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Escalation assessment for biomass generation in BG11 medium of *Chlorella minutissima*

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Abstract

Extract of *C. minutissima* confirms the production of biodiesel from lipid produced through microalgae. Also suggests the production of biodiesel, using microalgae, grown in presence of wastewater and exhaust gas from industries and vehicles. Apart from that this procedure also helps in waste remediation and utilization. In this technique nutrient and heavy metals are removed from wastewater as well as carbon dioxide is sequestering in form of fatty acid or biomass. Hence, three way benefits were achieved by outdoor culture of *C. minutissima*.

Keywords *Microalgae, Chlorella minutissima, biodiesel, bioremediation, clearer and renewable hydrocarbon fuel, fatty acids.*

Introduction

Climate change has reduced human security and threatened human wellbeing. An ignored reality of human progress is that human security largely depends upon environmental security. But on the contrary, human progress seems contradictory to environmental security. To keep up both at the required level is a challenge to be addressed by one and all. One of the ways to curb the climate change may be suitable scientific innovations, while the other may be the Gandhian perspective on small scale progress with focus on sustainability. Involvement of by-products of fossil fuels combustion like carbon dioxide, is recognized internationally a major cause [1,2] to change incessantly our global climate and yet now consumption of fossil fuel is predicted to grow incessantly[3,4].The development of sustainable sources of energy and chemicals is necessary to reduce greenhouse emissions responsible for global warming. Microalgae are microorganisms which can grow in photoautotrophic conditions utilizing cheap renewable resources (as CO_2 and sunlight) to produce new biomass. This biomass consists mainly of lipids, carbohydrates, and proteins, which can be used to produce biofuels and fine chemicals. Microalgae can ensure biomass and bioproduct productivity per hectare, which are higher than vegetable cultivations due to high content and high growth rate[5,29,38]. Nevertheless, microalgae based productions still present two main limits: the high costs (both fixed and operative), and the unfavorable energy balance, which is of particular relevance if biofuels are target products. In addition, high consumption of fertilizers is required to support microalgal elevated productivities[6,37]. Development of an integrated process in the biorefinery view could be a valid way to overcome these limits. In fact, microalgae are able to use organic carbon as energy source in heterotrophic and mixotrophic conditions [7,36]. This allows for their cultivation in media mainly made up of wastewaters that can be used to supply nutrients and reduce the global costs of the process [8,35]. Although improvements in the recovery of traditional fossil fuels, more attention needs to be paid to the search for clean and viable alternative renewable energy resources like biodiesel with the prospect of minimizing increases in atmospheric CO_2 by recycling carbon from the atmosphere [9,10,11].

Potential feedstock's for biodiesel production

There are various animal and plant oils which can be used for production of biodiesel like palm oil, maize, etc. among all microalgae can be the most potent feedstock for biodiesel production.

Micro-Algae

Chlorella minutissima is a eukaryotic alga, with relative fast growth and easy cultivation, and the levels of amino acids and polyunsaturated fatty acids in *Chlorella minutissima* are high, which could be potentially useful in health foods and pharmaceuticals [12,32].

There have been few studies focused on biofuel feedstock production using *Chlorella minutissima* [13,33]. Moreover, *Chlorella minutissima* is an extremely high-CO2-tolerent alga, which may be developed to use waste CO_2 for algal cultivation. Therefore, one freshwater strain, *Chlorella minutissima*, was chosen for the present investigation to find the best conditions of cultivation, including light source, light intensity, photoperiod and nutrient for high growth rate and cell density, oil content, and suitable FA profile for biodiesel production [14,34]. The production technology of microalgae is one of the most difficult and complex points need to be solved, including harvesting microalgae, oil extraction and energy conversion parts. In present scenario scientists and industrialist are able to manage this all difficulties one or two at a time but yet the failure comes in part of efficiency of the renewable fuel which affect the cost of large scale production of renewable fuel and hence biofuel is not fit properly in current quest of renewable energy[15,31]. Thus the fulfilment of this energy structure, the need of efficient renewable energy in the form of algal fuel up to certain extent is still the need of an hour.

Material and Methods

Materials

Sample Collection

Green Microalgae (*Chlorella minutissima*) were purchased from Centre for Conservation and Utilization of Blue Green Algae (CCUBGA) Division of Microbiology, ICAR, and Indian Agricultural Research Institute New Delhi- 110012.

The samples were subculture using BG11 medium by making media distilled water.

Table 1: Composition of BG11 Media

Components	BG11 medium
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
Ferric ammonium citrate	6.0 mg
$CaCl_2{\cdot}2H_2O$	0.036 g
$MgSO_4{\cdot}7H_2O$	0.075 g
Na_2CO_3	0.02 g
EDTA	1.0 mg
Citric acid	0.006 g
Trace metal solution	1 ml
DI Water	1 L

Methods

Biomass Processing: At first, the biomass was dewatered by filtration with a Whatman filter paper. Then the wet algal biomass was collected from the filter paper with a spatula and taken in Petri dishes. The biomass was then dried at 80°C for 2 hours and kept in the desiccator for whole night so that there is no water left in the sample. The dry mass was then grounded properly and weighed immediately. The drying step was skipped in case of wet extraction process [13, 20].

Soxhlet Extraction of Lipids: Extensive study and research were conducted earlier to determine the best extraction method over wet and dry algae biomass. In this experiment, the effect of three different solvents, that is, n-hexane [16] the mixture of chloroform and methanol at

2:1 v/v ratio [17, 30]. For this, 5 g of dry weight and 10 g of wet fresh algae paste were taken into the extraction chamber separately.

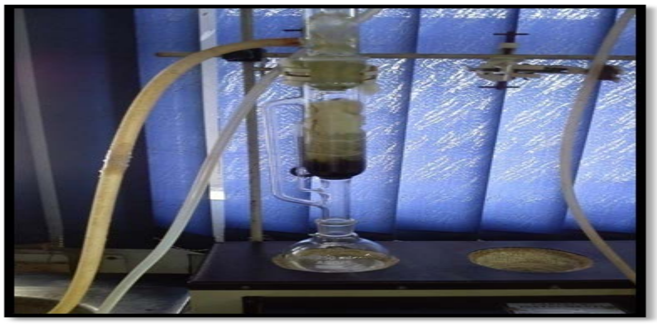


Figure 1: Soxhlet Extraction Apparatus

Both ends of the chamber were enclosed with cotton balls to withstand any solid algae discharge. The bulb was filled with 180mL of one of the three organic solvents and heated at 80°C. The solvent gradually started to vaporize and after condensation, it fell into the extraction chamber containing the algae and release lipids into the chamber. The solvent-lipid mixture was then reached to a critical height within the chamber and the siphoning process was initialized; that is, the mixture was drawn back to the bulb[18,29].

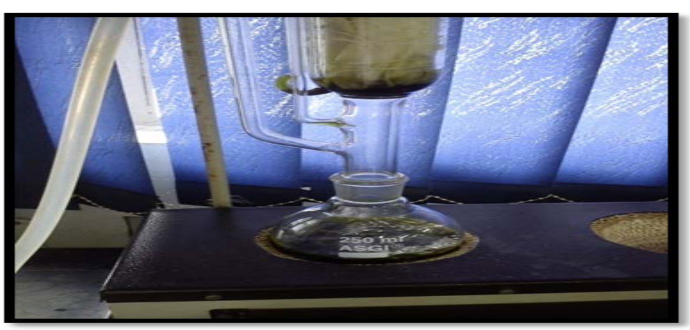


Figure 2: Lipid Extraction by Soxhlet Extraction Apparatus

The system recirculates the solvent by constantly boiling and condensing it. At the end of this extraction process, a mixture of solvent and oil was left in the bulb and the algae biomass was left in the chamber. The processing before evaporation was performed as described by [19,26,32]. The crude extract was taken into a separating funnel and washed with 1% aqueous sodium chloride solution (50 mL) twice. The aqueous layer was removed and the solvent layer was passed through a layer of anhydrous sodium sulphate by taking it in a glass funnel, blocked with cotton plug.

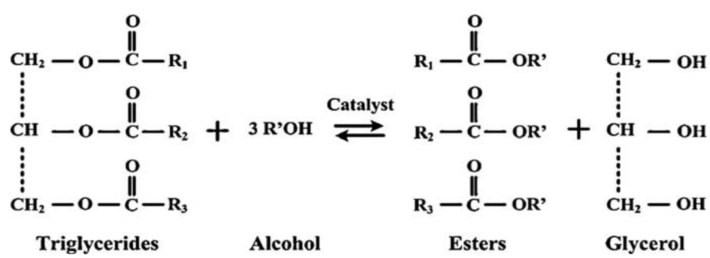
Transesterification

The aim of this technique is to transform the triglycerides which constitute the oil, to methyl esters [20,28] in which oils or fats are reacted with alcohol (like methanol or ethanol) by a catalyst (more often than not sodium or potassium hydroxide) to break long chain fatty acids in oil, separating the straight chain of esters from glycerol [21,25].

Principle

Transesterification is the course of action by which the glycerides, present in fats or oils react with an intoxicant in the bearing of a catalyst to form esters and glycerin [22].

Transesterification Reaction



Where R1, R2, and R3 are long chains of carbons and hydrogen atoms, sometimes called fatty acid chains.

Protocol

- Before proceeding to Transesterification, oil extracted from microalgae were strained and heated between 65 – 70ºC for 30 minute.
- Methanol (5:1 molar ratio methanol/oil) was blended with sodium hydroxide (0.25% w/w), until all of the NaOH was dissolve in methyl alcohol.
- This mixture was then adds to the extracted oil, and further heated to 60ºC, for 2 hours.
- Then the ester was be purified by washing with distilled water and citric acid and drying at 100ºC for 4 hours[13].

Results and Discussion

Contextual

Increment in world population results in increment of energy source, but our conventional source of energy is depleting very rapidly. To sustain or fulfil energy requirement urgent substitute of conventional energy is needed. One of the best answer is bio-energy i.e. energy derived from biomass. Among all biological energy, algal biodiesel is one of the most reliable and potent source of energy. Being a micro-organism, doubling rate of algae is very high and its oil accumulating capacity is also very high [22].

Table 2: OD readings of growth of microalgae *C. minutissima* .

Days	Sample 1	Sample 2	Sample 3
Day 1	0.008	0.011	0.021
Day 2	0.012	0.014	0.026
Day 3	0.322	0.216	0.251
Day 4	0.411	0.323	0.326
Day 5	0.635	0.446	0.492
Day 6	0.82	0.562	0.582
Day 7	0.9	0.642	0.663
Day 8	1.108	0.702	0.709
Day 9	1.254	0.752	0.849
Day 10	1.531	0.875	1.023
Day 11	1.72	0.937	1.156
Day 12	1.912	0.978	1.296
Day 13	2.042	1.144	1.553
Day 14	2.358	1.37	1.823
Day 15	2.346	1.427	1.82
Day 16	2.339	1.416	1.815
Day 17	2.332	1.403	1.807
Day 18	2.325	1.392	1.796

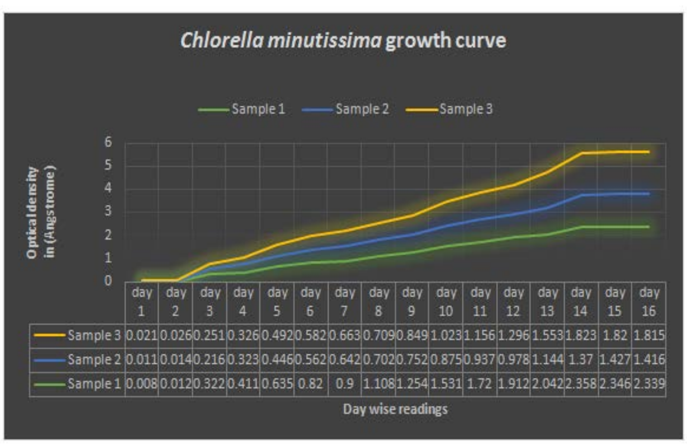


Figure 3: Growth curve of *Chlorella minutissima* at wavelength 670nm in BG11 medium

The image depicts the development curves for the culture media. These curves exhibit all characteristic growth forms. The exponential development in all culture media lasted for 6 to 13 days, travelled along by a stationary phase until the final stage of cultivation.

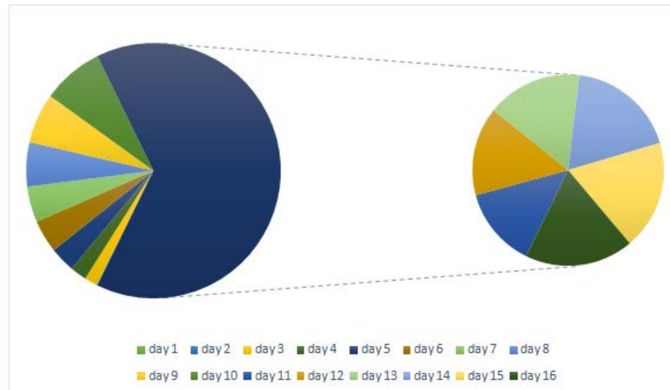


Figure 4: *Chlorella minutissima* growth's pie chart

The above pie chart clearly depicts that the more than 50% part grown in just 6 days, which shows the growing capacity of the microalgae *Chlorella minutissima.*

5.2 Large scale production of microalgae

For large scale microalgal biomass cultivation, scale up the culture of microalgae in BG11 medium in triplets in serial dilution starting from of volume 10 ml to 18 liter.

Figure 5: Large scale biomass generation/ cultivation

5.3 Filtration of Biomass

Filtration is good method for isolation of dry biomass. Algal culture poured in filter paper and after dewatering of certain amount weighted with the help of weighing machine and then dried into the hot air oven at 60°C for 24 hours.

Figure 6: Wet biomass of Microalgae *Chlorella minutissima*

Table 3: Wet Biomass of microalgae *Chlorella minutissima*

	Wet Biomass (weight in gm)
Container 1	29
Container 2	36
Container 3	31
Total	96*

*Total wet microalgal biomass = 96 gm in 50 liter

5.4 Determination of dry weight

Dry weight was determined by filtering a fixed volume of the algae suspension through a pre-weighed filter paper with a 0.11 μm pore size. The algal dry weight was calculated by subtracting the mass of the filter from the total mass [23].

Table 4: Wet biomass in different volumes.

Microalgae	Wet biomass wt (mg/100mL)	Wet biomass wt (mg/L)	Wet biomass wt (mg/10L)	Wet biomass wt (mg/50L)
Chlorella minutissima	192	1920	19200	96000

Table 5: Dry biomass in different volumes

Microalgae	Cell Dry wt (mg/100mL)	Cell Dry wt(mg/L)	Cell Dry wt(mg/10L)	Cell Dry wt(mg/50L)
Chlorella minutissima	134	1340	13400	67000

Dry biomass = Mass of filter paper after dry algae – Mass of filter paper without algae

= 2.59-1.25

= 1.34 gm/liter

5.5 Lipid isolation

For lipid extraction using Soxhlet apparatus, 5 g of dry weight was taken into the extraction chamber separately. Both ends of the chamber were enclosed with cotton balls to withstand any solid algae discharge.

The bulb was filled with 180mL of one of the three organic solvents and heated at 80°C. The solvent gradually started to vaporize and after condensation, it fell into the extraction chamber containing the algae and release lipids into the chamber. The solvent-lipid mixture was then reached to a critical height within the chamber and the siphoning process was initialized; that is, the mixture was drawn back to the bulb. The system recirculates the solvent by constantly boiling and condensing it. At the end of this extraction process, a mixture of solvent and oil was left in the bulb and the algae biomass was left in the chamber. The processing before evaporation was performed as described by Kumar *et al.* 2013.

The crude extract was taken into a separating funnel and washed with 1% aqueous sodium chloride solution (50 mL) twice. The aqueous layer was removed and the solvent layer was passed through a layer of anhydrous sodium sulphate by taking it in a glass funnel, blocked with cotton plug.

The total lipid extracted from microalgae is 15.57 gm after solvent extracted using Soxhlet method

5.6 Productivity of lipid

% of total oil recovered = Weight of crude oil extracted / Weight of dry algae biomass x 100

= (15.57/ 67) x 100

= 23.25%

5.7 Transesterification

Methanol **(**3:1 molar ratio methanol/oil) was mixed with sodium hydroxide 0.25%w/w, until all NaOH was dissolved in methanol. The ester was purified using distilled water and citric acid.

5.8 Biodiesel production

The total amount of biodiesel obtained from transesterification was 14.03 gm from dry biomass of 67 gm/50L so,

% of biodiesel production = (weight of biodiesel obtained / weight of dry biomass) x 100

= (14.03/ 67) x 100

= 20.94%

The need of whole race of human's mechanical energy's future depends on future of production technology of the biodiesel which can be achieved by dry biomass 1.34 gm / liter Which is in weight 10mg and in percent 5.58% higher than previous biomass generation done by Tang *et al.*, 2011.

Conclusion

In the current scenario, main blockage of bio-fuel creation from microalgae is that the present applied sciences do not permit an fiscal and sustainable bio-fuel production at modern day vigour prices[26,27] even though high biomass, lipid productivity, and nutrient elimination efficiency of wastewater grown microalgae make them promising as a feedstock for renewable vigour [24,30].

In addition, there is need of nutrient consumption charges of wastewater derived algal bio-fuels, bio-prospecting of extraordinary wastewater habitats to explore indigenous oil producing micro-algal lines. Oil-rich microalgae species are the most productive fuel crops, providing 10–100 times higher biomass and oil yield than land oil crops. Hence the objective was to analyze the consistency, sustainability and feasibility of biodiesel produced from microalgae *Chlorella minutissima*.

- Generation of dry biomass in ratio i.e. 1.34 gm / liter
- Production of the biofuel in 14.03 gm.
- Proficient in using the waste material like (water, land, air).
- Production cost is favorable for commercial production.
- Positive impact on local and global environment (land, plant & air, water and food cycle) animal and human race and therefore the objective was achieved.

Biodiesel has become further gorgeous in recent times because of its ecological reimbursement and the fact that it is made from renewable resources. Thus, biodiesel may be well thought-out as diesel fuel substitutes. It is one of the significant alternative energy sources and it becomes more and more strategic because of the critical environmental problems, decrease in fossil energy sources, increase in energy import bills causing from fluctuations of the oil price.

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