

# Extraction of biodiesel from wastewater using microalga *Chlorella vulgaris*

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## Graphical abstract



## Abstract

An experimental research on production of biodiesel from lipids of *Chlorella vulgaris* has been done through transesterification reaction process. The series of *Chlorella vulgaris* cultures has been carried out and the optimum growth time was determined. The biodiesel synthesis is processed by two steps i.e., isolation of *Chlorella vulgaris* lipids and the synthesis of biodiesel from lipids. The objective of the study was to develop a simultaneous wastewater treatment system and the production of renewable energy by utilizing the algal growth. Biofuel batch scale experiments with 10% and 15% dilution of municipal wastewater were carried out in this study. The total lipid content of algal biomass ranged from 7.5 to 28.5%. The approximate proportions of chloroform, methanol and water in the upper phase was found to be in the ratio of 3:48:47 by volume and the lower phase mixture proportion was found to be 86:14:1. The concentration of biomass reached a maximum value of 920 mg/L measured in terms of VSS. The nutrient removal, which was achieved to nearly 100% weight of biodiesel yield per biomass of

*Chlorella vulgaris*, was 49.7%. The viscosity values and heat of combustion of the biodiesel were 1.14 g/cm<sup>3</sup> and 42.50 KJ/g respectively.

**Keywords.** Biomass, *Chlorella vulgaris*, lipids, nutrient removal, transesterification

## 1. Introduction

Biodiesel is nothing more than vegetable or animal fat-based oil that typically contains long-chain alkyl esters (methyl, ethyl, or propyl). Biodiesel is made from lipids (such as vegetable oil, soybean oil, and animal fat) that chemically react with alcohol to produce fatty acid esters (Zhang *et al.*, 2013). This can be mixed with petrodiesel in any ratio or used alone in conventional diesel engines. You can use the biodiesel mixes as heating oil. The National Biodiesel Board's technical definition of biodiesel is mono-alkyl ester (USA). Blends of biodiesel and traditional hydrocarbons are frequently utilised in retail diesel fuel (Patiño *et al.*, 2021).

Biodiesel may also be used in its pure form (B100) or may be blended with petrodiesel at recommended concentrations for the use in injection pump diesel engines. B5 and B20 blends are used in the common rail engines with extremely high pressure (29000 psi) (Sari-Erkan, 2019). Biodiesel has some different properties when compared to petrodiesel and degrades the rubber gaskets and hoses found attached to vehicles and they naturally wear out which is non-reactive to biodiesel (Arora *et al.*, 2021).

Algae has the potential to produce hundreds of times more oil than a typical crop like rape seed, palms, soybeans, or jatropha and has a tendency to develop more quickly than other food crops. Algae differ from animal crops in that their harvesting cycle is discovered to be 1–10 days, allowing for several harvests in a short period of time (Costa *et al.*, 2021). Algal cultivation is typically done in photobioreactors or open ponds for research purposes, but open ponds have the drawback that the algae may be

exposed to contaminants such as heavy metals and pesticides (Naveenkumar *et al.*, 2022). Algal growth has the advantage that these can be grown even in unsuitable lands where conventional crops cannot be grown, including arid land and land with excessively saline soil, thereby minimising competition with agriculture (Jareonsin and Pumas, 2021).

The algal cells which contain lipids need to be extracted for further use. There are many ways for the extraction of oil from algae. The most popular method is the oil press method, which is similar to Olive press (Velusamy *et al.*, 2021). When the algae are pressed, nearly 75% of the oil can be extracted (Hamidian and Zamani, 2022). There are two-part process in extracting oil from algae, One of the method is the hexane solvent method (combination of using solvent along with pressing) which has the capability of extracting upto 95% of oil from algae. The algae are pressed and this process helps in squeezing out the oil (Raag Harshavardhan *et al.*, 2022). The remaining algae is then mixed with hexane, filtered and cleaned so that to ensure no chemical is left behind in the oil. The second method is the super critical fluids method which can extract oil upto 100% from algae (Torres *et al.*, 2018). The supercritical fluid used is carbon dioxide. A supercritical fluid is one which when pressurized and heated, changes its composition into liquid as well as gas (Malik *et al.*, 2022). Therefore, at that particular point carbon dioxide and algae are mixed together. The combination of the mixture turns the algae into oil completely. This could be achieved mainly by the use of carbon dioxide. But an additional equipment is needed for this method and thus making it less popular option (Murugesan *et al.*, 2022). When spilled or put into the environment, biodiesel in its pure, clumpy form induces much less harm than petroleum - based diesel. It is less combustible than petroleum diesel. The flashpoint of biodiesel is greater than 130°C, whereas petroleum diesel has a flashpoint of around 52°C (Shahid *et al.*, 2021). The extracted oil is refined using fatty acid chains in a process known as transesterification. This process occurs when an alcohol is mixed with catalyst such as sodium hydroxide. This forms the biodiesel fuel combined with glycerol (Haider *et al.*, 2023). The final obtained product is algae biodiesel fuel (Sampathkumar *et al.*, 2022; Shahid *et al.*, 2021). The process could be made cost-efficient by the reaction of an ester which is special type of chemical compound in which an acid has one of the hydroxyl groups or a molecule of hydrogen and oxygen bonded together replaced by a molecule of oxygen (Sundar *et al.*, 2022). Thus, through a specific chemical reaction, the oil is obtained from algae turns into diesel.

## 2. Materials and methods

### 2.1. Materials

Initial collection of wastewater was performed by hand using plastic containers to collect water from the holding pond surface. This wastewater is then fed into the Bench scale digester in the lab. The approximate volume of wastewater in the digester was 90 litres *Chlorella Vulgaris* microalgae which was obtained by culturing in wastewater

is cultivated and harvested (Figure 1). The cultivation of microalgae was done in small scale in mini tanks at a temperature of 30°C (Ragunathan *et al.*, 2022). The algal suspensions were homogenized at the end of the culture cycle in order to disintegrate the cells of the algae and to give an ease for the extraction of oil. The algae suspensions are passed through the centrifugal separator for the filtration. The Folch method and the Bligh and Dyer method are the most common methods of biodiesel production from algae. Solvents like methanol and chloroform are employed in the ratios of 2:1 and 1:2 v/v in both methods.



Figure 1. Cultured algae

### 2.2. Experimental methodology

The collected wastewater is first fed into the anaerobic digester, which was designed on a lab scale. The digester is provided with insulation of 2cm in order to maintain the temperature inside and to avoid temperature swings (Agarwal, 2007). A tube is inserted for feeding the digester and to pump the effluent out to feed the algal tanks. The approximate volume of wastewater in the digester was 90 litres. The digester is loaded with wastewater in batches once in a week.

#### 2.2.1. Algal growth containers

The anaerobic digester effluent is transferred to glass tanks, which serve as the bioreactor for algae growth. Volume of water was 2 litres in two reactors and 5 litres in other two reactors (Demirbas, 2005). The penetration of light from the top helps in stimulating the growth of algae. Air sparging pipes were placed in each reactor to provide mixing and gas exchange. Additional carbon dioxide was used at a rate of 0.015 liter per minute or approximately 1% the volume of air provided. Lux meter is used to determine the amount of sunlight passing into the bioreactor. The experiment were first performed in semi continuous cycle of feeding the effluent from the digester to the bioreactors. The experiment was performed in batch mode which allows for the observation of growth of the algae (Dennic *et al.*, 2010).

#### 2.2.2. Working of semi continuous experiment

Initially the algae bioreactors were operated for a period of 48 days in a semi continuous mode. The retention time taken initially was 5 and 10 days. The different algae reactors with capacities of 5 liters and 10 liters were started with 0.5 liters and 1.5 liters of wastewater, which were

then diluted with tap water to make a total volume of 5 liters and 10 liters in the bioreactors (Deoliveira and Prado, 2006). The algae inoculum was already cultured is fed into the reactor at approximately 125ml in 5 liters bioreactor and 250ml in 10 liters bioreactor respectively. The daily loading was 0.5 liter and 1.5 liters of wastewater to achieve the different retention times. Apart from adding the wastewater to the reactor tap water was also added to compensate for evaporation and to maintain the 5 liters and 10 liters capacity in the bioreactor (Table 1). If the bioreactor was not found green with algae the wastewater loading should be increased until the bioreactor attains a healthy green color (Figure 2). The semi continuous experiments were performed so as to design the batch experiments effectively (Knothe, 2005).

**Table 1.** The loading rate for Semi continuous experiment

Reactor	Retention time (days)	Wastewater loading (L)	Tap water loading (L)	Routine loading of wastewater (L/day)
R1	5	0.5	4.5	0.5
R2	5	0.5	4.5	0.5
R3	10	1.5	8.5	1.5
R4	10	1.5	8.5	1.5



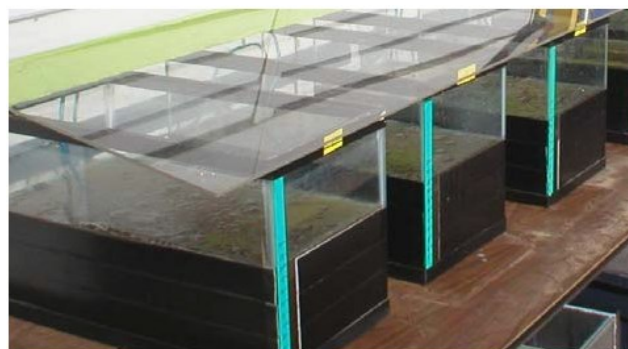
**Figure 2.** Photo bioreactors

### 2.2.3. Batch experiment

The experiment was carried out in two batches with two dilutions of wastewater in order to ensure that the light falling on the waste water does not get observed and also to ensure the proper growth of algae. The tap water was used to dilute the wastewater 10% in one set of tanks and 15% in another set of tanks (Table 2). The tap water was sparged with air for nearly 30 minutes inoculums which was cultured was provided in the tank from previous experiments. The inoculums concentration was 125 mg/liter and 250 mg/liter VSS in 5 liters and 10 liters capacity bioreactor respectively. Therefore 0.5 liter and 1 liter of Inoculums were used in 5 liters and 10 liters capacity bioreactor (Knothe and Steidley, 2005). The three constituents, tap water, algae inoculums, and anaerobic digester effluent, were mixed in a specific proportion to produce a uniform feed (Figure 3).

**Table 2.** Media of algae bioreactor

S. No	Description	5 litres bioreactor	10 litres bioreactor
1	Inoculums	0.5 L	1 L
2	Wastewater	0.5 L	3 L
3	Tap water	4 L	6 L



**Figure 3.** Bioreactors with 10% and 15% wastewater dilution

### 2.2.4. Operating conditions

Mixing was continuously provided by air and carbon dioxide sparging mechanical mixing was additionally performed by using glass stir rods which are bundled together. After mixing the temperature and PH was recorded and at the time of sampling tap water is added each time when the sampling has been done (Figure 4). The temperature was normally found to be 22°C (Thompson and He, 2006).

### 2.3. Processing of algae

The algal suspension which was homogenized is filtered through centrifuge separator. The obtained suspension is then dried to a constant weight using oven drying and then stored at 18°C until use. Sulfuric acid of 98% purity is used as a catalyst in the transesterification process. Methanol is used as the reacting alcohol which has its purity of 99.9% (Bozbus, 2003).

### 2.4. Lipid production by Folch method

The pure solvent from the upper phase and pure solvent from lower phase are to be collected in this method. The reagents used are Chloroform, methanol and the mixture of Chloroform and methanol in the ratio of 2:1 by volume. Chloroform, methanol and water are mixed in the separatory funnel in the ratio of 8:4:3 by volume. The mixture is undisturbed and allowed to stand for about 6-12 hours. Biphasic system is obtained when the mixture is undisturbed. The two phases are collected in the separate glass bottles and stored at 18°C (Krisnangkura and Pairintra, 2006). The approximate proportions of chloroform, methanol and water in the upper phase was found to be in the ratio of 3:48:47 by volume and the lower phase mixture proportion was found to be 86:14:1. The mixture which is obtained from upper and lower phase of the separatory funnel can directly be prepared in the above recommended proportions (Tat and Gerpen, 1999). The composition of pure solvent obtained from upper phase contains 0.02% calcium chloride, 0.017% magnesium chloride, 0.29% sodium chloride or 0.37% potassium

chloride with the help of composition the solution can be prepared in two ways, one of the method for the preparation of the solvent is to shake the glass stoppered vessel with the salt along with upper phase pure solvents and the solution goes complete. The other method for the preparation of upper and lower phase pure solvent is to use 0.04% of calcium chloride, 0.034% aqueous magnesium chloride, 0.058% aqueous sodium chloride or 0.74% aqueous potassium chloride instead of water should be used (Shu et al., 2007).

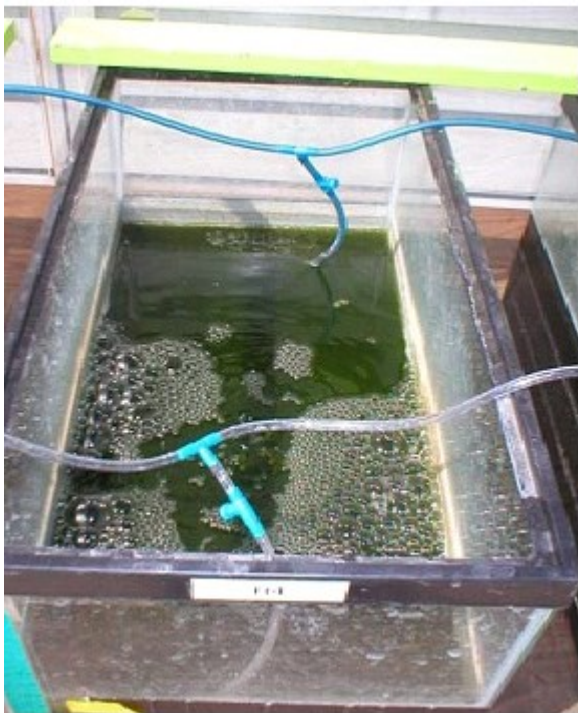


Figure 4. Operating condition of bioreactor

The following is the step-by-step procedure to obtain the lipids from the microalgae:

- The algal suspension is homogenized with the mixture of chloroform and methanol which should be in the ratio of 2:1 to a final volume of 20 times the volume of the tissue sample (1g in 20ml of solvent mixture). The mixture is then agitated in a shaker for about 15-20 minutes at the room temperature.
- The homogenized mixture is filtered either by using a filter paper of centrifugal separator for the recovery of liquid phase.
- The solvent is washed with 0.2% volume of water or 0.9% volume of NaCl solution. The mixture is agitated slowly and allowed to settle down. The two phases are separated without disturbing the settled liquid phases.
- The lower phase of the liquid obtained contains lipids which could be separated by the process of evaporation.
- The oil which is extracted was weighed so that to determine the total lipid content per dry algal biomass and analyzed. The properties of *Chlorella*

*Vulgaris* is characterized the sulfuric acid concentration is varied throughout the study (Figure 5).



Figure 5. Lipid's extraction experimental setup

### 2.5. Transesterification of oil

The biodiesel synthesis from *Chlorella Vulgaris* lipid was done by transesterification using methanol (1:12) with KOH as catalyst and the mixture is stirred for 15 minutes. The transesterification process takes about a total of 180 minutes. The temperature should be maintained during the reaction. For the effective results, the process is left undisturbed for 3-4 days for the formation of two phases (Figure 6). The phases are separated using pipette and anhydrous Na<sub>2</sub>SO<sub>4</sub> is added to the separated methyl ester. The supercritical reactants which are formed are taken in an oven at a temperature of 70°C. The pure biodiesel is thus obtained (Joshi and Pegg, 2007).



Figure 6. Extract of Transesterification Process

## 3. Results and discussion

The results obtained in various experiments (Semi continuous and batch) has been presented in this section R1 reactor refers to the 5L capacity bioreactor and R2 reactor refers to the 10L capacity bioreactor in the section.

### 3.1. Biomass growth in semi continuous experiment

The pH was found be about less than throughout the experiment. Initially the pH was found higher due to the uptake of CO<sub>2</sub> by the algae and in places of lower pH, the CO<sub>2</sub> sparging leads to lower pH. The solids concentration is observed after 10 days, which is usually considered as lag phase. The biomass concentration in the R2 reactor was found to be found 1.0g/L VSS on day 36 (Figure 7). The TSS

is found to be increased which is due to the fact that the anaerobic digester may have not performed properly. The aerobic bacteria tend to determinate the process which is also a main cause for the slow growth of algae. The dairy wastewater has high suspended solid concentration which causes the opacity. Hence, in batch experiments, the wastewater was diluted with 10% and 20% of water (Dmytryshym *et al.*, 2004).

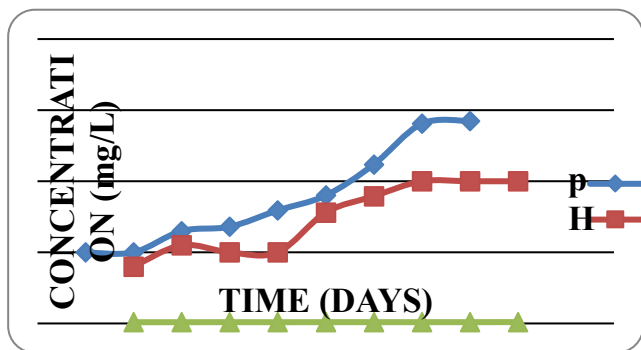


Figure 7. pH and biomass for semi continuous experiment

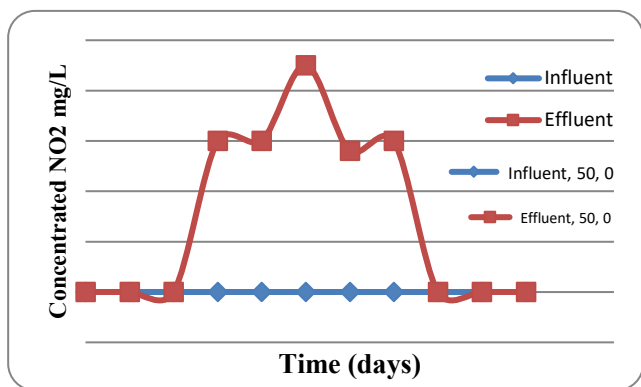


Figure 8. Concentration of Nitrite

3.2. BOD during semi continuous experiment

The samples obtained from the experiment were tested for its BOD. The BOD measured includes the respiration and degradation of the algae present in the sample. The BOD was observed to be about 150mg/L with a standard deviation of 10.9 mg/L.

3.3. Nutrient removal in semi continuous experiment

The influent and effluent concentrations were observed, and the nutrient removal can be determined in the experiment. The nitrite was almost undetectable in the influent whereas in effluent about 4.5 mg/L of nitrite ions at day 25. The nitrite covalent in the effluent is due to the nitrification process in the middle of the experiment (Figure 8).

The nitrate concentrations were observed in both influent and different during the experiment. The highest concentration influent contains relatively small amount of nitrate, and the concentration of 0.68 mg/L was achieved. The effluent contains very small amount of nitrate ions and hence the value of nitrate is almost neglected.

The Phosphate is one of the most extensive of the nutrients analyzed. The influent concentration gradually increases to the peak of about 28mg/L, but the effluent concentration remained low which is less than 2.0mg/L (Jain and Sharma, 2010).

3.4. Initial lipid content during the semi-continuous experiment

Lipid was extracted from the samples from two algae bioreactors. The total lipid percentage was nearly 25% by weight on the 25th day. The actual lipid productivity cannot be determined in the initial stages and therefore batch experiments were conducted (Table 3).

Table 3. The percentage of lipid in Biomass during semi continuous experiment

S.No.	Algal Bioreactor	VSS (mg/L)	Lipid %
1	R1(5L capacity)	550	18%
2	R2 (10L capacity)	690	25%

3.5. Results of experiment

The experiment which was performed in batch mode and the wastewater was diluted with 10% and 15% of water and no additional loadings were used (Zhu *et al.*, 2020). In order to increase the growth rate of algae, bioreactor is sparged with CO<sub>2</sub>. The average pH was maintained as 7.9 for both the dilutions. The results are obtained to a greater extent than the semi-continuous experiment (Chisti, 2007; Abdelfattah *et al.*, 2022).

3.5.1. Initial characteristics of wastewater

The wastewater is tested for its various parameters such as ammonia, nitrate, nitrites and other such concentrations (Table 4). The characteristics of the wastewater are tabulated as follows,

Table 4. Initial characteristics of Diluted wastewater

S.No	Wastewater characteristics	15% Wastewater	10% wastewater
1	TSS (mg/L)	200	140
2	VSS (mg/L)	185	105
3	pH	6.9	7.2
4	Ammonia(mg/L)	27.8	15.42
5	Nitrate(mg/L)	0.01	0.04
6	Nitrite(mg/L)	0.007	0.04
7	TKN	80.7	35
8	Phosphate(mg/L)	2.07	1.85

3.5.2. Results of biomass and growth curve for batch experiments

The microbial growth curve and biomass concentrations are analyzed. The biomass concentrations was found to be about 490-550 mg/L of VSS from day 6-10 for 10% dilution of wastewater and similarly the maximum biomass concentration was recorded as 950 mg/L for 15% dilution of wastewater from day 10 to 13 in the batch reactors. The production of biomass was found to be higher in 15% dilution due to the higher nutrient concentration (Figure 9). The maximum specific growth rate can be calculated by plotting a graph between the natural log and concentration with respect to time. The lower value in the growth rate

may be due to the higher turbidity that was found initially which can be noted in the TSS value and hence decreases the available light (Miao and Wu, 2006).

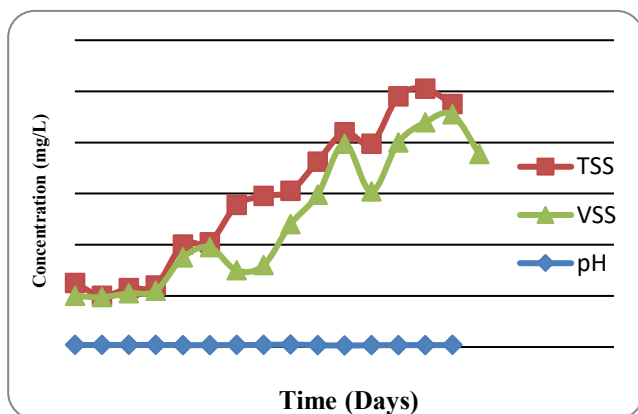


Figure 9. Biomass and pH for 15% wastewater dilution

#### 4. Conclusion

The productivity of Oil from the algae was observed in the batch type a reactor is comparatively higher than of terrestrial crops. The rate of production per year may scale upto 1200-2200 gal/acre/year with the same environmental conditions. The production efficiencies obtained from the daily wastewater grown algae were relatively remarkable uncontrolled conditions. The nutrient removal was found to be effective in the wastewater in batch experiments which reduces the ammonia concentration from 30mg/L to less than 1 mg/L and the phosphate removal efficiency reaches nearly 100%. The utilization of nutrients helps in reducing the fertilizer cost of the project. Furthermore, the significant nutrient uptake by the biomass suggests that the biomass waste left over after oil extraction has the potential to be used as crop fertilizer. This study represented a significant advancement in the production of biodiesel fuel from algae, exhibiting the ability to remove nutrients and increasing productivity. In order to validate the annual productivity of lipids and biodiesel fuel, year-round study should be conducted to analyze the seasonal variations. As a result, the biodiesel fuel can meet the demands of the future and can be effective solution for nation's global warming and energy independence. The yield that may be easily produced is substantially increased when algae are used as the feedstock for the manufacture of biofuel. A sustainable modern can be achieved as a result of simultaneous wastewater treatment and the production of biofuel.

#### Conflict of interest

The authors declare no conflict of interest.

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