The potential of *chlorella vulgaris* using fish effluent as a substrate for biodiesel production

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ABSTRACT

In this experiment, the physiochemical characterization of biodiesel produced from *chlorella vulgaris* cultured from fish wastewater from aquaponics systems was examined in relation to its water parameters. This effluent was collected from a biofilter that was connected to a fish tank used for culturing *chlorella vulgaris;* these parameters include electrical conductivity, total dissolved solids, nitrate nitrogen concentration, and pH The algae cultured in a 10-litre photobioreactor produced 30g of wet biomass, and 1.35g of biodiesel was produced using direct transesterification. FTIR physio characterization reveals ester formation at a wavelength of 1743 cm1. The physical properties of the diesel were tested and compared with Indian biodiesel standards. The diesel has a good flash point of 132 and a calorific value of 901 kJ/kg.

Keywords: chlorella vulgaris, water parameters, Culture, Physio Chemical Characterization, Algae

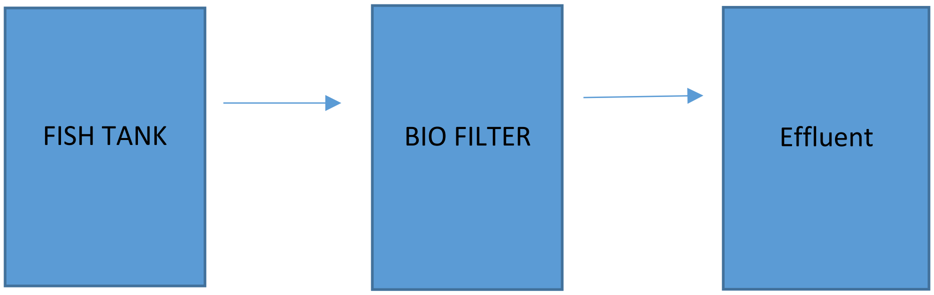
1. Introduction

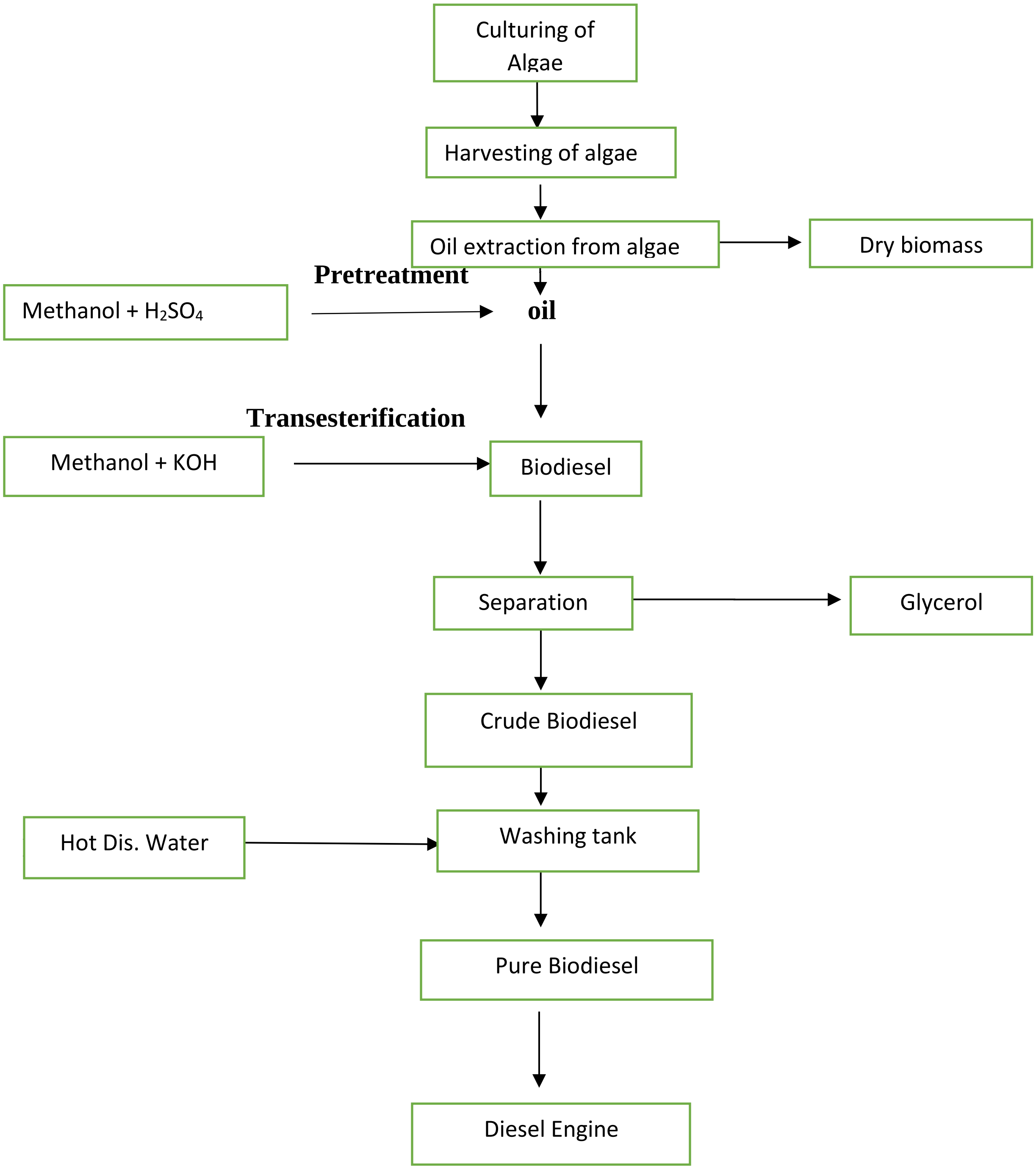
Biodiesel is a liquid fuel that is referred to as liquid biofuel," obtained from the chemical processing of vegetable oil, animal fat, or algae oil with alcohol. It can be used in a diesel engine alone or blended with diesel oil(Romano & Sorichetti, 2010), the best alternative to fossil diesel is biodiesel, which is also more environmentally safe than petroleum diesel.(Enwereuzoh et al., 2020b), this fuel is made from renewable resources such as non-edible vegetable oils, waste feedstock such as residual cooking oil, animal fats, or tallow, and aquatic biomass (microalgae or seaweed). Edible rapeseed oil is one of the popular feedstocks used in Europe for biodiesel production. Due to the environmental pollution associated with fossil fuels, the entire world has shifted its focus towards using renewable sources of energy in producing biofuels. Frequent usage of petroleum will intensify global warming, which is a problem caused by CO_2 emissions; from there arose the idea of utilizing plant oils or edible or non-edible oils as a fossil fuel substitute (Santaraite et al., 2020).

Microalgae have several advantages over other feedstocks for biodiesel since they grow quickly, can be cultivated on non-arable land, can be grown in wastewater, do not replace conventional edible crops, can be produced nearly every day, and their production is not seasonal. Its waste might be utilised for other uses or as animal feed.(*Baig et al. 2018*). As the demand on farmed fish grows, a growing global population will cause even bigger volumes of effluent emitted from aquaculture (*FAO.,2016)* ,wastewater from aquaculture is a significant source of organic nutrients, particularly nitrogen and phosphorus. This nutrient-rich aquaculture effluent can replace the massive quantities of synthetic fertilizers necessary for the growth of microalgae as a feedstock for biofuel(Markou & Georgakakis, 2011).

The majority of research on biodiesel production using algae as a feedstock has concentrated on biodiesel yield, the goal of this study was to use algae species *chlorella vulgaris* as a feedstock for biodiesel production, cultivated in aquaculture wastewater from aquaponics systems, to see the biomass yield, the growth rate, and also the physical characteristics of the diesel produced.

2.0 METHODOLOGY:





10ml of *Chlorella v* algae culture was collected from the university **Bharathidasan University,** A 200-liter tank was connected to the biofilter, which was constructed using a 40-liter tank containing a sponge filter, mechanical filter, ceramic rings, and bio balls, the effluent was collected from the bio filter for algal cultivation, a biofilter containing ceramics rings, a sponge filter that helps in filtering the uneaten food by the fish, and bio-balls used to hold beneficial bacteria, 10 liter Photobioreactor was also constructed for the culturing of the algae. The solid form of the uneaten feed will be trapped in a mechanical filter. 2) Ammonia, which is excreted by fish, needs to be oxidised to nitrate by nitrifying biofilters. 3) The dissolved carbon dioxide expelled by the fish must be removed, and oxygen must be added to the nitrifying bacteria in the fish(Goddek & Keesman, 2020). Some parameters, which include EC, TDS, temperature, and pH, were monitored daily. The nitrate concentration of the wastewater was also determined, and the alkalinity of the wastewater was analyzed before culturing.

2.1: Collection of the fish effluent and preparation

The flow rate from the fish tank to the biofilters was maintained constant throughout this experiment, and the hydraulic retention time (HRT), which is the average amount of time that liquids and soluble compounds stay in a reactor or tank (David et al., 2019), was determined. The HRT was constant throughout the experiment because a constant flow rate was maintained. The effluent was collected from the biofilter tank, and the temperature, TDS, and EC were measured, as well as the pH, Nitrate concentration, and the alkalinity of the effluent was determined to monitored to further correctly determine some of the important properties that have changed in the wastewater. An autoclave was used to sterilize the effluent, at 121^{O}C for 15 min as reported by (Lizzul et al., 2013) After sterilization, the effluent was allowed to settle, and then it was filtered.

2.2: Maintenance of Algal culture/ Scale up.

1 ml of the pure algal culture was inoculated with 9 ml of fish effluent for 5 days, during these days, the culture was exposed to a photoperiod of 8 hours, which is between 9 a.m. and 5 p.m., and then to the dark condition of 16 hours at a temperature of 25 ºC (DeniZ, 2020), At 6 days, the 10 ml culture was subcultured into a 100 ml flask, and again, this culture was allowed to grow for 5 days, 100 mL of culture was placed into 900 mL of the fish effluent medium, at this stage, the flask was designed so that aeration was introduced for 8 hours. At the same time, it was exposed to photoperiod for 8 hours, and its optical density was measured each day to find the growth.

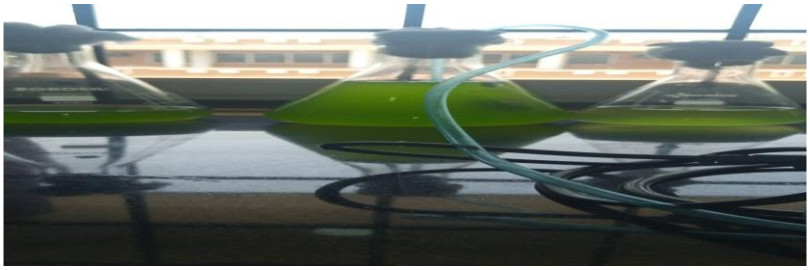


Figure 2: Algal culture in 1liter flask

Absorbance was used to measure the growth of algae culture in the bioreactor, spectrophotometer was used at 750nm(*B978-0-12-817536-1.00009-6.Pdf*, n.d.).

2.3: Harvesting of Algae Biomass

Microalgae was cultivated at log phase (Enwereuzoh et al., 2020a) using flocculation method (Vandamme et al., 2013), (Milledge & Heaven, 2013) for the harvesting of the biomass from the 10 liter photo bioreactor.

2.3: Preparation of Biodiesel from Algal Biomass

The direct transesterification reaction (Johnson & Wen, 2009) and (Enwereuzoh et al., 2020) were used to produce biodiesel (FAME) from cultivated algae biomass. In this method, wet biomass was used as feedstock, and water (80%, w/w) contained in the biomass was used for extraction. The biodiesel produced was washed to remove the contaminants in the fuel (such as glycerin, potassium salt, and methanol) using hot water (*Viewcontent.Pdf*, n.d.), and then dried.

Figure 2: Algal Culture in photobioreactor

2.4: Diesel characterization

FTIR, as proposed by Rosset and Perez-Lopez (2019), was used to analyses the functional groups present in the diesel produced

2.5: Diesel properties Test

The ASTM and Indian standards were followed in this experiment to determine the physiochemical characteristics of the diesel produced. Some of these standards, a sample of the diesel produced was taken to Sri Venkateswara Engineering Consultancy for the test, the results were compared with the ASTM Standards (*Alternative Fuels Data Centre: ASTM Biodiesel Specifications*, n.d.).

3.0: RESULT AND DISCUSSION

3.1: Collection of the fish effluent and preparation

A 200-liter tank was used to grow fish, with 15 catfish stocking the tank, and a 40-liter tank was used as a biofilter. The flow rate from the fish tank to the bio filter was maintained at 2250 ml/min, and then the effluent was collected for the culturing of algae from the bio filter. This filter tank contained a ceramic ring, bioballs, and a sponge filter. Figure 1-5 shows the water chemistry parameter for the filtrate in the biofilter. This parameter of effluent was taken during the culturing of 10 ml, 100 ml, 1 litre, and 10 litre cultures

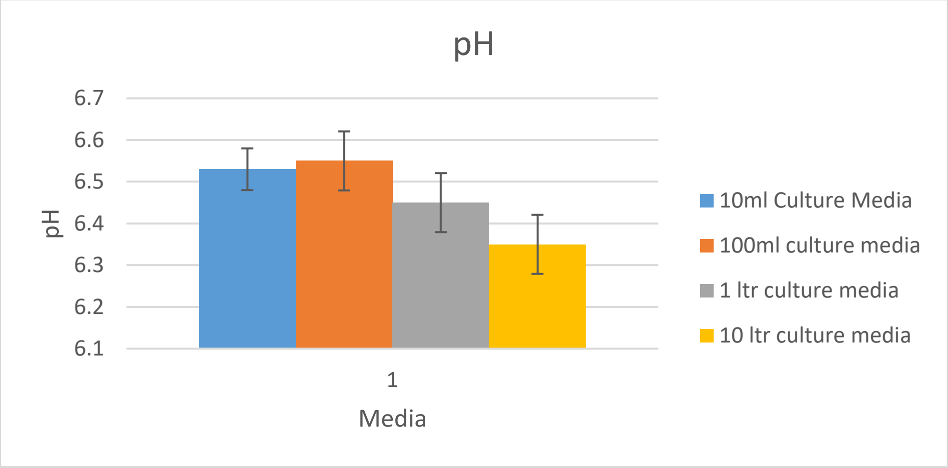


Figure 1: Different pH for Culture Media

According to figure 1, pH of 6.55±0.07 was the highest pH in 100ml culture media, as reported by (*Bergstrom_McKeel_Patel_2007.Pdf*, n.d.) pH has an impact on algal abundance. In one study, algal abundance increased when the pH was decreased from 6.6 to 5.0. The pH of our effluent in various concentrations of culture media favored algae growth. From figure 2, the electrical conductivity obtained from this experiment shows that the conductivity is high, meaning more ions are present in the effluent, the conductivity of water increases with the number of ions that are present. Similarly, water is less conductive the fewer ions there are in it(*Conductivity, Salinity & Total Dissolved Solids*, n.d.). The presence of higher EC in the effluent indicates the presence of more ions, which favors algal growth as shown in figure 4 and 5

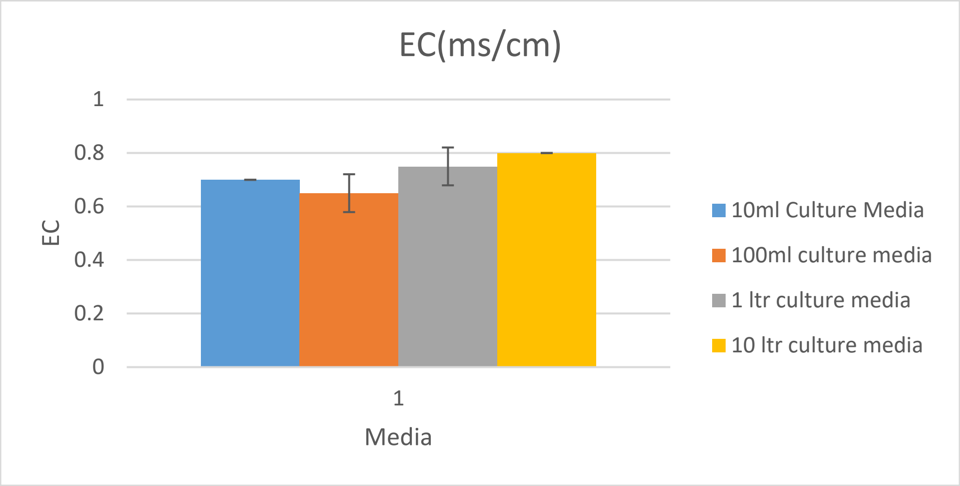


FIGURE 2: Different EC for Culture Media

From Figure 3, which shows the total dissolved solids present in the effluent, the 10 litre media has the highest TDS of 560 ppm, and it gives a very good growth of the algae in the photo bioreactor, as shown in Figure 4 and 5 TDS refer to any dissolved minerals, salts, metals, cations, or anions. Total dissolved solids (TDS) are the inorganic salts that are dissolved in water, mostly calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulphates. There are also some trace amounts of organic materials (*Total Dissolved Solids*, n.d.) .The effluent in this experiment shows a good concentration of dissolved solids, and these dissolved solids aid in algal growth.

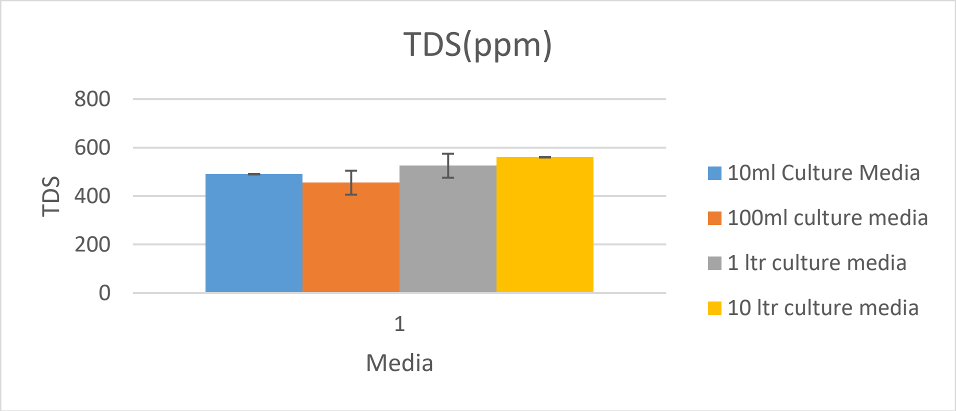


FIGURE 3: Different TDS for Culture Media

3.1.1: Nitrate Nitrogen (NO3 -)

The Nitrate Nitrogen The concentration of nitrate nitrogen was determined using a method proposed by *(APHA Method 4500-NO3)*, a spectrophotometer was used to measure the concentration of Nitrate Nitrogen at 220nm, for each of the samples of the medium, the concentration was determined. Table 1 shows the different concentration of Nitrate Nitrogen in the sample medium.

Culture	**10ml**	**100ml**	**1 ltr**	**10 ltr**
Nitrate Nitrogen (NO3 -)	**2.816mg/l**	**2.826mg/l**	**2.910mg/l**	**2.981mg/l**

Table 1: Nitrate Nitrogen Concentration

The effluent from the 10-ml culture has a nitrate nitrogen concentration of 2.816mg/l, while for the 10-litre culture in the bioreactor, the nitrate nitrogen concentration increases to 2.981mg/l. Considering the TDS of 10 litres, which is higher, this causes the nitrate nitrogen concentration to be high, so our effluent is good for algal growth.

3.2: Maintenance of Algal culture/ Scale up.

The algal species were scaled-up from 10ml to 100 ml, then 1000 ml to 10 L in a Photobioreactor. The reactor was designed to provide an optimum light intensity of 60.5µmol^{-1}m^{2}s^{-1} and aeration was provided by using an aeration pump. The growth rate was taken from 1litre culture and 10litre culture, figure 4 shows the growth rate of algae in 1litre, the OD was taken from 1st day of inoculation of culture to 5th day, the growth was increasing from 1st day to 5th day

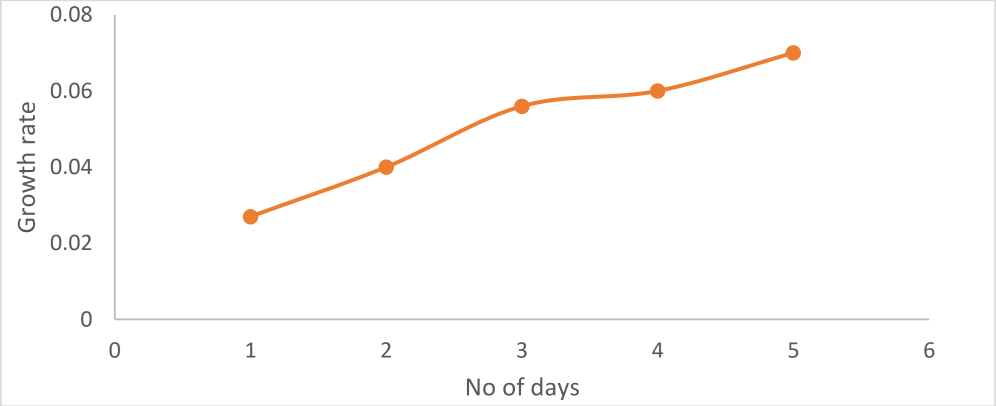


Figure 4: Growth rate for 1liter Algal Culture

The culture was scaled up to a 10-liter photobioreactor, the OD was taken until 15 days before harvesting, and figure 5 shows the growth rate for the 10-liter culture. Two batches of a 10-litre culture were cultured and harvested.

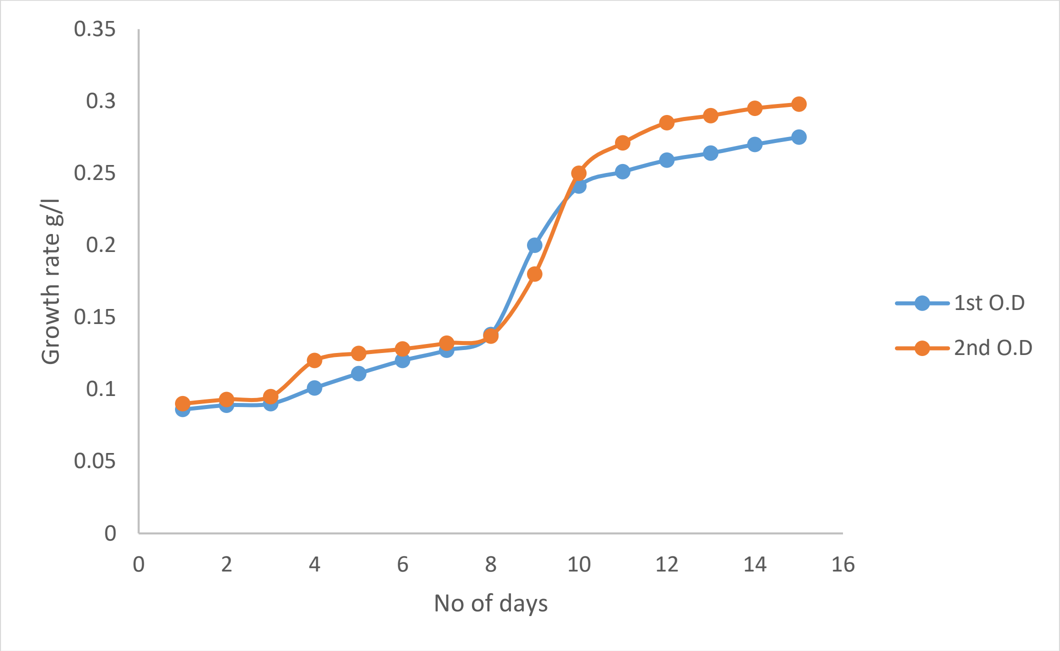


Figure 5: Growth rate for 1liter Algal Culture

The maximum growth rate is at day 15; it is shown in Figure 5, where the first culture attained a maximum growth rate of 0.298g/l and the second culture attained a maximum growth rate of 0.275g/l in day 15, At 15 days from Figure 5, the culture was in the log phase before it reached the stationary phase, where there would be no growth.

From the results of the water parameter, it will be concluded that fire-shallow effluent treated in a bio filter, the ceramics ring, and bio ball help in treating the effluent, and the ES, TDS, and pH of this effluent make it good for culture medium.

3.3: Harvesting of Algae Biomass

The method used is called the flocculation method; the pH was varied within the 10-litre culture in the reactor. Initially, the pH of the culture in the reactor was 7.3. Using 1m KOH, the pH was increased to 10. After adjusting to this pH, the entire biomass slowly settles down. About 80–85% of the biomass is removed using a micro filter. The remaining 15% of recovery was done by bringing pH back to its original range of 7.3.

The amount of wet biomass recovered from the 10-litre reactor from the two batches of the experiment is shown in figure 6.

Batch	Algae Biomass(g)
1	28.5
2	30

Table 2: Algae Biomass

3.4: Preparation of Biodiesel from Algal Biomass

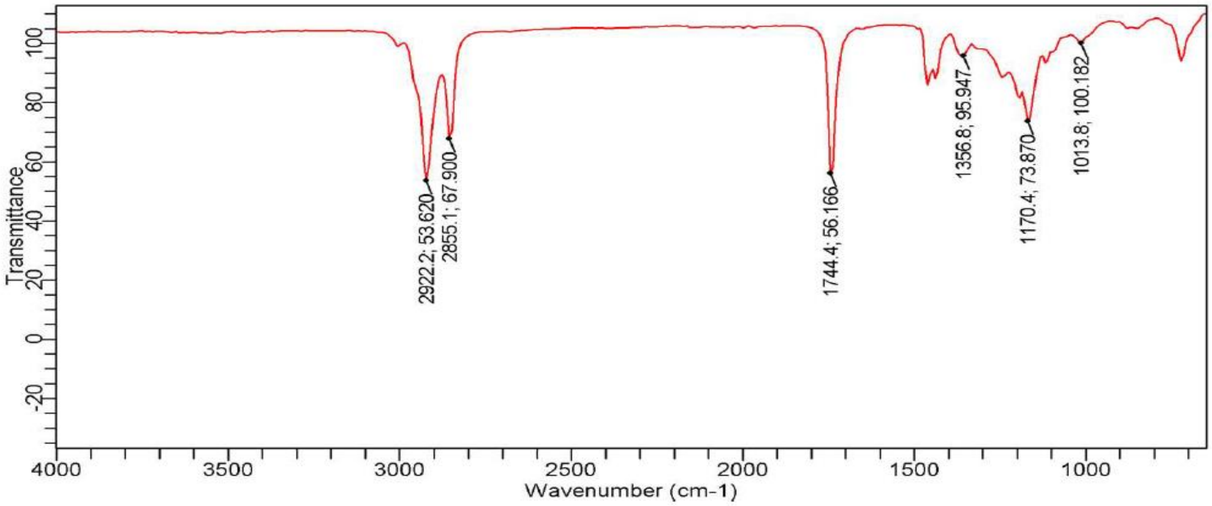
Using the method of direct transesterification, the wet algae biomass that was cultivated was used to produce biodiesel. Methanol was used as a catalyst. Table shows the amount of diesel produced after washing and drying the biomass

Algae Biomass(g)	Oil extracted(g)
28.5	1.28
30	1.35

Table 3: weight of oil extracted from the algae Biomass

3.5 Physio chemical properties of Biodiesel

FTIR was conducted at Veltech University, and a sample of the diesel was sent for analysis. Figure 6 shows the functional groups presence in the biodiesel. From figure 6, At a wavelength of 1356 cm1 and 1743 cm1, ester production results from the esterification process. Two more wavelengths, 2855.14 cm1 and 2922.23 cm1, which show the functional group of the CH bond as a result of esterification,



Peak	Wave Number	Vibration type
1	1013.83	C-H Bonding of alkyl
2	1170.38	C-C-O Stretching Carbonyl ester
3	1356	O-C-C Stretching of ester
4	1744.3	C=O Ester Carbonyl Stretching
5	2855.1 and 2922.25	C-H Alkyl stretching

Table4: wavenumber and vibratio

3.6: Physical properties of biodiesel

According to table 5, the kinematic viscosity of the diesel was 6.40, whereas the limit range for Indian standards was between 2.5 and 6, and the experimental diesel produced in this study had a kinematic viscosity of 6.40, indicating a 0.4 difference. Additionally, our diesel had a flash point of 132, whereas the standard (P21) had a min limit of 120, indicating that the biodiesel produced had a good flash point, which is one important

Fuel properties	Algae Biodiesel	Biodiesel Standard in India or ASTM	Limit	
			Min	Max
Kinematic Viscosity @40^{O}C in cSt	6.40	ISO3104/P25 (Indian standard)	2.5	6
Flash point	132	P21 (Indian standard)	120	-
Fire point	142			
Density(kg/m^3)	39.847	ISO3675/P32 (Indian standard)	860	900
Gross calorific Value Kj/kg	901			

Table5: Physical properties of biodiesel

4.0: Conclusion

The water parameters of the aquaculture waste water supported the growth of the algae, and the nitrate nitrogen in the fish waste water has enough concentration to be used as a culture medium in culturing algae, and also the species used, Chlorella *vulgaris,* has grown in this media and has also produced enough oil to produce biodiesel. The FTIR measurements also indicate the conversion of oil into fatty acids methyl esters. In the physical properties of the diesel produced, it has less density compared to the standard, which indicates that future experiments should be carried out to see the cause

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