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Isolation, Characterization of algal Chlorophyll and Hydrocarbon content in algae found in National Capital Region

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Key words: Algae, Biomass, Hydrocarbon, Fatty acids, Chlorophyll.

Abstract

The need to develop and improve sustainable energy resources is of eminent importance due to the finite nature of our fossil fuels. This project deals with a third generation renewable energy resource like algae biomass. Different samples of algal biomass were collected from different localities of NCR. The different Algae were identified as *Ulothrix, Ulva, Cladophora, Rhizoclonium.* Physicochemical properties of algae such as chlorophyll, carotenoid and Hydrocarbon and lipid content were estimated and compared. Total chlorophyll, carotenoid, Hydrocarbon and lipid content are found to be highest in *Cladophora sp*. The lipid profile shows that C16:0 and C18:1 fatty acids have been found to be present in highest percentage in Cladophora sp. among the algae isolated and purified.

Introduction

Algae are aquatic, autotrophic organisms that chiefly contain three important biopolymers; protein, carbohydrates and lipids. Algae have been shown to be potential organisms for fuel production since they have higher photosynthetic efficiency, higher biomass production, higher lipid containing and higher growth rate (Chisti, Y, 2007). Algae can be compared to sunlight driven factories which can produce large biomass and this biomass can be harvested throughout the year. Algae have a tremendous capacity of fixing atmospheric CO2 (Swamayama et al, 2012) into hydrocarbons which finally lead to higher lipids and thus result in giving higher Biodiesel yields (Kumar et al , 2011). This conversion rate is higher than most of the energy crops (Li et al, 2008) and algae yield more oil per hectare as compared to any crop, allowing a fast turnaround for biofuel production. Even though hydrocarbon estimation studies have been done in some specific studies (Rekha et al, 2012; Rodofi et al, 2009) there is still a requirement of identification and isolation of new algal cultures which could be potential biofuel producers.Thus, the present study involves isolation and characterization of commonly found fresh water algae and their potentiality and sustainability as biofuel producers.

Materials & Methods

Collection of Algal Strain

Algal strains were collected from different freshwater ponds in and around NCR. Algal Strains were inoculated in CHU 13 Medium (pH 7.5) and Incubated at 25^0c in 1.2 klux irradiation with 16:8 hours light and dark cycle. Purification of the strains was done by dilution method and purity was checked microscopically. The strains were observed under microscope and depending on their morphological features were classified as: *Ulothrix sp., Ulva sp., Cladophora sp. And Rhizoclonium* Species.

Biomass Estimation

The cultures were harvested and the cells were washed with distilled water after centrifugation at 5000 rpm. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight.

Chlorophyll Estimation

A known volume of culture was centrifuged (8000 rpm) for 10 min and the pellet was treated with 10 ml volume of methanol and kept in water bath for 30 min at 60^0C. Absorbance of the pooled extracts was measured at 663 and 646 nm and chlorophyll (a+b) was estimation using equation.

Chlorophyll a (µg/ml) = $12.21 (A_{663}) - 2.81 (A_{646})$
Chlorophyll b (µg/ml) = $20.13 (A_{646}) - 5.03 (A_{663})$
C total = $17.32 A_{663} + 7.18 A_{646}$

Estimation of Carotenoids

A known quantity of algal dry biomass was homogenized and extracted repeatedly with acetone. The pooled extracts absorbance was read at 470nm and total carotenoid contents were quantified according to this formula. (Lichtenthaler, 1983)

Carotenoid (ug/ml) = $(1000 A_{470} - 3.27 [chl a] – 104 [chl b])/ 227$

Hydrocarbon Estimation

Hydrocarbon was extracted in hexane after homogenizing the dry biomass (2 g) in a mortar and pestle and incubated at 50ºC for 2-3 hours. Supernatant was recovered after centrifugation. Supernatant was dried in a pre weight beaker (Tripathi,U, 2001). The difference in weight of the beaker would be the hydrocarbon content (gravimetric method) which is expressed as gm/l (Lichtenthaler, 1983)

Total Lipid Extraction and Analysis

The total lipid content was determined by gravimetric method where chlorophorm; methanol ratio of 2:1 was used for extraction of the total lipids. The solvent layer was separated and freeze dried to obtain the total lipid content.

Results

The study involves isolation of freshwater and brakish water algae found in Noida. The underground water of Noida is hard and has a high amount of salts. Thus, both the fresh water and brackish water algae are found in the ponds in and around Noida. The different algae were initially purified and observed under the microscope to be identified and characterized. All the four algae namely, *Ulothrix* sp, Ulva sp, *Cladophora* sp, and *Rhizoclonium* sp. were classified under Chlorophyta Phylum of green algae.

Table 1: Chlorophyll and Carotenoid content of fresh water algae

S. No.	**Algal Species**	**Chlorophyll Estimation (ug/ml)**		**Carotenoids Estimation (ug/ml)**
		Chl (a)	**Chl (b)**	
1	***Ulothrix***	22.86	10.50	9.2
2	***Ulva***	41.47	23.96	20.2
3	***Cladophora***	56.20	22.04	17.7
4	***Rhizoclonium***	27.80	14.11	10.3

Table 2: Biomass and Total lipid content of fresh water algae

S. No.	**Algal Species**	**Biomass Estimation (g/l) (w/w)**	**Hydrocarbon Estimation (g/l) (w/w)**	**Total lipid content %**
1	***Ulothrix***	2.88	0.7	6.8
2	***Ulva***	3.97	1.6	18
3	***Cladophora***	4.94	2.9	26
4	***Rhizoclonium***	2.92	1.1	8.2

Table 3: Fatty acid profile of fresh water algae

Fatty acid	***Ulothrix***	***Ulva***	***Cladophora***	***Rhizoclonium***
12:0	-	3.1	1.4	1.6
14:0	4.5	0.4	10.1	5.9
16:0	16.2	46.8	32.4	26.8
16:1	10.4	-	5.4	5.2
16:2	-	-	2.9	1.6
18:0	0.6	12.1	4.5	6.9
18:1	1.3	10.6	24	15.2
18:2	1.2	5.2	9.1	9.6
20:0	2.1	8.8	1.1	1.1
20:1	1.1	0.2	0.5	-
20:2	-	-	0.1	-
22:0	-	2.5	2.1	1.6
22:1	1.4	-	--	-
22:2	-	-	-	-
24:0	-	-	0.3	-

These four algae were then analyzed for their Biodiesel producing quality. The parameters included in the study were Chlorophyll content, carotenoid content, total Biomass, Hydrocarbon content, Total lipid content and fatty acid analysis. The chlorophyll a, b and carotenoid content are depicted in Table. 1 and Fig.1 and the Biomass and Lipid content are depicted in Table 2. and a comparison is shown in Fig. 2.As shown the total chlorophyll, carotenoid, Hydrocarbon and lipid content are found to be highest in Cladophora sp. and next highest in Ulva sp. The lipid profile shows that C16:0 and C18:1 fatty acids have been found to be present in highest percentage in Cladophora sp. among the four algae isolated and purified.

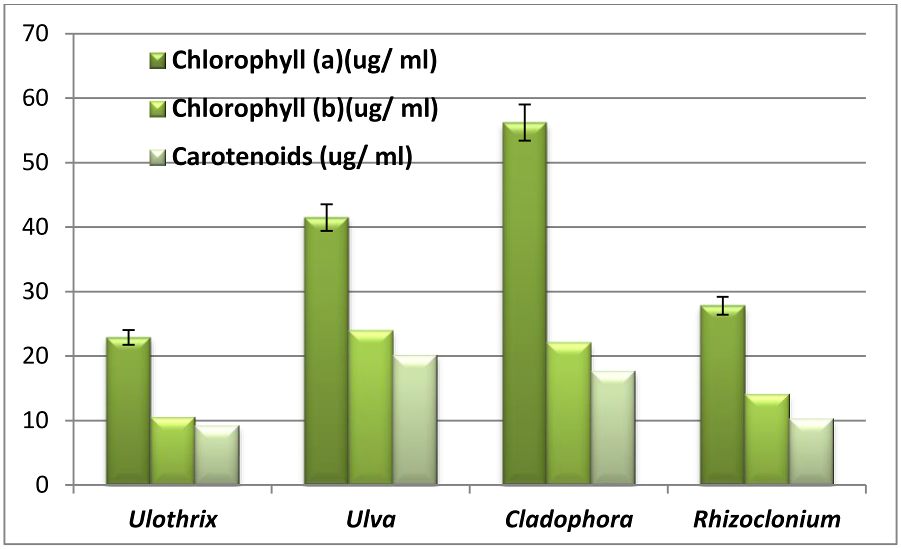

Fig: 1 Comparative Estimation of Chlorophyll (a), (b) and Carotenoids

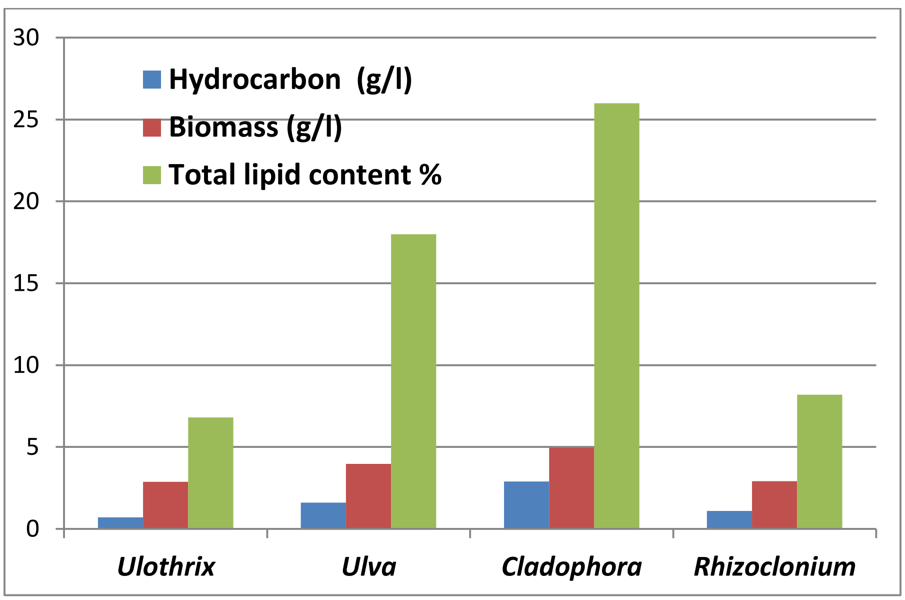

Fig: 2 Comparative Estimation of Hydrocarbons, Biomass and total lipid content

Discussion

The present study was undertaken to identify and purify algal Blooms which could be considered as potential cells for higher lipid and in turn for higher biodiesel production. It has been proven that algae contain and produce larger amount of hydrocarbon and lipids as compared to crops per hectare (Singh et al 2002).

Algal and microalgal lipids are now known as the oils of future for sustainable biodiesel production. However, relatively high production cost is due to low lipid productivity , which has been one of the major obstacles impeding their commercial production. Thus, search and requirement of high lipid producing alage and microalgae is the need of the day.

In the present study it has been found that the level of chlorophyll 'a' in Cladophora sp. (56.20 ug/ ml) was rather high in comparison with the other three algae. The chlorophyll 'a' level in Ulva sp. was also found higher (41.47 ug/ ml) than the other two species. Because of the variety of the cell wall structures of those two species, a well performed homogenization and a complete extraction facilitated the isolation of the pigments.
Higher amount of Chlorophyll normally would lead to higher amount of Photosynthesis activity and thus the algae would be likely to produce higher amount of Biomass.
As a result, in comparison between all four algal cultures *Cladophora* reported highest amount of Chlorophyll (38.36 ug/ ml), Hydrocarbon 9.94 g/l and Biomass 2.9 g/l. Thus, it can be further used in Biofuel production.
Microalgae are the largest primary producers of any aquatic ecosystem and it can be considered as a source of high-lipid material for the production of biofuel because the photosynthetic conversion of microalgae is an efficient and no alternative competent and carbon dioxide released is fixed.
In conclusion, the Algal Cultures isolated and studied in this study can be of used in production of Biodiesel since they have high amount of hydrocarbons. With further understanding on the influence of cultural conditions on hydrocarbon production, the algae can be exploited for outdoor cultivation also and thus could result in production of biomass in large scale and further commercial exploitation of the algal strains.

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