pipe will be inside the culture medium . The air pumps have to be put inside the plastic box . The flood light has to be placed in front of the culture medium . After placing the culture medium in the right place we have to pump carbon dioxide from the cylinder

Figure 8. Lowcost Photobioreactor setup

to the plastic box . The air pump inside it will pump the $CO_2$ into the culture medium through the rubber pipes . The $CO_2$ from the cylinder has to be pumped a few times after each 3/4 hours . The process has to be continued for the next two weeks. In between this time the growth of microalgae will reach in its peak . After this time the wet mass can be collected from the culture medium.

## 3.5 At The Day of Production


Figure 9. At the day of production

In this figure we can see the situation at the day of production . The colour of the culture medium is almost white . This is the scenario immediate after mixing every element in the culture medium . The photobioreactor setup can also be seen in this figure . We used aluminium foil as the lid of the culture medium to make it a bit more contamination proof .

## 3.6 Two Weeks After Production

Figure 10. Two weeks after production

In this figure it can be seen that the colour of the four culture mediums have become dark green .This means we have got a very good production from this batch . The whole culture medium has become full with microalgae . From here we will have to get rid of the unwanted water and retrieve the wet mass .

## 3.7 Retrieving the Wet Mass

To collect the wet mass from the culture medium we have to get rid of the unwanted water at first . For that purpose we have to use a flocculant . Flocculants are such chemicals that helps the clumping of particles in water . Aluminium Chloride was used as the flocculant . It helps to create a sediment of the wet mass at the bottom of the culture medium .

Figure 11. Sediment of the wet mass

The floculant Aluminium Chloride was used at a rate of .5 gm/L .In the next step using syphon the water from the top is removed until only the wet mass is left. Then the wet mass is collected from the medium to find the dry mass.

## 3.8 Producing Biomass/ Dry Mass from the Wet Mass

After collecting the wet mass it has to be dried to get the desired dry mass or biomass of the microalgae . The drying process of the wet mass is done in the oven . The collected wet mass has to be put in an oven in (70-80) °C temperature for one day . And then from there we can get the dry mass .

Figure 12. The desired biomass

From mass production huge amount of drymass can be get and from there the oil processing activities begins .

# Chapter 4 . Result Analysis

The mass production of microalgae was done several times during the experiment . From the earlier part it was done in a smaller scale in 2L jars . Some of the earlier cases the production was ruined because of the bacterial attack . But as we progressed, we were able to find out the specific amount of anti bacterial that was needed and after that the contamination problem was solved . Then we started to increase our production. We went from producing to 2 litre jars to produce in 100 litre drums during the experiment .The results we got from those smaller scale production and those larger scale production were same . We got drymass at a rate of 50 gm from 100 litre production . And later to produce oil from that we can get 30 ml oil from 200 gm of microalgae dry mass .

At first we used $CO_2$ cylinders to pump $CO_2$ to the culture medium for the production purpose . Then we put the microalgae species *c. vulgaris* in two culture medium simultaneously . In one medium we used air pump to pump $CO_2$ from the cylinder to the culture medium . In another aim pump we pumpes natural air to the culture medium . The wavelength data of each of the culture medium was collected simultaneously each day for fourteen days . The result that we got was quite surprising . The growth rate of the microalgae in the culture medium that has got natural air supply was far more than the one that got $CO_2$ supply . We got the same result when we did the experiment for the microalgae species *c. sorokiniana* . From there we can come to the conclusion that natural air supply is enough for the growth rate of microalgae in the culture medium , we don't need to supply addition $CO_2$ from outside .

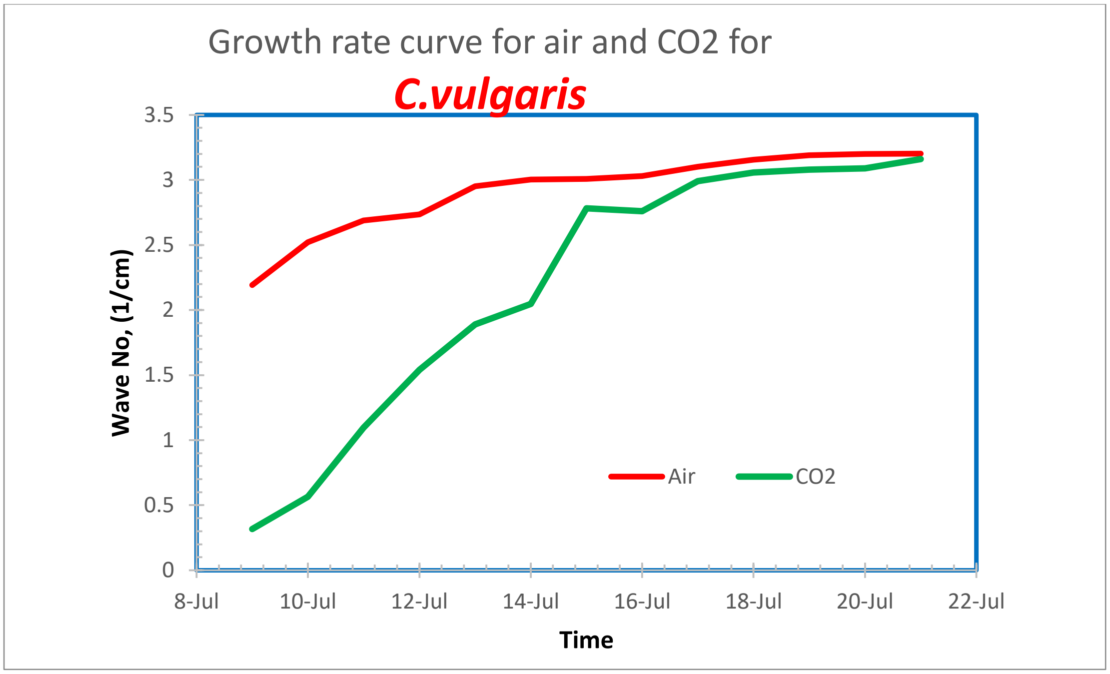


Figure 13. Growth rate curve for air and $CO_2$ for *c. vulgaris*

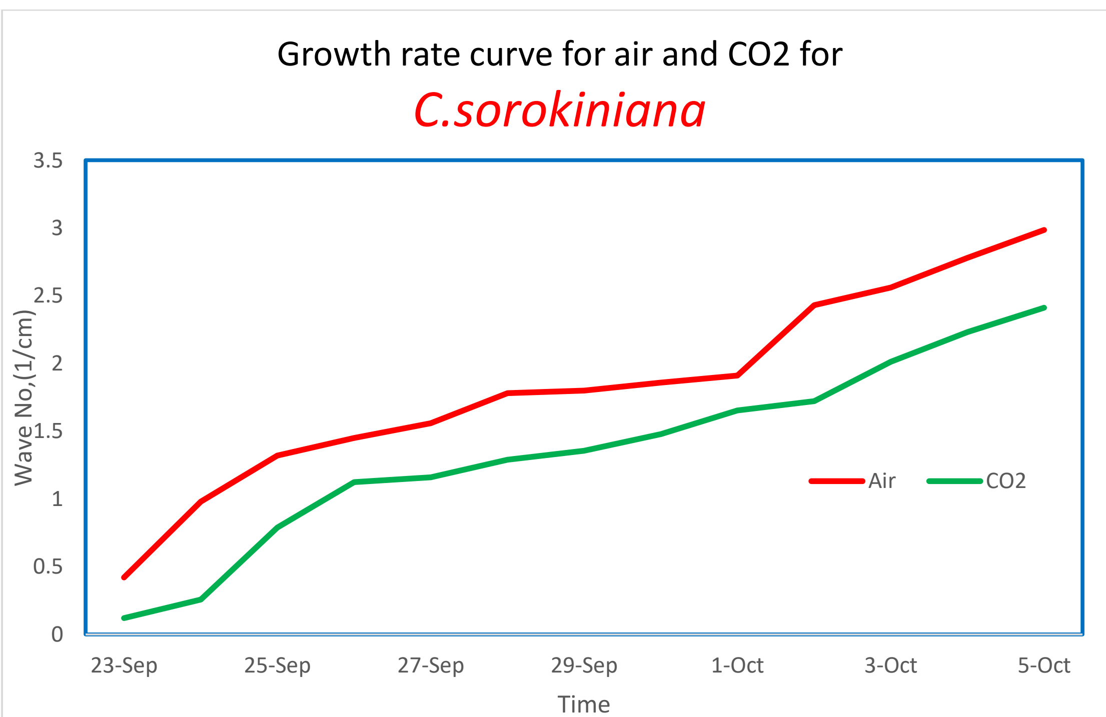


Figure 14. Growth rate curve for air and $CO_2$ for *c. sorokiniana* .

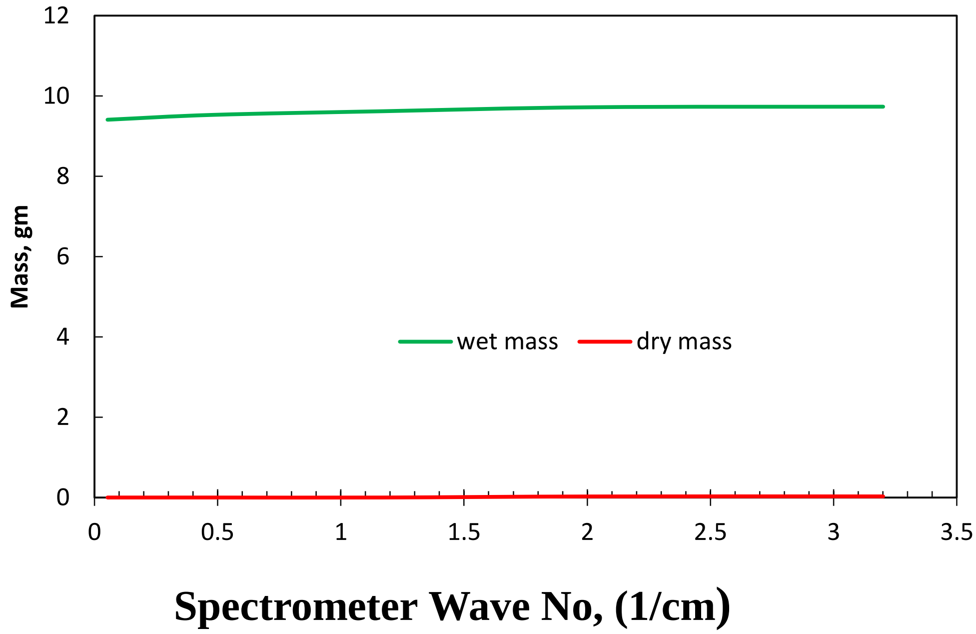


Figure 15 . Spectrometer Wave Number vs wet and dry mass for air

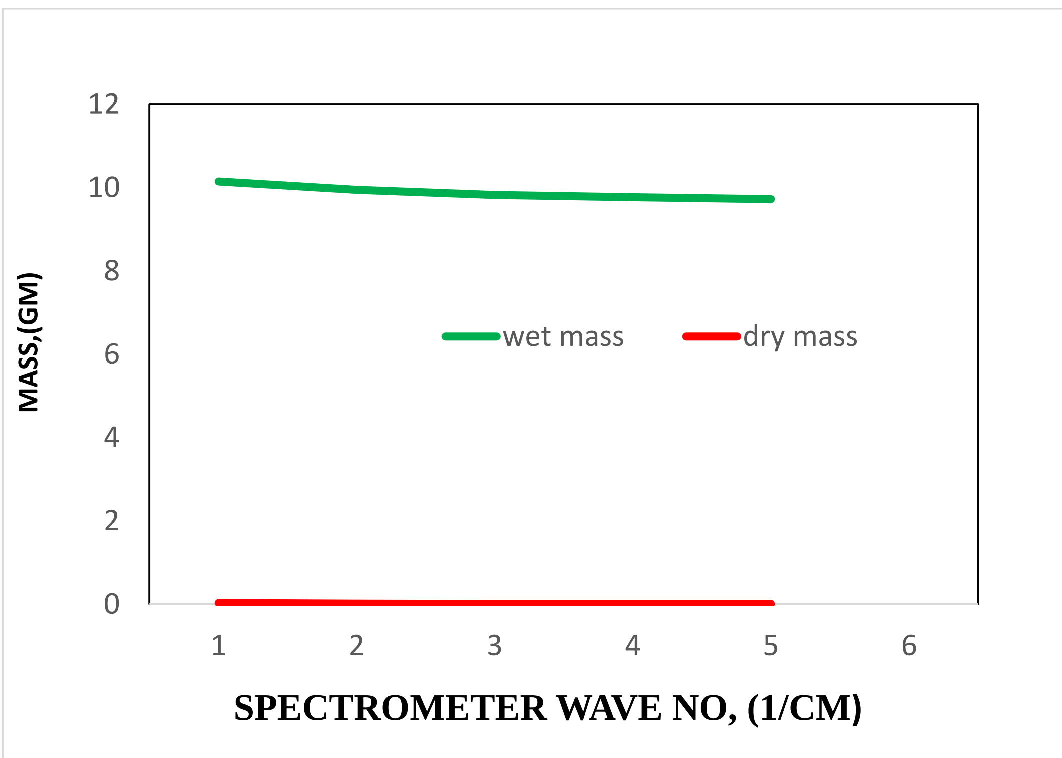


Figure 16. Spectrometer Wave Number vs wet and dry mass for $CO_2$

Here figure 13 and 14 we can see the growth rate curve of *c. vulgaris and c. sorokiniana* . for air and $CO_2$ . And in both the cases the growth rate in the presence of air is more than the growth rate in the presence of $CO_2$ . In the next two figures , fugure 15 and 16 we can see the Spectrometer wave number vs wet and dray mass of air and $CO_2$ simultaneously .

# Chapter 5. Conclusion

In our study we found out an effective and innovative production process for the production of microalgae . In the beginning  we faced some problems such as contamination mainly because of bacterial attack . But we were able to solve that problem by using anti contaminant as Penicillin and Amoxicillin at a specific amount . We also faced problem while selecting a photobioreactor . Then we made a lowcost photobioreactor  to solve the problem . While doing experiment we also found out that we don't need to provide extra $CO_2$ to the culture medium . as the rate of production in the natural air was greater than the rate of production in the $CO_2$ mixed air . We have found out how to get the wet mass from the culture medium  using flocculant . We tried flocculant at different rates to try to find out the exact amount we needed for sedimentation . From this experiment the amount of biomass that we got was better than other experimental studies but still not a great amount comparing to the amount of production we were doing . As we got biomass at a rate of 50 gm from 100 litre production . But this problem can be solved if it can be done commencially in pond system . In that case the amount of biomass we can get will be enough to produce good amount of oil  that we can use instead of fossil fuels . . If it can be done in pond system then we will need light only at night because of the availability of sunlight at daytime .This will reduce the cost to a great extent too.

## Future Scopes

To secure future fuel supplies (66% of global energy), biofuels represent almost the only viable option. Micro-algal biofuel systems have strengths in terms of delivering clean and sustainably produced fuels for the future while eliminating the food versus fuel and forest versus fuel concerns associated with first generation biofuels and lignocellulosic processes based on wood feedstocks. Microalgal biodiesel is technically feasible. It is the only renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum. Economics of producing microalgal biodiesel need to improve substantially to make it competitive with petrodiesel This conclusion is supported by detailed economic feasibility studies that demonstrate that microalgal production systems have considerable economic potential [41], not only for the production of fuels but also for food supplements (for a rapidly increasing population).Producing low-cost microalgal biodiesel requires primarily improvements to algal biology through genetic and metabolic engineering. Use of the biorefinery concept and advances in photobioreactor engineering will further lower the cost of production. Microalgal production system are therefore well placed to offer significant potential for near-term community benefit that will assist the maturation of a stand-alone industry over the next decade.

# References


[1] Jones CS,Mayfield SP. Algae biofuels : versatility for the future of bioenergy. CurrOpin Biotechnol2012;23(3):346–51.

[2] Alam , Date , Rasjidin ,Mobin , Moria ,Baqui . Biofuel from algae – is it a viable alternative? ProcediaEng2012;49:221–7.

[3] Gupta , Tuohy . Biofuel technologies: recent developments. Berlin Hei- delberg: Springer; 2013.

[4] Maness , Yu , Eckert , Ghirardi . Photobiological hydrogen production: efforts to scale up the capacity of green algae and cyanobacteria to use sunlight to convert water into hydrogen gas for energy use. Microbe 2009;4(6):275–80.

[5] Netravali , Chabba . Composites get greener. Mater Today 2003;6(4):22–9.

[6] Chisti . Biodiesel from microalgae. Biotechnol Adv 2007;25(3):294–306.

[7] Medipally , Yusoff , Banerjee , Shariff . Microalgae as sustainable renewable energy feedstock for biofuel production. BioMed Res Int 2015, [519-513].

[8] Rittmann BE. Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioe ng 2008;100(2):203–2012.

[9] Mata TM, Martins A, Caetano N. Microalgae for biodiesel

[10] Song , Fu J Shi . Exploitation of oil-bearing microalgae for biodiesel. Chin J Biotechnol 2008;24(3):341348.

[11] UNEP. Towards sustainable production and use of resources: Assessing biofuels 2009.

[12] Popp , Lakner , Harangi-Rákos , Fári . The effect of bioenergy expansion: food, energy and environment. Renew Sustain Energy Rev 2014;32:559–78.

[13] REN21. Renewable energy policy network for the 21st C. Global Status Report. Paris; 2009.⟨http://www.ren21.net/Portals/0/documents/activities/gsr/RE_GSR_2009_Update.pdf⟩.

[14] Hoogeveen , Faures , Giessen . Increased biofuel production in the coming decade: to what extent will it affect global freshwater resources?. Irrig Drain 2009;58:148–60.

[15] Satyanarayana , Mariano , Vargas J. Microalgae, a versatile source for sustainable energy and materials. Int J Energy Res 2011;35:291–311.

[16] Hu , Sommerfeld , Jarvis , Ghirardi , Posewitz , Seibert , Darzins . Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 2008;54:621–39.

[17] Dismukes , Carrieri , Bennette , Ananyev , Posewitz . Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 2008;19:235–40.

[18] Wen . Microalgae as a feedstock for biofuel production. Virginia Cooperative Extension publication; 2009. p. 442–886 ⟨http://pubs.ext.vt.edu/442-886⟩.

[19]Cazzola . Algae for biofuels production Process description, life cycle assessment and some information on cost. OECD/IEA; 2010.

[20]Saranya , Prabavathi , Sudha , Selvakumar , Sivakumar . Perspectives and advances of microalgae as feedstock for biodiesel production. Int J Curr Biol Appl Sci 2015;4(9):766–75.

[21]Spolaore , Joannis-Cassan, Duran, Isambert. Commercial applications of microalgae. J BiosciBioeng2006;101(2):87–96.

[22]Borowitzka . Commercial production of microalgae: ponds,tanks,tubes and fermenters .JBiotechnol1999;70(1–3):313–21.

[23]Razzak SA,Hossain ,Lucky ,Bassi , deLasa. IntegratedCO2 capture, waste water treatment and biofuel production by microalgae culturing —a review. RenewSustainEnergyRev2013;27:622–53.

[24]Pruvost , Cornet ,Le Borgne, Goetz, Legrand. Theoretical investigation of microalgae culture in the light changing conditions of solar photobioreactor production and comparison with cyanobacteria .Algal Res-Biomass Bio- fuels Bioprod2015;10:87–9

[25] Rama raj , Tsai ,Chen . Carbon dioxide fixation of fresh water micro- algae growth on natural water medium. Ecol Eng2015;75:86–92.

[26] Singh , Yadav , Deeps hildia, Singh .Biofixation of carbon dioxide using mixed culture of microalgae. Indian J Biotechnol2015;14(2):228–32.

[27] Suali , Sarbatly . Conversion of microalgae to biofuel. Renew Sustain Energy Rev 2012;16(6):4316–42.

[28]Vandna , Ravindra , Pankaj , Kumar . Microalgae as emerging source of

energy: a review. Res J Chem Sci 2015;5(3):63–8

[29] Manoj Kumar Enamalaa, Swapnika Enamalab, Murthy Chavalic . Production of biofuels from microalgae - A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renewable and Sustainable Energy Reviews 94 (2018) 49–68.

[30] Eyasu Shumbulo Shubaa, Demeke Kifle . Microalgae to biofuels: ‘Promising’ alternative and renewable energy, review . Renewable and Sustainable Energy Reviews 81 (2018) 743–755

[31] Muhammad Rizwan, Ghulam Mujtaba, Sheraz Ahmed Memon . Exploring the potential of microalgae for new biotechnology applications and beyond: A review. Renewable and Sustainable Energy Reviews 92 (2018) 394–404

[32] Lin-Lan Zhuanga , Dawei Yu , Jian Zhang . The characteristics and influencing factors of the attached microalgae cultivation: A review . Renewable and Sustainable Energy Reviews 94 (2018) 1110–1119.

[33] Jassinnee Milano , Hwai Chyuan Ong , H.H.Masjuki , W.T.Chong. Microalgae biofuels as an alternative to fossil fuel for power generation . Renewable and Sustainable Energy reviews 58(2016)180–197.

[34] El-Sayed Salama, Mayur B. Kurade, Reda A.I. Abou-Shanab . Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renewable and Sustainable Energy Reviews 79 (2017) 1189–1211

[35] F. Iasimone, Panico, De Felice . Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics. Journal of Environmental Management 223 (2018) 1078–1085

[36] Kit Wayne Chew , Shir Reen Chia , Pau Loke Show , Yee Jiun Yap. Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review . Journal of the Taiwan Institute of Chemical Engineers 91 (2018) 332–344

[37] M. Motijur, M.G. Rasul , N.M. S Hassan , M. N. Nabi. Recent Development in the Production of Third Generation Biodiesel from Microalgae . Energy Procedia 156(2019) 53-58 .

[38] Muhammad Imran Khan, Jin Hyuk Shin and Jong Deog Kim. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Khan *et al. Microb Cell Fact (2018) 17:36*

[39] Srikanth Reddy Medipally, Fatimah Md. Yusoff, Sanjoy Banerjee, and M. Shariff . Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production . BioMed Research International Volume 2015, Article ID 519513.

[40] Wan Nadiah Amalina Kadira , Man Kee Lama , Yoshimitsu Uemura , Jun Wei Limb ,

Keat Teong Lee . Harvesting and pre-treatment of microalgae cultivated in wastewater for

biodiesel production: A review. Energy Conversion and Management 171 (2018) 1416–1429

[41] Stephens. et al. (2010) An economic and technical evaluation of

microalgal biofuels. Nat. Biotechnol. 28, 126–128