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Fatty Acid and Hydrocarbon Composition in *Asteracys Quadricellulare* (Behre)

KEYWORDS: Lipids, Fatty acids, Hydrocarbons, Green algae

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ABSTRACT *Microalgae have been cited as a promising feedstock for biodiesel production because of their rapid growth rate and high intracellular content of lipids. Asteracys quadricellulare (Behre) isolated from the water bodies of Madurai was maintained in Chu10 medium. Nile red fluorescence indicated more number of lipid bodies and the lipid content (26.2±1.14% dry weight) was maximum on the 30th day of growth. Fatty acid analysis by GC indicated more amount of saturated and monounsaturated fatty acids (25.6 - 31.2%). Greater proportion of saturated fatty acids and the presence of monounsaturated fatty acids in A.quadricellulare (Behre) indicated its potential for production of oil. Hydrocarbon analysis by GC-MS indicate the presence of hydrocarbons ranging from C10 to C27. Normal alkanes of carbon number less than C10 and more than C28 are rarely present in green algae.*

INTRODUCTION

Microalgae contain lipid and fatty acid as membrane components, storage products, metabolites and source of energy. They are considered as one of the most promising feedstocks for biodiesel production due to their short cell cycle, high oil content (20%-50%) and strong adaptive capacity to environment [3]. Under optimal conditions of growth, algae synthesize fatty acids principally for esterification into glycerol-based membrane lipids, which constitute about 5–20% of their dry cell weight (DCW) [6]. Some microalgae present a larger fatty acid spectrum, when compared to oleaginous plants, containing a molecular structure with even more than 18 carbons [1]. Algal fatty acids have been recognized as an attractive source of oil for biofuel and pharmaceuticals [11]. Depending on the species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils. Algae differ in their fatty acid composition due to varying amounts of illumination in different habitats. The total saturated fatty acid increased, while monounsaturated and polyunsaturated fatty acid decreased with increasing irradiance and light duration [7]. This study mainly focuses on the lipid, fatty acid and hydrocarbon content of *Asteracys quadricellulare* (Behre).

MATERIALS AND METHODS

Nile red assay

A.quadricellulare (Behre) isolated from the water bodies of Madurai was maintained in Chu10 medium with a photoperiod of 12 hours light/ 12 hours dark, light intensity of 2000 lux at a temperature of 25^{0}C. Lipids were extracted from the algal culture at an interval of 10 days until the 30th day of growth [2]. 0.1 mg of Nile red (9-diethylamino-5H- benzo [α] phenoxa phenoxazine-5-one) was dissolved in 1ml of acetone. To 4.0ml of the algal culture, 0.04ml of Nile red solution was added and mixed well for five minutes. Nile red stained cells were observed in a Fluorescence microscope [8].

Fatty acid analysis by GC

The composition of the different chain length FAMEs in the algal culture was determined using GC. GC analysis was performed at different days intervals in Gas Chromatograph 2010 Plus using Flame Ionization Detector (FID). Injector and Detector temperature was set at 225^{0}C and 250^{0}C respectively. One microlitre was injected in split mode (35:1) at a flow rate of 184.9 ml/min with Nitrogen as the carrier gas onto a FAMEs-RTX-2330 column (length 105.0m, Film thickness 0.20 µm, total run time 40min). Peak areas were integrated using the GC solution software. The fatty acids methyl esters were identified by internal standards.

Hydrocarbon analysis by GC-MS

The hydrocarbon samples were analyzed by GC-MS. 100ml of algal culture was taken and centrifuged at 10,000 rpm for 20 minutes. The algal pellet was extracted in 5ml of hexane. The sample was centrifuged at 10,000 rpm for 15 minutes and the supernatant was recovered [5]. GC-MS analysis was performed using GC Clarus 500 Perkin Elmer mass detector with Elite-5MS column (30×0.25mm×0.25µm film thickness). Nitrogen was used as the carrier gas at a flow rate of 1ml/min. The injection port was maintained at 250^{0}C. Oven temperature programming was done from 110 to 280^{0}C at 100^{0}C/mins and it was kept at 280^{0}C for 5mins. Mass scanning range was from 45-450(m/z).

RESULTS AND DISCUSSION

Nile red staining for lipid determination

Lipid content was maximum (26.2±1.14 % dry weight) on 30th day of growth. During lag phase of the culture *A.quadricellulare* (Behre) had very less amount of lipid. Regression analysis indicated a linear relationship between algal biomass and lipid accumulation and R^2 value 0.968, showed an increase in algal biomass and lipid accumulation. Nile red is a lipophilic fluorescent dye used for intracellular lipid determination in prokaryotic and eukaryotic cells, capable of detecting neutral lipids. After 10 minutes, nile red stained cells were observed under blue filter of fluorescence microscope. Both yellow and red fluorescence cells were observed. Red fluorescence indicates the presence of chloroplast. Nile red permeated the algal cell walls, stained the lipid droplets and emitted yellow fluorescence. *A.quadricellulare* (Behre) showed a large number of lipid bodies inside the cell and within the gelatinous matrix, as indicated by the yellow colour under blue light (Figure 1). There is a linear correlation between nile red fluorescence and the total lipid content of microalgae, where an increase of lipids in aging algal cells was due primarily to neutral lipids rather than glyco or phospholipids [4]. Nile red staining is a high throughput, rapid screening technique to determine the neutral lipid content of many algal strains [8].

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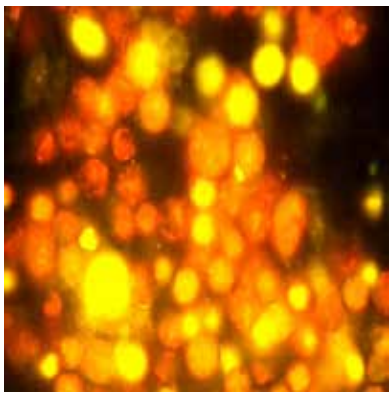

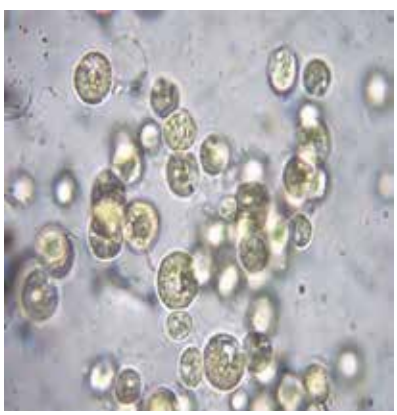

Figure (1): Light Microscopic observation and Nile red staining of *A.quadricellulare* (Behre)

Fatty acid composition

Algae synthesize fatty acids as building blocks for the formation of various lipids. The most commonly synthesized fatty acids have chain lengths that range from C_{16} to C_{18}. The function of fatty acids in algae is related to the cell membrane, energy storage and metabolic processes [12]. The content of the fatty acid methyl ester in the final product was calculated quantitatively by comparing the peak areas of fatty acid methyl esters to the peak area of the internal standard and also retention time. The fatty acid content of the algal biomass was compared with harvesting time. Fatty acid composition from 10^{th} to 30^{th} day of growth are represented in Figure 2. Tatsuzawa [16] observed the main saturated fatty acid was 16:0 (3.03%), and the amount of 18:0 was very low (0.51%). Piorreck [13] reported that, during early stages of growth, green algae produced relatively large amounts of polar lipids and polyunsaturated C_{16} and C_{18} fatty acids. During the stationary phase of growth, the dominant lipids produced by algae were neutral and consisted primarily of saturated 18:1 and 16:0 fatty acids. The percentage of saturated fatty acids increased with days of growth. The total saturated fatty acid content ranged from 20 to 25%. A reverse trend was observed with mono and polyunsaturated fatty acids. Amount of fatty acid level differed from exponential phase to stationary phase. The biochemical composition of cells in the exponential phase may differ from those in the stationary phase. A study done by Pratoomyot [14] identified that, at the stationary phase microalgae had a high amount of fatty acids than in the exponential phase. Due to nutritional limitation, cell division gradually decreased and the cells began to store fatty acids. GC analysis of purified lipid fraction was found to contain more amounts of neutral and glyco lipids and a fewer amount of phospholipids. Smith [15] reported that, neutral lipids would increase if the culture became nutrient limited.

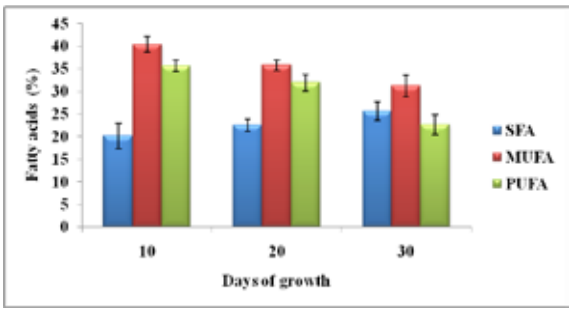

Figure (2): GC analysis of total Fatty acid composition (%) in A.quadricellulare

(Note: SFA – Saturated Fatty Acids; MUFA – Mono Unsaturated Fatty Acids;

PUFA – Poly Unsaturated Fatty Acids)

Hydrocarbon composition by GC-MS

The GC-MS study of major peaks revealed the presence of straight chain and branched hydrocarbons ranging from C_{10}-C_{27}. Short chain hydrocarbons ($<C_{20}$) were mostly present in this strain in comparison to long chain hydrocarbons ($>C_{19}$). But the percentage of long chain hydrocarbons (C_{21} – 25.11%) was higher than short chain and branched hydrocarbons [9]. GC-MS analysis showed that the hydrocarbons such as 2 - methyl Eicosane and 1- Iodo-2-methyl Undecane were the major components among the hydrocarbons produced by *A.quadricellulare*. Normal alkanes of carbon number less than C_{10} and more than C_{28} are rarely present in green algae [10]. Hydrocarbons identified by GC-MS are summarized in Table 1.

Table (1): Identified hydrocarbons by GC-MS

RETENTION TIME	IDENTIFIED HYDROCARBONS	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA %
4.14	Heptane, 2,2,3,3,5,6,6-heptamethyl	$C_{14}H_{30}$	198	1.70
8.22	1-Iodoundecane	$C_{11}H_{23}I$	282	0.43
9.01	1- iodo Tetradecane	$C_{14}H_{29}I$	324	1.28
10.60	1-iodo Decane	$C_{10}H_{21}I$	268	0.43
11.61	R(-) 3,7-Dimethyl-1,6-octadiene	$C_{10}H_{18}$	138	0.85
16.11	2-Bromo dodecane	$C_{12}H_{25}Br$	248	4.26
17.50	Heptacosane	$C_{27}H_{56}$	380	14.89
18.91	2-methyl Eicosane	$C_{21}H_{44}$	296	25.11
20.33	1- Iodo-2-methylundecane	$C_{12}H_{25}I$	296	23.40
21.73	2-ethyl-2-methyl Tridecanol	$C_{16}H_{34}O$	242	16.17
23.12	3,8-dimethyl Undecane	$C_{13}H_{28}$	184	9.36

CONCLUSION

Nile red is a promising fluorescence dye in rapid quantification of cellular lipid microalgae. Linear regression analysis

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showed a significant relationship between dry weight and lipid weight. Greater proportion of saturated fatty acids and the presence of monounsaturated fatty acids in *A.quadricellulare* (Behre) indicated its potential for production of oil. Higher biomass productivity and cell lipid content determines the economic feasibility of this algae for biodiesel production.

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