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PRODUCTION OF SINGLE CELL OIL FROM MICROALGAE GROWN IN WASTEWATER AND CONVERSION OF THE SAME TO BIODIESEL

Bhagyashree Magdum, Trupti Phapale, Anil C. Ranveer
Department of Technology, Shivaji University, Kolhapur, India.

ABSTRACT: Biomass produced is reused and allows efficient nutrient recycling. Sustainable production of renewable energy is being debated globally since it is increasingly understood that first generation biofuels, primarily produced from food crops and mostly oil seeds, compete for arable land, freshwater or biodiverse natural landscapes and are limited in their ability to achieve targets for biofuel production. This study was undertaken to evaluate the remediation of wastewater by the green alga Chlorella spp and the potential of these algae (biomass) to produce sustainable biofuel. Microalgae were grown in BG11 media, harvested by different techniques like centrifugation, filtration and flocculation and lipids extracted by soxhlet extraction. The experimentation shows that centrifugation is more efficient technique to harvest microalgae, followed by flocculation and filtration. By centrifugation, biomass harvested is 0.982g and 0.39g of Chlorella MA-8 and MA-13 respectively. Flocculation was carried out by three flocculants like FeCl3, (NH4)2SO4 and Chitosan. This activity depicts that FeCle3 is more effective when compared to other two flocculants. The lipid profiling was done by GC-MS analysis. It showed that lipid which is extracted from microalgae contains linoleic acid.

KEYWORDS: *Chlorella* spp, single cell oil, harvesting methods, lipid profiling

***Corresponding Author: Dr. Anil C. Ranveer** Ph.D.
Department of Technology, Shivaji University, Kolhapur, India.
*Email Address: anilranveer@gmail.com

1.INTRODUCTION

Algae are among the most potentially significant sources of sustainable biofuels in the future of renewable energy. Thorough analysis reveals Chlorella vulgaris to be one of the most effective microalgae in terms of production of single cell oil as well as waste water treatment [1]. Investigations have been done widely to evaluate the effects of different algal media components to get optimized cell count of Scendesmus dimorphus [2]. Wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling [3]. Additional challenges of micro-algae harvesting come from the small size of micro-algal cells, the similarity of density of the algal cells to the growth medium, the negative surface charge on the algae and the algal growth rates which require frequent harvesting compared to terrestrial plants [4]. Algae can be harvested by a number of methods; sedimentation, flocculation, flotation, centrifugation and filtration or a combination of any of these. For microalgal oil extraction, solvent mixtures containing a polar and a non-polar solvent could extract a greater amount of lipids [5].

2. MATERIALS AND METHODS

Microalgae cultures used are Chlorella sp strain MA-8 and MA 13 (The strains are kindly provided by Department of Microbiology, University of Agricultural Sciences, Dharwad, Karnataka). The suitable medium for growing microalgae is BG11 media. After three weeks, microalgae when attains stationery phase (which is suitable stage for harvesting) is harvested by different harvesting methods.

Harvesting techniques:

i) Centrifugation: The broth was centrifuged at 8000 rpm for 15min. By discarding the supernatant, weight of biomass was noted.

ii) Filtration: The media containing microalgae was filtered by Whatman filter paper 41 and the membrane was weighed, which gave weight of biomass.

iii) Flocculation: Flocculation was carried by using three flocculants. They were chitosan,
1M Ferric chloride, and 1mM Ammonium sulfate.

Extraction of oil:

Soxhlet extraction: Dried microalgal powder (~ 1 g) was placed in a sample holder of soxhlet extraction apparatus. The solvent used for lipid extraction was hexane. The extractions were allowed to proceed for about 4 h with subsequent removal of solvent by rotary evaporator. The fatty acid profile of extracted oil was studied by GC-MS analysis.

Wastewater treatment: Wastewater sample was collected from nearby location and its physico-chemical properties like pH, nitrogen content, phosphorous content and COD. To this water, inoculum of Chlorella MA-8 and MA-13 was added and incubated under controlled conditions. The flasks were shaken occasionally to avoid sedimentation of cultures at the bottom. As and when the culture attained stationary phase i.e., after three weeks, the cultures were harvested from the water by centrifugation. The amount of biomass yield was determined by taking the weight of the biomass. The supernatant was collected analyzed for total nitrogen content (ammonium and nitrate content), phosphorous content and COD.

3. RESULTS AND DISCUSSION

Single cell oil is successfully produced and extracted from Chlorella spp MA-8 and MA-13 strains of microalgae. The results of different harvesting methods and fatty acid profiling by GC-MS analysis is discussed below. By visual properties, it is found that, Chlorella sp MA-8 is having higher chlorophyll content than Chlorella sp MA-13.

Optimization of harvesting methods:

Microalgae were harvested by three different methods like centrifugation, filtration and flocculation. The amount of biomass obtained from these techniques is tabulated in the Table 1

Table 1: biomass obtained from various techniques

Microalgae Species	Amount of biomass collected in grams				
	Centrifugation	Filtration	Flocculation		
			Chitosan	$FeCl_3$	$(NH_4)_2SO_4$
Chlorella MA-8	0.983	0.12	0.72	0.98	0.95
Chlorella MA-13	0.97	0.21	0.81	0.91	0.902

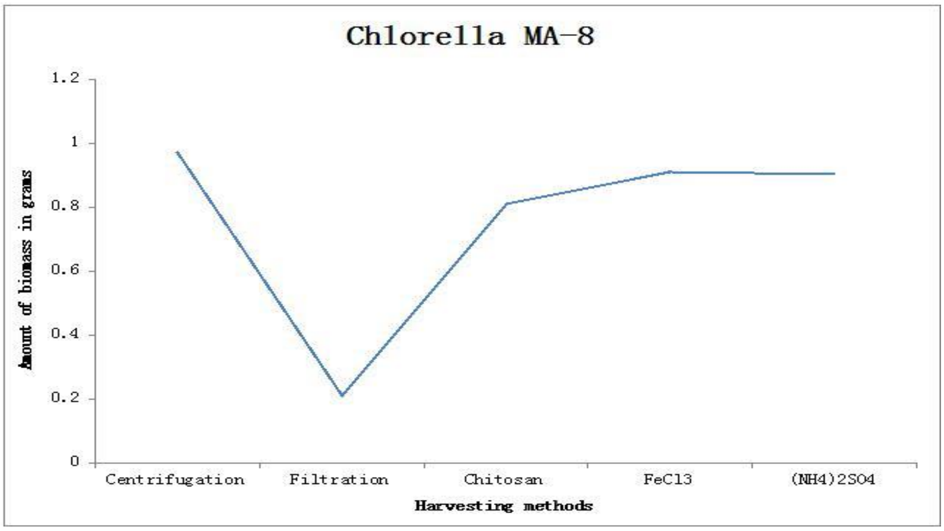

Figure 1: Amount of *Chlorella* MA-8 biomass (g) harvested by different methods

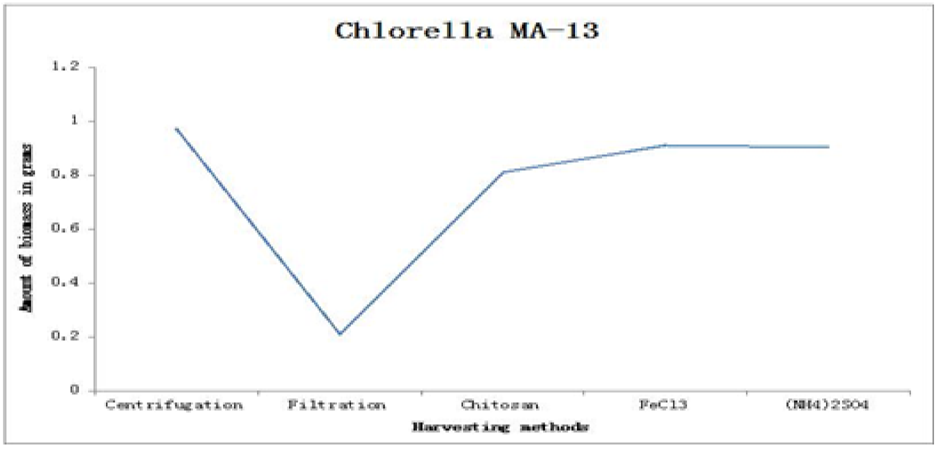

Figure 2: Amount of *Chlorella* MA-13 biomass (g) harvested b different methods

This experiment reveals that centrifugation is prohibitive for bulk uses such as for biofuel and feed as its cost of operation is high compared to other techniques there is more yield of biomass and centrifugation is found to be effective for harvesting microalgae in small scale as it has harvested biomass up to 0.983g of biomass for 100ml of broth. From this study we can claim that centrifugation is the best method of harvesting microalgae when compared to other harvesting methods like filtration and flocculation. However, on larger scale, flocculation would be the preferred method of microalgae harvesting.

GC-MS Analysis of lipid:

Considering the standard graph s GC graph of methyl esters which is referred from Moldoveanu et al, 2014, it is clear that the oil extracted from microalgae contains oleic acid and linoleic acid. Our experimentation results reported that Chlorella sp MA-8 has 39.06% of oleic acid and 60.94% of linoleic acid. Similarly Chlorella sp MA-13 contains 45.63% of oleic acid and 54.37% of linoleic acid. From the literature it is noted that the most abundant FAs in the lipid extracts (approx. 70% of total FAs), such as C16 hexadecanoic (or palmitic) acid, C18:1 (n-9) oleic acid and C18:2 (n-6) octadecadienoic (or linoleic) acid.These FAs are normally treated as the major components for microalgal biodiesel production. The US Department of Energy indicates that a perfect biodiesel should only comprise mono-unsaturated fatty acids. It is also shown that it is possible to operate an engine with a methyl ester containing more than 30% of unsaturated acids. The European Standard EN 14214 limits linolenic acid methyl ester content in biodiesel for vehicle use to 12% (mol). By considering all these information, we can confirm that the oil the extracted from Chlorella sp MA-8 and Chlorella sp MA-13 has all requirements for biodiesel to be in its stable form and also accounts for ignition property of biodiesel.

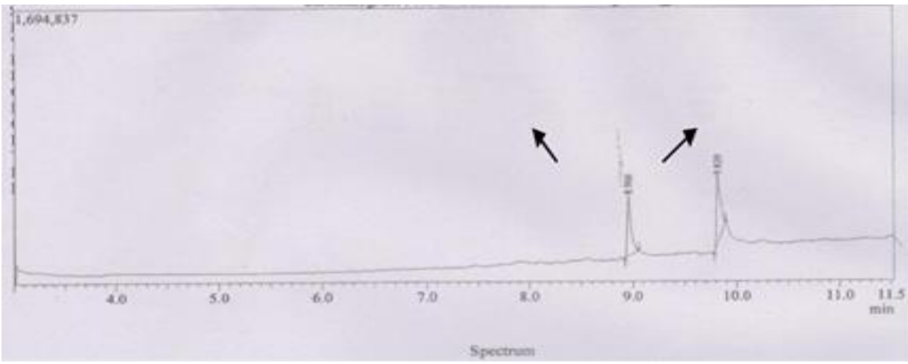

Figure3: GC of *Chlorella* MA-13

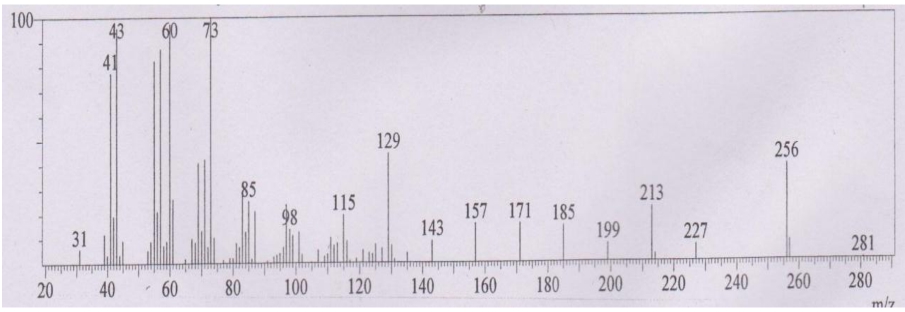

Figure4: MS of *Chlorella* MA-13

Table 2: Fatty acid composition of oil extracted from *Chlorella* MA-13

Fatty acid	Oil Area %	Name of fatty acid
C18:2	45.63	Oleic acid
C18:1	54.37	Linoleic acid

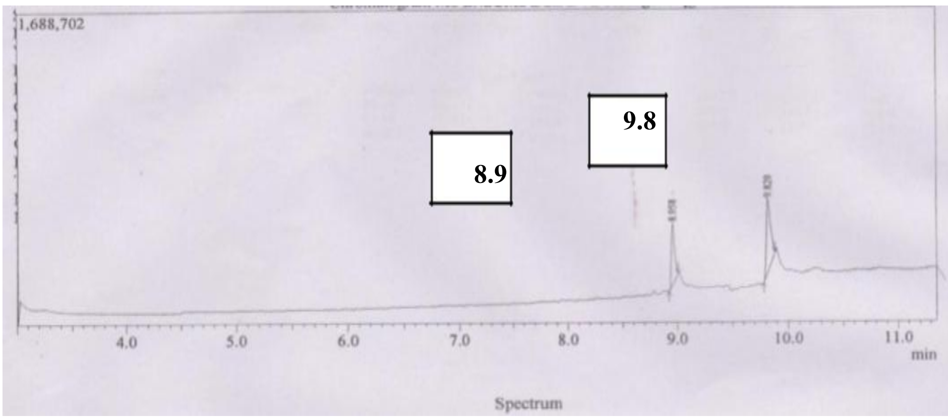

Figure 5: GC of *Chlorella* MA-8

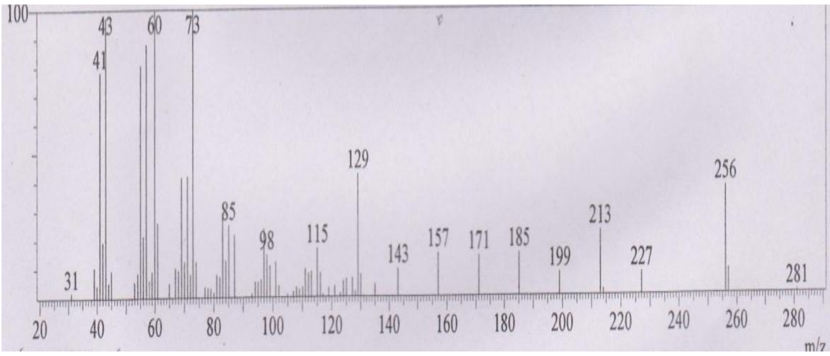

Figure 6: MS of *Chlorella* MA-8

Table 3: Fatty acid composition of oil extracted from *Chlorella* MA-8

Fatty acid	**Percentage area of oil**	**Name of fatty acid**
C18:2	39.06	Oleic acid
C18:1	60.94	Linoleic acid

Wastewater treatment:

The biomass of microalgae obtained by growing in waste water is depicted in the table 5.4. This clearly explains that *Chlorella* MA-8 has grown better when compared to *Chlorella* MA-13. It can also be noticed that, *Chlorella* MA-8 has grown much better in wastewater than in BG11 media. This may be due to the complex nutrients available in waste water.

Table 4: Amount of biomass obtained when grown in polluted Unkal Lake water

Sr. No.	**Microalgal strain**	**Amount of biomass (g)**
1.	*Chlorella MA-8*	1.05
2.	*Chlorella MA-13*	0.9

The physico-chemical parameters of treated and un-treated waste water were analyzed and the results are listed in the table 4 to table 6

Table 5: Physico-chemical parameters of un-treated waste water

Sl. No.	Parameters	Results (mg/ml)
1.	Chemical Oxygen Demand	55.0
2.	Nitrate as NO_3	5.6
3.	Phosphorous	17.00

Table 6: Physico-chemical parameters of waste water grown by *Chlorella* MA-8

Sl. No.	Parameters	Results(mg/ml)
1.	Chemical Oxygen Demand	37.00
2.	Nitrate as NO_3	4.60
3.	Phosphorous	3.53

Table 7: Physico-chemical parameters of waste water grown by *Chlorella* MA-13

Sl. No.	Parameters	Results (mg/ml)
1.	Chemical Oxygen Demand	49.00
2.	Nitrate as NO_3	5.18
3.	Phosphorous	14.75

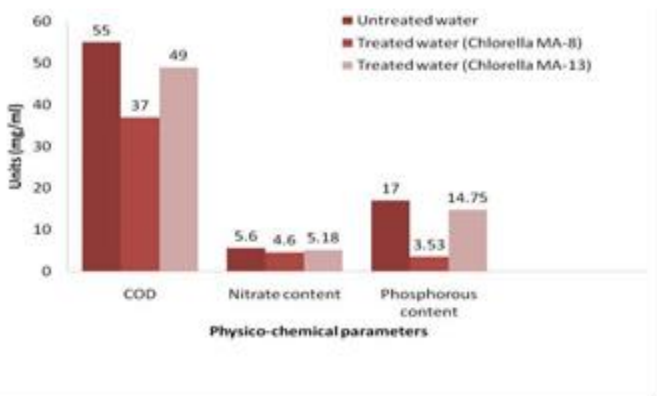

Figure 7: Efficiency of wastewater treatment by *Chlorella Sp*

Nitrogen and phosphorous plays an important role as major nutrient for growth of microalgae. These microalgae utilize nitrogen and phosphorous present in the wastewater and grow, which accounts for water treatment. The COD of waste water before treated with microalgae was 55mg/ml, nitrate content 5.6mg/ml and phosphorous content 17mg/ml. When the treated water was analyzed for the same parameters, it showed good results. Physico-chemical parameters of water treated from *Chlorella* MA-8 showed COD of 37mg/ml, nitrate content 4.60mg/ml and phosphorous content of 3.53mg/ml. Similarly physico-chemical parameters of water treated from *Chlorella* MA-13 showed COD of 49mg/ml, nitrate content 5.18mg/ml and phosphorous content of 14.75mg/ml. This experiment clearly depicts that the polluted waste water is successfully treated by microalgae as there is drastic decrease in nitrate content, COD and phosphorous content from untreated water to treated water and *Chlorella* MA-8 is more efficient in doing this. Even though nitrate was not much consumed by alage, but there is tremendous decrease of phosphorous content. This clearly shows that these strains of *Chlorella* can be used for treating polluted waste water in an economical and eco-friendly condition.

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