

**Algae as a bioremedy for wastewater treatment and stock of natural oil**

**Submitted in partial fulfillment of the requirements**

**of the degree of**

**MASTER OF TECHNOLOGY**

**in**

**CIVIL ENGINEERING**

**by**

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*Transforming Education Transforming India*

**School of Civil Engineering**

**LOVELY PROFESSIONAL UNIVERSITY, PHAGWARA**

**2017**

## DECLARATION

I, Arjun Sareen (11502413), hereby declare that this thesis report entitled “**Algae as a bioremediation for wastewater treatment and stock of natural oil**” submitted in the partial fulfilment of the requirements for the award of degree of Master of Civil Engineering, in the School of Civil Engineering, Lovely Professional University, Phagwara, is my own work. This matter embodied in this report has not been submitted in part or full to any other university or institute for the award of any degree.

**Date:**

**Place:**

## **CERTIFICATE**

Certified that this project report entitled “Algae as bioremediation for wastewater treatment and stock of natural oil” submitted individually by student of School of Civil Engineering, Lovely Professional University, Phagwara, carried out the work under my supervision for the Award of Degree. This report has not been submitted to any other university or institution for the award of any degree.

### **Signature of Supervisor**

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## **ACKNOWLEDGEMENT**

This study is important for the sustainability of the fossil fuel as we are going to replace it with bio-fuel by using algae plant cultivated in wastewater and will check the properties of the generated bio-fuel. I would like thank my mentor Mr. Suhas srivastava for helping me in the journey of my project. My parents helped me a lot with their guidance and financially too. My friend circle gave their precious time and support in fulfilment of this project. In the end, I am feeling myself very lucky to have magnificent influence of all people.

**Signature of Student**

**Arjun Sareen**

## **ABSTRACT**

Algae are the most abundance species in this planet earth. So to make its potential use a study was conducted to evaluate its potential of nutrient stripping and production of natural oil for the production of bio-fuel. Native algal strain were isolated from the dairy farm and then cultured in the same dairy farm wastewater in an open pond system. The main aim was to reduce the concentration of nutrients (N &P) up to the permissible limits defined by CPCB. So this strain was capable to remove more than 70% of TKN and about 94% of P. It took HRT of 10 days to lower down the concentration of nutrients up to the permissible limits as per by the CPCB for the disposal in inland surface water. Moreover result for the biomass production was also good as it produced about 850g of biomass from 10L of sample and about 17% of the natural oil content. so according to the data analysis, dairy farm can produce about 11,900 L of natural oil and can be further use for the production of bio-fuel.

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## **LIST OF ABBREVIATIONS**

<b>ABBREVIATION</b>	<b>FULLFORM</b>
CPCB	Central pollution control board
GHG	Green house gases
MM	Millimetre
CM	Centimetre
M	Metre
L	Litre
SEC	Second
GTOE	Giga tonnes of oil equivalent
MLPD	Million litres per day
KLPD	Thousand litres per day
BOD	Biochemical oxygen demand
TKN	Total kjeldahl nitrogen
P	Phosphorus
%	Percentage

# CHAPTER 1

## INTRODUCTION

Our earth has lots of non-renewable energy sources and we are using lots of them blindly. So that are depleting with the passage of time. Here we are talking about one of the most needed non-renewable source Fossil fuel. Every sector/system depends upon this energy source. From a small kitchen to big industry, developing to developed country, they need fossil fuel for their running purpose. But the resources are very limited so they depleting day by day. To meet everybody's fuel demand, we need to relocate them or we need their replacement. The table 1.1 is showing the different fuels and their demand year by year.

**Table 1.1** worldwide energy demand (Gtoe)

Energy sources	1990	2020
Coal	2.31	4.82
Oil	2.83	4.61
Natural gas	1.73	3.54
Nuclear	0.411	1.012
Hydro	0.521	1.24
Renewable	0.2011	0.802

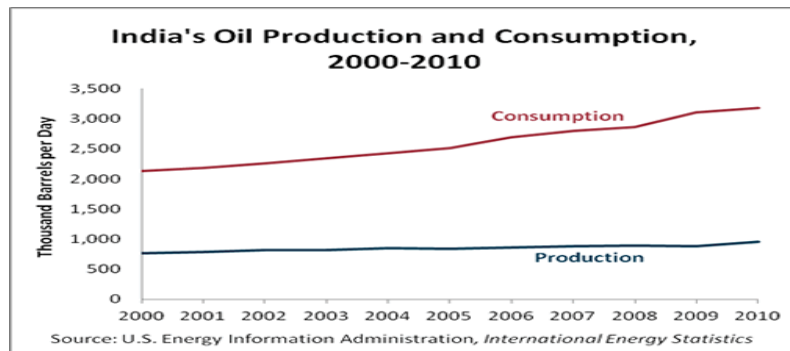
**Ref:** - Environmental pollution control engineering by C.S Rao

In the above table GTOE shows the Giga tonnes of oil equivalent, as demand goes on increasing with years and will make economical unbalance and crisis.

### India's Fossil Fuel Scene

As we are a developing country, we are using traditional sources of energy as firewood, dung cakes and agriculture wastes and other commercial resources as coal, oil and hydro-electrical power. But consumption of modern resources of energy is growing very fast since past three

decades. In India, systematic surveys and exploration for assessing and proving coal reserves by Geological Survey of India have been fairly reliable. The assessment of 1992 places the proved recoverable reserves at 64800 million tonnes. At present the production of coal is increasing at exponential rate of 6.6% per year. It will attain a peak sometime between the years 2040 and 2080 and after that it will decline. Table 1.2 is showing crude oil production.



**Figure 1.1** India’s crude oil production

So to overcome this problem or to replace the fossils, we are going to replace it with Bio-fuel derived from the Algae.

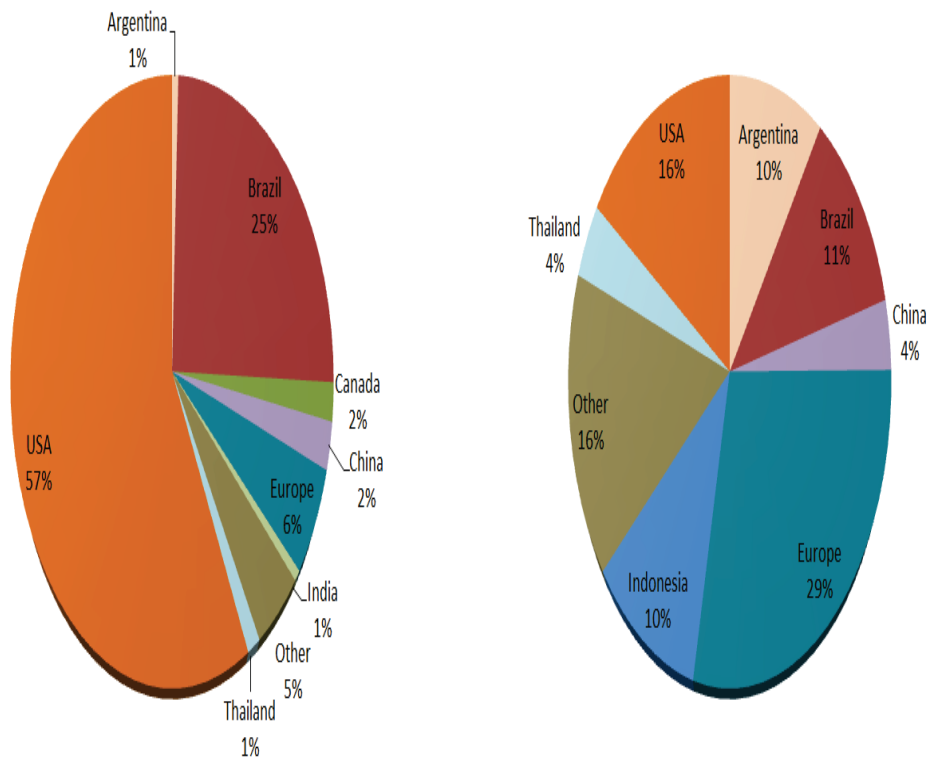
## 1.1 Bio-fuel

Bio fuel, as from the name is a type of fuel generated from the living organisms but not from the geological processes but from the biological processes such as agriculture, anaerobic digestion.

Bio-fuels can be taken as the better replacement for the fossil fuel as they can be produced easily and any where if one has a good knowledge about procedure and can be replace fossil diesel or gasoline up to some extent to meet the energy requirements in the transportation and other industrialization sector . Bio-fuels could give us positive remarks for energy security of our nation, air quality and GHG minimization, employment generation and rural development.

### 1.1.1 Current status of Bio-fuel

If we check the status of bio-fuel production currently then we can get 127.7 billion litres, 74% of which ethanol fuel and 23% biodiesel globally. Figure 1.1 is showing some statistics.



**Figure 1.2** Bio-fuel Production by countries.

### 1.1.2 Need of Bio-fuel in India

As India is a developing country and we are making our economy backbone strong. The Urbanization and industrialization is taking place everywhere in our country at very large scale. As all these developments are going at rapidly, the demand of fossil fuel is increasing at a very high rate. Acc. To current stats the oil demand in India will reach more than 8.1 mbpd in 2036. But current domestic production of crude oil is meeting only 18% of the national requirement. So to overcome the crises we are going to make use of bio-fuel. Bio-fuels are considered as the strong alternatives among this family.

### 1.1.3 Bio-fuel from Algae

To get a bio-fuel, plant must have the lipid contents in it. Lipid content of plants gives the natural oil from which we can drive the bio-fuel. There are many plants in our ecosystem from which we can get natural oil. But among them, Algae has the highest lipid/natural oil content about 48%-50% of total biomass. The main advantage of bio-fuel

from algae is that algae can be produce anywhere and can be produce in large quantity in a very short span of time.

## **1.2 Algae for the nutrients stripping**

As we know nitrogen and phosphorous are the main nutrients present in the waste water. And if we do not cure them they can cause eutrophication in the natural water bodies and make them contaminated and affect the aquatic life badly. So as we know N and P is the main food for the algae so cultivation of algae in the waste water can reduce the amount of nitrogen and phosphorous from the waste water through controlled conditions required for the growth of the algae. By using algae as nutrient stripper we have two advantages. First is that we are reducing the amount of nutrients from the waste water and other is we are producing algal biomass which can further give us algal oil by which we will generate bio fuel. But main disadvantage of this method is that it requires a large area of land for the construction of algal pond setup. But as the technologies are rising day by day we can also find some alternatives for this. Nitrogen and phosphorus are the main nutrients present in the waste water. If they are within the permissible limits or not present in high quantity, further can be used for the irrigation purpose after doing primary sedimentation of effluent. If they are present in very high concentration then there is different biological and chemical process for the reduction of the nutrients (N&P) like aeration tanks, BNR (biological nutrient removal). But main problem with these techniques is that they can only remove nutrients upto the their standards for the secondary treatment removal efficiency. After the secondary treatment they easily disposed to inland surface water or for the irrigation purpose. But due to incomplete removal of the nutrients they cause eutrophication in the water bodies on the surface as well as some salts/nutrients can spoil the properties of soil and then the quality of the groundwater as they can easily percolates into the ground water. The main sources of the nutrients are Nitrogen in sewage effluent arises primarily from the metabolic inter conversions of extra derived compounds, whereas 50% or more of phosphorus arises from the synthetic detergents. The principal forms in which they occur in wastewater are ammonia, nitrite, nitrate and orthophosphate. Together these two elements are known as nutrients and their removal is called as nutrient stripping.

### 1.2.1 Algae

Algae are the aquatic plants do photosynthesis and combine the water and  $\text{CO}_2$  to form sugar for energy and for growth. Algae produce oxygen, a useful by product but when sunlight is not available at night they quickly respire. This respiration uses the stored sugars and oxygen to form  $\text{CO}_2$  which depletes the oxygen in the pond.



**Figure1.3** Algae on water surface

There are two classifications of algae: macro-algae and micro-algae. Algal biomass contains three main components: carbohydrates, proteins and lipids/natural oils. The biomass from algae can also be burned similar to wood or anaerobically digested to produce methane biogas to generate heat and electricity. The tables mentioned below showing some information regarding algae and their oil yield comparison with other crops. The advantage of algae is that it grows rapidly and very high in volume and sequester the carbon dioxide from the environment. But if we want more yield for the bio fuel then choose the micro algae species.

**Table1.2** Oil content of

Microalgae

Microalga	Oil content (% dry weight)
<i>Botryococcus braunii</i>	25-75
<i>Chlorella sp.</i>	28-32
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca sp.</i>	16-37
<i>Nitzschia sp.</i>	45-47
<i>Phaeodactylum tricornutum</i>	20-30
<i>Schizochytrium sp.</i>	50-77
<i>Tetraselmis suecia</i>	15-23

**Table1.3** Oil yields based

crop type

Crop	Oil yield (gallons/acre)
Corn	18
Soybeans	48
Canola	127
Jatropha	202
Coconut	287
Oil Palm	636
Microalgae	6283-14641

Generally grow with the right conditions for example with adequate nutrients (phosphorous and nitrogen), good light level, alkaline pH, temperature. Sewage water can be the good source of nutrients for the algae growth as sewage water contains lots of nitrogen and phosphorous in it but after the primary clarifying process.

#### **Algal growth parameters are following:**

- Carbon and nutrients algae are autotrophs as they can synthesize organic molecules themselves from the inorganic nutrients. Some algae may be heterotrophy as well as mixotrophs depending upon the nutrients and the light availability. A stoichiometric formula for the most common elements in an average algal cell is  $C_{106}H_{181}O_{45}N_{16}P$ . Algal culture offers a cost effective approach for removing nutrients from the wastewater mainly in tertiary wastewater treatment. Algae have a high capacity for the inorganic nutrient uptake and they can be grown in mass culture in outdoor solar bio reactors. Biological processes appear perform well compared to the chemical and physical processes which are in general are too costly to be implemented in most places and may lead to secondary pollution. The widely used algae cultures for nutrients removal are species of chlorella, Scenedesmus and Spirulina. It was pointed out through research that Scenedesmus is very common in all kinds of fresh water



bodies which play an important role as primary producers and contributes to the purification of eutrophic water.

- Light conditions affect directly the growth and photosynthesis of algae. They obtained energy from light. The light energy is converted to chemical energy in the photosynthesis. It was reported that in outdoor ponds, more than 90% of the total incident solar energy can be converted into heat and less than 10% into chemical energy.
- Temperature is one of the crucial parameter for the growth of algae in countries where fluctuation of the temp. is high. Increased temperature is good for the growth of algae up to certain range, after that critical temperature growth is ceased. The temperature at which the organism collected.
- pH is also an important factor that affects the growth of algae. In the algae cultivation, pH value usually increases because of the photosynthetic CO<sub>2</sub> assimilation. pH value will affect the availability of inorganic carbon.

**Cultivation methods for the algae are below:**

- Open pond system cultivation is the more preferable due to its low cost and can be done in large scale cultivation and it is easier to manage. It is more durable than large closed reactors. Open pond cultivation can be carried out in natural or artificial lake and ponds. There are many types of pond that had been designed and experimented before for the optimum cultivation of algae.
- Closed photo bioreactors can be grouped into two major classes: covered raceway and tubular reactors. Closed photo bioreactors usually have better light penetrating characteristics than the open ponds; the light path is usually less than 30mm, which makes make it possible to sustain high biomass.

**Harvesting techniques are mentioned below:**

- Filtration is the most simple and cost effective harvesting method. Filtration can be carried out either in small or large scale. Filtration can be done by using filter paper in laboratory scale or using coarse screening in a large scale harvesting of algae.
- Using sedimentation or floatation, the biomass can be concentrated already in the water, which in turn can be decanted. Sedimentation without addition of chemicals is

the most common method in full scale facilities. Flootation process operates more rapidly and efficiently than sedimentation.

### **1.2.2 Composition of typical wastewater**

Water sources receive pollution from many different sources which vary both in strength and volume. The composition of the wastewater is a reflection of the life style and type of industries. It is the complex mixture of natural organic and inorganic materials as well as manmade compounds. Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids and volatile acids. The inorganic constituents include large concentration of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals.

## **CHAPTER 2**

### **OBJECTIVES**

- Generation of bio-fuel to reduce the excess load on the usage of fossil fuel.
  - To check the nitrogen and phosphorous take up capability of the algae for the different species.
  - To enhance the carbon neutral ecosystem.
  - Establishment of our ecosystem free from the eutrophication.
  - To introduce the generation of bio-fuel as an application of wastewater.
-

## CHAPTER3

### LITERATURE REVIEW

**Woertz et al, 2009** Algae can be grown on the waste water as media and can be the best source of the lipids for the production of the bio-fuel. Algae can remove nutrients from the wastewater efficiently either from dairy wastewater or municipal wastewater up to 99%.

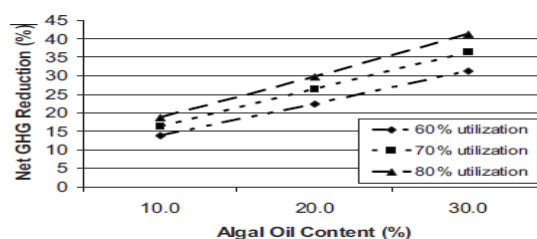
**GuanHua et al, 2009** High potential of the biodiesel production can be achieved by achieving the high content of the lipids in microalgae through the heterotrophic cultivation. By finding out the cheaper oleaginous substances and by rectifying the transesterification we can achieve our target of producing the bio-fuel at very high rate with low price.

**Yusuf Chisti et al, 2007** Microalgae appears to be the only natural oil content that can full-fill the demand of fuel in place of fossil fuel in coming years and can minimize the impact of the carbon dioxide in our ecosystem by reducing its quantity and can reduce the global warming like circumstances. And to enhance the good productivity of the biodiesel, microalgae should be cultivated through photobioreactors as they provide very controlled condition required by the algae for their growth.

**Dingnan Lu et al 2016** The addition of septic sludge to the microalgae resulted in more favourable initial carbon to nitrogen ratio(c/n)(11:1 to 27:1). And it also helps in decreasing the quantity of hydrogen so that it will give us good amount of methane gas.

**Muthukumar et al 2012** 60.26% of bio diesel yielding from 0.752 g/l contains 30% oil content from N.salina. And 50% yield from the 0.527g/l contains the 20% oil content. We found the N. Salina and C. Marina very good for the production of the biodiesel.

**Brune et al 2009** Recovery of fuel gas carbon dioxide can be done by the algae biomass. So cultivation of algae biomass can reduce the GHG and can be proved friendly for the



**Figure 3.1**GHG reduction v/s algal oil content

**Natsima et al 2010** Waste water from the piggery farm can be utilized to grow algae and can be used to enhance the good fertility of the soil for the irrigation and we can enhance the good crop yield. Acc. To study, this wastewater contained the 30kg/day and 5kg/day of N and P content respectively.

**Himeshwariya et al 2012** Microalgae can be used for the sustainable production of the bio-fuel as it has a capacity to store high content of lipid/natural oil. It can also provide us the high amount of bi products as it has high photosynthetic efficiency, higher biomass production and can grow faster than other terrestrial crops. Moreover it is giving standardized biodiesel to us.

Properties	Biodiesel from microalgae	ASTM biodiesel standard
Density (kg/L)	0.864	0.86–0.9
Viscosity (mm <sup>2</sup> /s, cSt at 40 °C)	5.2	3.5–5.0
Flash point (°C)	115	Minimum, 100
Solidifying point (°C)	–12	–
Cold filter plugging point (°C)	–11	Summer maximum, 0; winter maximum < –15
Acid value (mg KOH/g)	0.374	Maximum, 0.5
Heating value (MJ/kg)	41	–
H/C ratio	1.81	–

**Figure 3.2** Comparison of biodiesel and ASTM

**Nazim Muradov et al 2015** Some of the fungal and micro-algal strains has the ability to treat the waste water and can generate the high biomass and further used for the bio-fuel generation easily.

**Michael Morweiser et al 2010** The latest photo bioreactors has improved efficient light utilization, are foot prints and minimization in cost of installation. Light path lengths of only small cm can able to enhance the high biomass productivity.

**Lenka. Blinova et al 2015** The growth rate and the content of lipid depends upon the optimal cultivation conditions like concentration of carbon dioxide, temperature, light conditions, pH, design of photobioreactors for culturing.

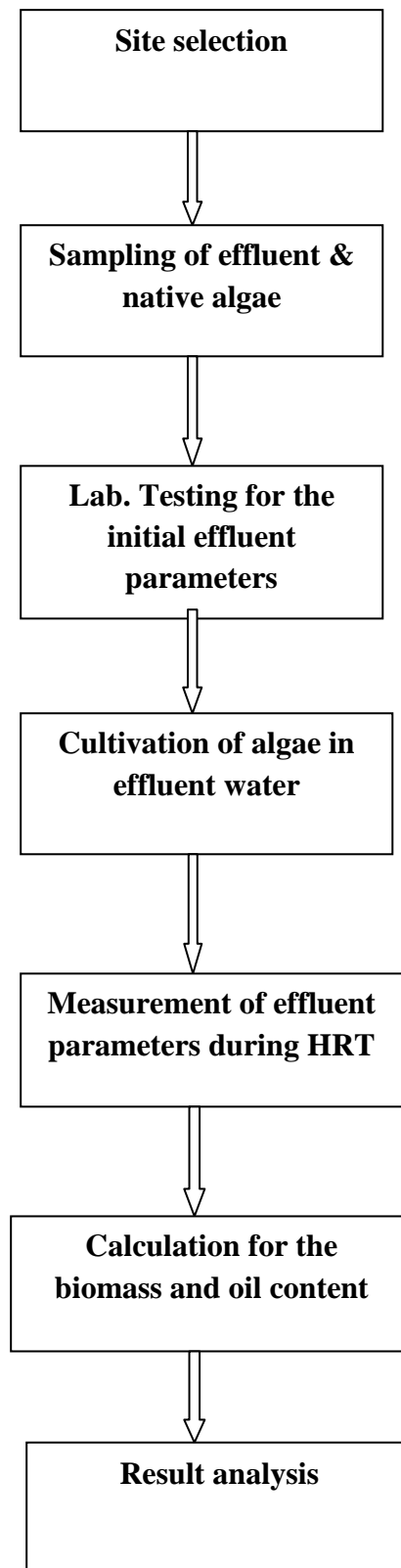
**N abelel, Raouf 2012** The high concentration of nitrogen and phosphorus in most wastewaters, these water may possibly be used as cheap nutrients sources for the algal biomass production

**Harshad rathod 2014** For effecting algal growth and treatment efficiency, it is likely that the major factors are carbon and light.

**S. Hena 2015** 500 billion m<sup>3</sup> industrial wastewater could produce 37 million ton of algal oil.

## CHAPTER 4

### METHODOLOGY



**4.1 Site selection:** A dairy farm was selected as a site (Milk plant) which is producing milk and milk products under well equipped setup and well trained staff. This milk plant is producing about 725KLD wastewater from different processes like milk pasteurization making of curd and other milk products.

## 4.2 Sampling:

### 4.2.1 Sample of wastewater

Sample of the effluent was taken after the primary sedimentation of 10 L volume. The sampling method was grab sampling means taken directly at the same time in bucket.

### 4.2.2 Sample of Algae

As an alga is the main remedy for my project for wastewater treatment, native algae specie in place of other cultured algae species. Naturally cultured algae from the milk farm in polythene bag was taken and added to system.

## 4.3 Tests performed:

Here we going to test some common parameters of wastewater to be checked and Phosphate content present in the water. We are calculating these parameters because among them the presence of phosphorous and nitrogen is very important for rapid production of algae. We are also checking for the BOD and DO before the cultivation of algae in waste water so that we can get information about the effect of algae on the BOD and DO of waste water. After that we will comprises them with standards given by the CPCB. Table4.1 is showing some standards of wastewater to be disposed in our ecosystem after secondary treatment.

**Table4.1** Parameters by CPCB for disposal of effluent into inland surface water

S. No.	Parameter	Inland surface water
1.	BOD	30 mg/l
2.	TKN	100 mg/l
3.	Total phosphorus	5 mg/l



### 4.3.1 BOD Test

Biochemical oxygen demand is the oxygen demand by microbes for the decomposition of biodegradable organic matters. Generally we find the BOD for 5 days at 27 degree Celsius.

#### Apparatus required

1. Incubator
2. Burette
3. Pipette
4. Conical flask
5. BOD bottles

#### Chemical required

Manganese sulphate, sodium azide, potassium iodide, sodium hydroxide, starch, sodium thiosulphate.

#### Procedure

1. Water sample was collected in two BOD bottles.
2. One BOD bottle was incubated in BOD incubator for the 5 days at 20<sup>0</sup> c while DO of another BOD was determined on the very first day.
3. After 5 days another BOD bottle was removed from the incubator and DO was determined.
4. Difference in DO was calculated, gave the measure of 5 day BOD.

#### Calculation

$BOD (mg/l) = \{(DO1 - DO5) * \text{diluted sample}\} / \text{volume of sample taken}$

DO1 = DO in water sample in 1<sup>st</sup> day.

DO5 = DO in water sample after 5 days.

$DO (mg/l) = \text{volume of sodium thiosulphate consumed in ml} \times \text{Its Normality} \times 0.008 \times 10^6$   
/ volume of sample in ml.

### 4.3.2 Nitrogen Test

Here I used the Kjeldahl method to find out the nitrogen content in the waste water. The steps are as following

- Sample was digested in strong sulphuric acid in the presence of the catalyst which is used to accelerate the reaction and conversion of the amine nitrogen to ammonium ions.
- The ammonium ions then converted into the ammonia gas by the addition of alkali, it is then heated and distilled.
- The ammonia gas is led into the trapping solution where it dissolves and becomes an ammonium ion once again.



**Fig4.1:** kjelo Plus apparatus for calculating TKN

**Calculation:**

$$\% \text{ Nitrogen} = \frac{14 \times (\text{normality of acid}) \times (\text{Titrant Value burette reading}) \times 100}{\text{Sample ml} \times 1000}$$

$$\text{PPM} = \% \text{ of Nitrogen} \times 10,000$$



**Fig4.2:** color showing after the Kjelo plus apparatus output



**Fig4.3:** End point after the titration for TKN

#### 4.3.3 Total phosphorus test:

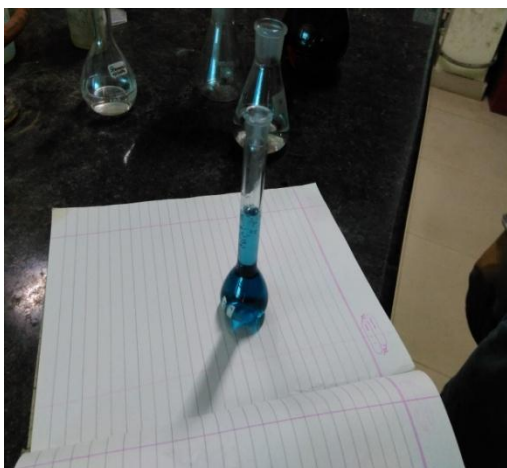
- Take 25 ml of sample in 250 ml flask and add pinch of activated charcoal.'
- Add 50 ml of 0.5N  $\text{NaHCO}_3$  solution into it and shake the flask for 30min.
- Filter the suspension through a whatman no. 42 filter paper and take 5 ml of the extract in 25 ml volumetric flask.
- Add 5 ml of ammonium molybdate and a little quantity of distilled water and shake the content of volumetric flask
- Add 1 ml of  $\text{SnCl}_2$  in 25 ml volumetric flask and make the volume up to 25 ml with distilled water.
- Take the absorbance through spectrophotometer in 660 nm wavelength.
- **Standard phosphate solution** Dissolve 4.388 g of dried anhydrous potassium hydrogen phosphate in distilled water to make the volume 1L. Take 10 ml of this solution and add distilled water to make 1 L of stock solution containing 1 mg/L. prepare standard phosphorus solution of various strengths in range of 0.0 to 1.0 mg/L by diluting the stock solution with distilled water. And draw a graph and get equation for the further concentration of the phosphorus.







**Fig4.4:** Mixing of the chemicals required for the calculation of the phosphorus



**Fig4.5:** solution to get the absorbance by spectrophotometer



**Fig4.6:** spectrophotometer

#### 4.4 Cultivation of algae:

To cultivate the algae biomass I used an aquarium as open pond system for small production. As this was transparent, so sun light easily transmitted at every corner through glass. To acquire the most biomass of algae for the much production of the bio fuel by the native species of algae, I obeyed some specific standard condition. These parameters are below.

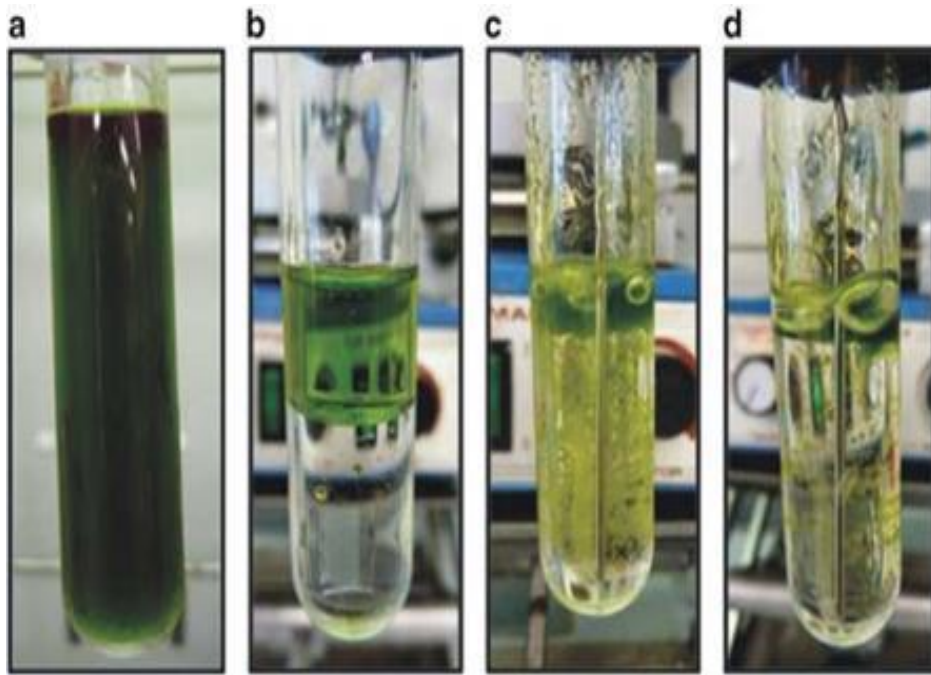


**Fig 4.7:** Open pond system (aquarium)

- **Light** As algae are the phototropic as well as auto tropes, they need a well quantity of light for the growth purpose. So to reduce the production cost the sunlight with 12:12 N: D ratio was used for the algae growth. As per the concept with the increase in the depth of the culture system the penetration capacity of sun rays can get decreased so glass chamber was used and didn't took too much depth. We can also use fluorescent bulbs as a source of light but for the cost effectiveness they were not used.
- **Culture medium or nutrients** these are the most important parameters to select the best culture media for the growth. Algal sample was taken from the native place of the wastewater so idea came that it can also grown in this wastewater sample. So this added my project cost efficiency because for culturing high sensitive specie we have to handle it more scientifically in the lab and it could increase our production cost. So i took native sample of algae and wastewater as the culturing media directly.
- **pH** The pH range is between 7 to 9 for the most of the algae species. In case of low pH condition we can add some salts to the water or can add acids in case of higher pH for the optimal growth. In this case the pH was maintained.
- **Mixing** The mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients. In this case because this culture system was not big so mixing was done by hands.
- **Temperature** lower than 16 degree Celsius slow down the growth whereas those higher than 35 degree Celsius are lethal for a number of species. In case of higher temperature system can be cooled down by a flow of cold water over the surface of the culture vessel.

**4.5 Extraction of algae biomass from water** To extract the biomass from the system filtration method was used and the water for further testing and sent the biomass for the oil extraction.

**4.6 Extraction of natural oil from algae** separation of the algal oil from the algal biomass is done by the Soxhlet instrument. The biomass was sent to the ABCA consultancy Chandigarh, Punjab to extract the natural oil.



**Fig4.8:** Process during extraction of natural oil



**Figure 4.9** Soxhlet apparatus



## CHAPTER 5

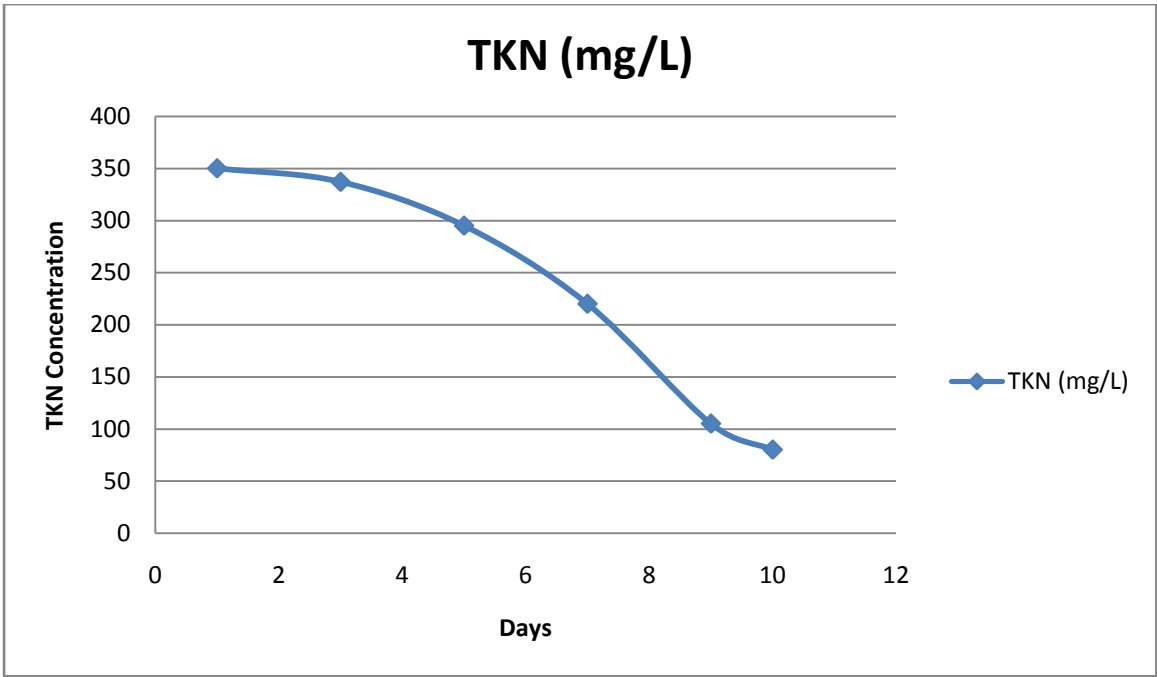
### RESULTS AND DISCUSSIONS

**Table5.1:** Initial waste water parameters

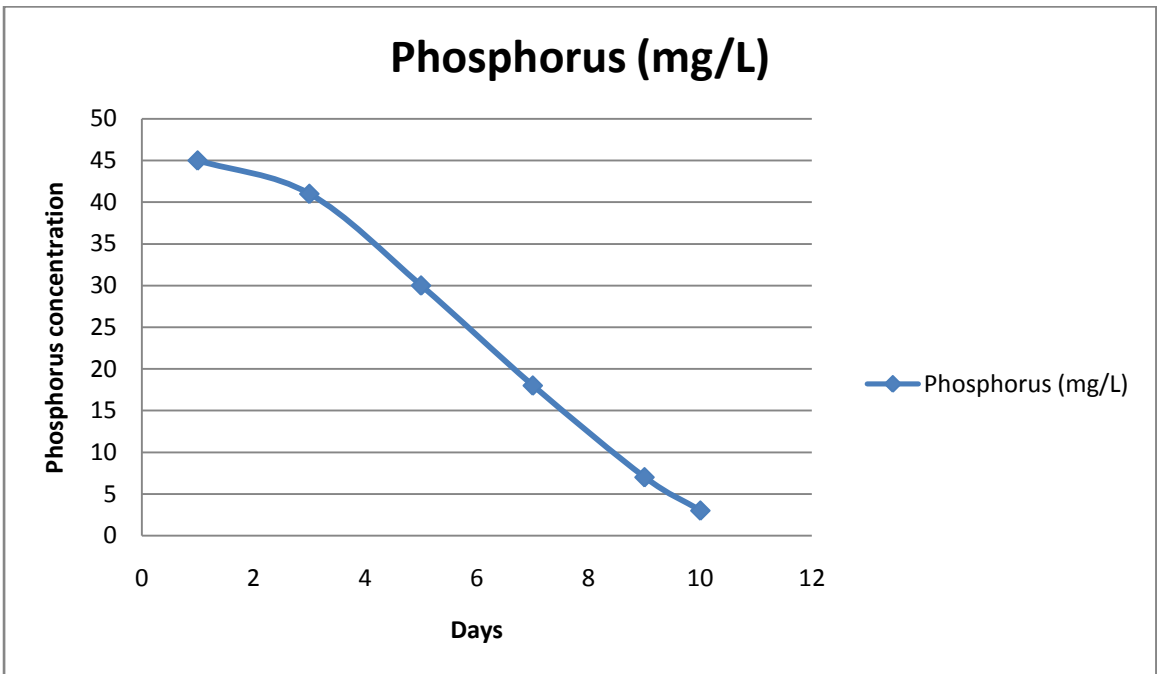
S.No	Parameters	Obtained values (mg/litre) approx.
1	BOD	1200
2	TKN	350
3	Phosphorus	45

**Table5.2:** Intermediate waste water parameters (TKN & Phosphorus)

S.No	Cultivation period	TKN (mg/litre)	Phosphorus (mg/litre)
1	Day 1	350	45
2	Day 3	337	41
3	Day 5	295	30
4	Day 7	220	18
5	Day 9	105	7
6	Day 10	80	3



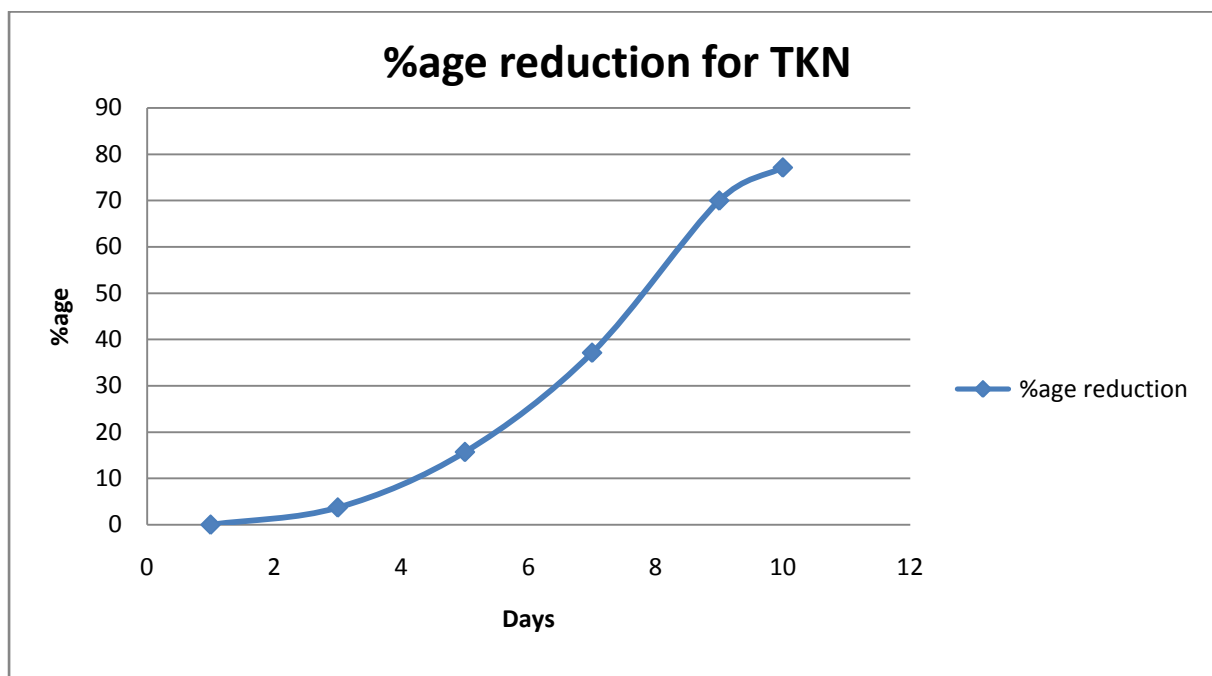
**Graph5.1:** Cultivation period (days) vs. TKN stripping



**Graph5.2:** Cultivation period (days) vs. Phosphorus stripping

**Table5.3:** Percentage reduction for TKN

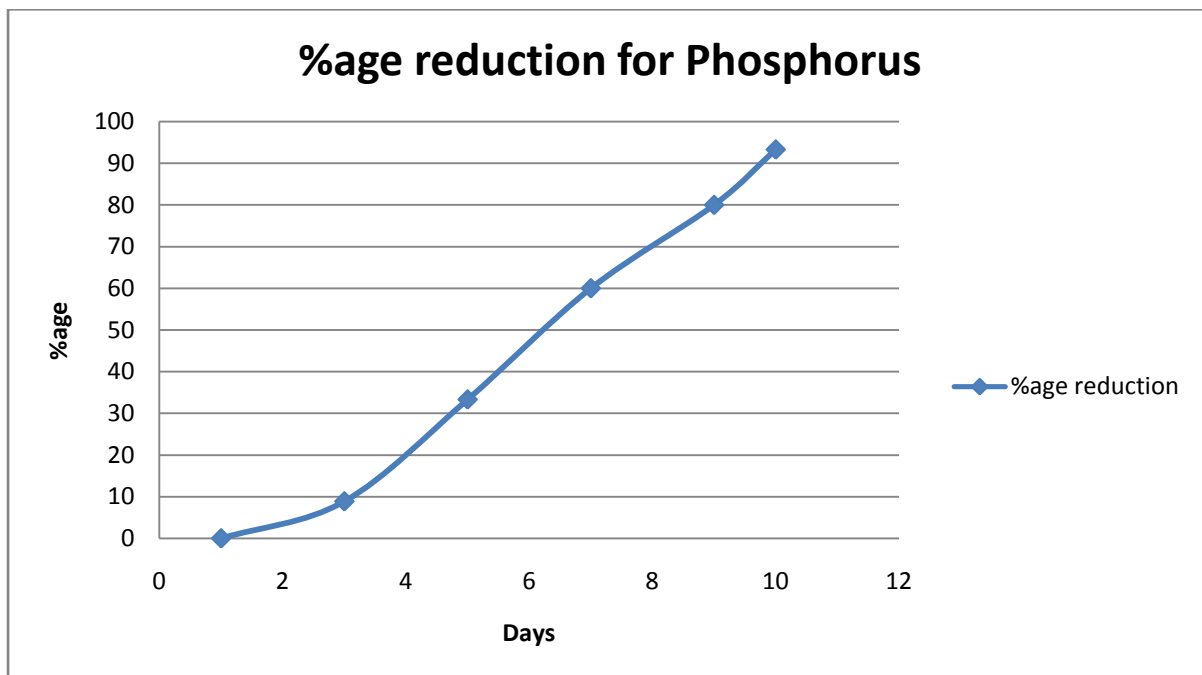
S.No	Cultivation period (days)	TKN reduction (mg/L)	Percentage reduction
1	Day 1	-	-
2	Day 3	13	3.71
3	Day 5	55	15.7
4	Day 7	130	37.14
5	Day 9	245	70
6	Day 10	270	77.14



**Graph5.3:** Cultivation period (days) vs. %age reduction for TKN

**Table5.4:** Percentage reduction for Phosphorus

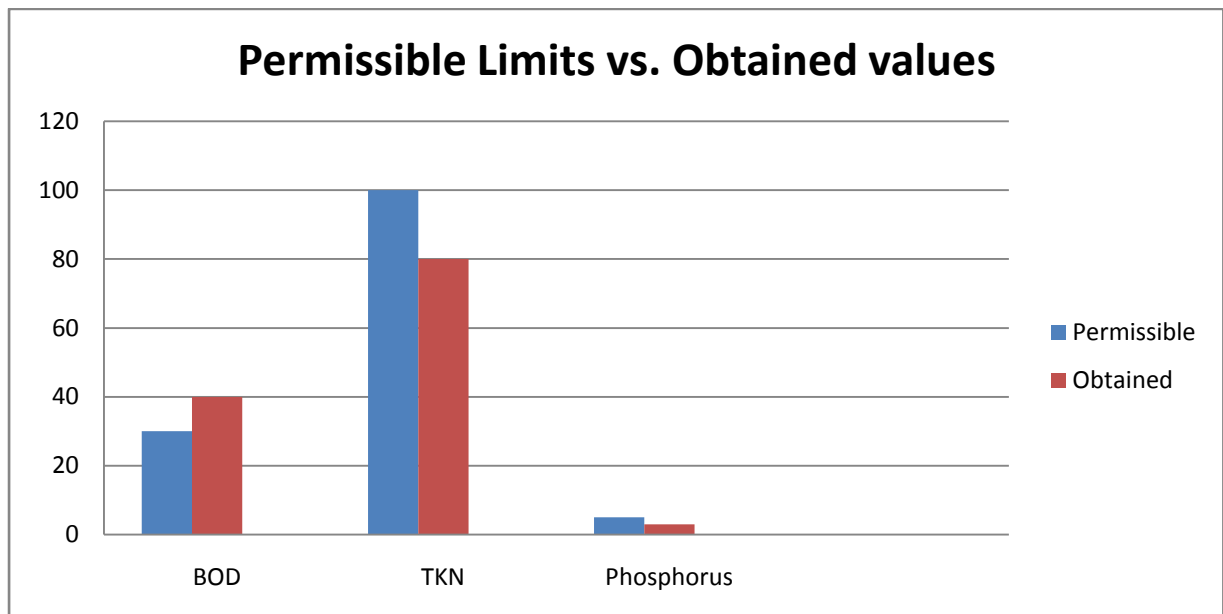
S. No	Cultivation period (days)	Phosphorus reduction (mg/L)	Percentage reduction
1	Day 1	-	-
2	Day 3	4	8.88
3	Day 5	15	33.3
4	Day 7	27	60
5	Day 9	38	80
6	Day 10	42	93.3



**Graph5.4:** Cultivation period (days) vs. Percentage reduction for Phosphorus

**Table5.5:** Optimum Reduction in initial parameters

S.No	Parameters	Obtained values	%age reduction
1	BOD	40	96.6
2	TKN	80	77.14
3	Phosphorus	3	93.3



**Graph5.5:** Comparison of permissible limits and obtained values for various parameters of effluents

**Table5.6:** Comparison of nutrients stripping with other algae treatment system

<b>S.No</b>	<b>Authors</b>	<b>TKN reduction (%age)</b>	<b>Phosphorus (%age)</b>	<b>Algal species used</b>
1	Martimez et al (2000)	80-99	92-95	S.condemus
2	Lincolon et al (1996)	99	96	Arthropira
3	Green et al (1995)	99	95	Chlorela
4	Current project	70.14	93.33	Native species at dairy industries

Results for the nutrients stripping were positive as their concentrations were getting reduced up to the permissible limits. After doing this, the extracted biomass was sent for the natural oil testing. The test was performed by the Soxhlet apparatus i.e. a natural oil extractor. The total biomass of the algae produced was 850 g (approx.) and when send was for the extraction it gave approx about 165 ml of the algal oil / natural oil which is about 17% of the total effluent.

## CHAPTER 6

### CONCLUSION AND FUTURE SCOPE

#### 6.1 Conclusion:

This project is about the nutrients stripping by algae and producing natural oil from the cultivated biomass. In this research I used native species of algae which I took from nearly available by ETP. Till now numerous researches have been made for processing the same work in which they used some other algal species. After completing the laboratory testing I got positive results for required parameters and the efficiency of this native species for my aim was good. This species can reduce the up to 70% of TKN and about 94% of phosphorus in the 10 days of cultivation period. And through his method of treatment I was easily able to reduce the effluent parameters up to disposal limits of effluent in inland surface water. Moreover I got about 17% of the natural oil content. In future we can also make some increment in the efficiency of this native species by doing some modifications.

#### 6.2 Future scope:

- This type of native can also be used for other type of industries where the effluent is very high with N and P concentration.
- Further we can make use of biomass as feed for the biogas plant and can check the production percentage of methane gas as biomass contains carbohydrates and proteins.
- After checking the toxicity of the biomass it can be further used as the fertilizers.
- Native species can be further checked for the individual species through cell morphology.
- In future we can check its CO<sub>2</sub> sequestration capacity as it takes CO<sub>2</sub> and present in abundance in our planet.

## REFERENCES

1. D. E. Brune, T. J. Lundquist, and J. R. Benemann. (2009). Microalgal Biomass for Greenhouse Gas Reductions: *JOURNAL OF ENVIRONMENTAL ENGINEERING*. 135(11): 1136-1144
2. D. E. Brune, T. J. Lundquist, J. R. Benemann. (2009). Potential for Replacement of Fossil Fuels and Animal Feeds. *JOURNAL OF ENVIRONMENTAL ENGINEERING*. 135(11): 1136-1144
3. DingnanLul and Xiaoqi Jackie Zhang. (2016). Biogas Production from Anaerobic Co digestion of Microalgae and Septic Sludge *Journal of Environmental Engineering*, 0733-9372.
4. GuanHua Huang, Feng Chen, Dong Wei, XueWu Zhang. (2009). Biodiesel production by micro algal biotechnology. *Journal of Applied Energy*. 87 (2010) 38–46
5. Nazim Muradov, Mohamed Taha. (2015). Fungal-assisted algal flocculation: application in wastewater treatment and bio-fuel production. *Journal of Biotechnology for Bio-fuels*. 8:24
6. S. Hemaiswarya, Rathinam Raja, Isabel S. Carvalho,R. Ravikumar, Vasudeo Zambare Debmalya Barh. (2012). An Indian scenario on renewable and sustainable energy sources with emphasis on algae. *Journal of Appl Microbiol Biotechnol*. 96:1125–1135
7. Sergeeva YE, Galanina LA, Andrianova DA, Feofilova EP.(2008). Lipids of filamentous fungi as a material for producing biodiesel fuel.*journal of Appl Biochem*.44:523–7.
8. Woertz,A. Feffer, T. Lundquist, Y. Nelson. (2009). Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Bio-fuel Feedstock. *Journal of Environmental Engineering*. 135(11): 1115-1122
9. Yusuf Chisti.(2007). Biodiesel from microalgae. *Journal of Biotechnology Advances* 294306



