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RESEARCH ARTICLE

MICROWAVE TREATMENT FOR HIGH LIPID PRODUCTION IN SCENEDESMUS OBLIQUUS

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Abstract

Microalgae with high oil content are only alternative for decreasing fossil fuel supplies, but more work remains to be done to improve the lipid content of microalgae strains. In this study, strain improvement is done using microwave radiation in Scenedesmus obliquus to increase the production of triacylglycerol, which is the main source of biodiesel. Microalgal culture were exposure to varied microwave irradiation over different time periods. Maximum increase of 2.22-fold in biomass and 2.5-fold in triacylglycerol was observed for microwave irradiation of 25mins and 20mins respectively. The percentage of some monounsaturated fatty acids increased in gas chromatographic examination of neutral lipid fractions from total lipids of microwave irradiated samples, which is considered as one of the preferable properties of biodiesel.

Introduction:-

Energy cannot be accomplished effectively without petroleum, normal gas, coal, hydro power and atomic energy; and they turned into the fundamental regular hotspots for the energy[1]. Petroleum and its by-products usage are increasing day by day because of the increment in population and industrialization[2]. The world is entering a time of declining non-renewable energy assets, prominently known as 'peak oil', while energy concern is expanding[3]. The world's oil production is expected to decline in the middle of one and ten decade, approaching energy emergency, both government and private industries are looking at options of energy[4]. Biodiesel, in the recent years became renowned everywhere in the world because of its accessibility, renewability, non-toxicity, improved gas emissions and its biodegradability. Biodiesel is converted into liquid fuels so that it can be used by automobiles and for heating purposes[5]. Biodiesel is produced by mixing a vegetable oil or animal fat with a short-chain alcohol, such as methanol, ethanol, or butanol and a catalyst[6]. These sources are also limited therefore scientists came up with an idea of producing biodiesel with natural sources like plants, vegetables like soybean oil, sunflower oil, palm oil etc can be used to produce biodiesel[7]. It also has some disadvantages like the amount of biodiesel produced with a huge quantity of these oils was very low, hence biodiesel production from microalgae came into existence[8.] The microalgae for biodiesel are aquatic unicellular algae, photosynthetic, have high growth rate, population density and under optimum conditions algae can grow and double its biomass in less than 24 hours[9]. Microalgae consist of large amount of lipid approximately 50%, some species of algae like Chlorella sp. contains 28-30% of lipid, Nitzschia sp. contains 45 – 47% of lipid and Nannochloropsis sp. contains 31 – 68% of lipid and Schizochytrium sp. contains50 – 77% of lipid[10]. Microalgae produce and store neutral lipids as unsaturated fats, phospholipids, glycolipids and it can be utilized as feedstocks for biodiesel production[11]. In our study, the growth, oil content and biodiesel production

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from microalgae Scenedesmus obliquus were examined and an attempt was made to increase the TAG production through microwave irradiation[12].

Materials and Methods:-

Culturing and harvesting of Scenedesmus obliquus:

Scenedesmus obliquus were procured from NCIM-Pune, India (Accession No. 2897). The culture was allowed to grow in FOG medium at 25°C for 4 days under fluorescent light illumination, with the aeration of 2ppm. Later, the microalgal culture was harvested using flocculation method by the addition of alum (hydrated potassium aluminum sulphate) and centrifuged at 7000rpm for 5mins. After harvesting, the wet biomass was dried in hot air oven at 100°C overnight. The dried biomass was grinded to fine powder using motor and pestle and weighed[13].

Microwave irradiation:

Scenedesmus obliquus, was subjected to mutational analysis for the enhanced production of TAG. Randomly generated mutants were screened for TAG production 500ml of the culture with continuous stirring on magnetic stirrer was exposed to microwave light for different time intervals (5mins, 10mins, 15mins, 20mins and 25mins). The inoculums were taken out at respective time intervals and inoculated into 1litre of FOG medium (pH-7.5) and grown for 4 days at 25°C[14].

Total lipid extraction using BUME method:

200 mg of biomass was incubated overnight in the mixture of chloroform and methanol (2:1). This solution was then subjected to phase separation in a 125ml separating funnel. The bottom chloroform phase containing total lipids was collected into a beaker of known weight (W1). The collected chloroform phase was completely evaporated and final weight of beaker (W2) was taken. The total amount of lipids was estimated by calculating the difference in weights of the beaker. Weight of total lipid = W2 - W1[15.]

Lipid fractionation using column chromatography:

Lipid fractionation was performed by following standard Frostegard method, the column (BioRad; 1.5cm inner diameter and 20 cm length) with silica gel (230-400 mesh) was equilibrated with chloroform. The extracted total lipid sample was applied to the column in the chloroform solvent (1mL). The neutral lipid fraction was then eluted with chloroform (3 times the column volume), followed by glycolipids fraction elution with acetone: methanol (9:1) and phospholipids fraction elution with methanol. Solvent in all the eluted fractions was evaporated to 1ml for preparation of FAMEs for GC analysis. Fatty acid analysis was performed by injecting 0.5 µL of the sample with nitrogen as a carrier gas. The following temperature program was adopted for detection of FAME: Initial temperature 100^{o}C, 1 min hold; ramp at 10^{o}C min^{-1} until 180^{o}C with 1 min hold; ramp at 10^{o}C min^{-1} until 240^{o}C, with a 2 min hold[16].

Results and Discussion:

Microwave treatment for Scenedesmus obliquus showed the highest increase in biomass yield of 2.22-fold for the sample treated for a period of 25mins, where as the samples treated for 10 and 5 minutes showed an increase in biomass yield of 1.2-fold and 1.48-fold, respectively. The remaining two samples exposed to microwave for 15 and 20mins has shown a slight decrease in biomass yield of 1.13 and 1.3-fold respectively when compared to control. The biomass yield for different microwave exposure times was shown in Figure 1.

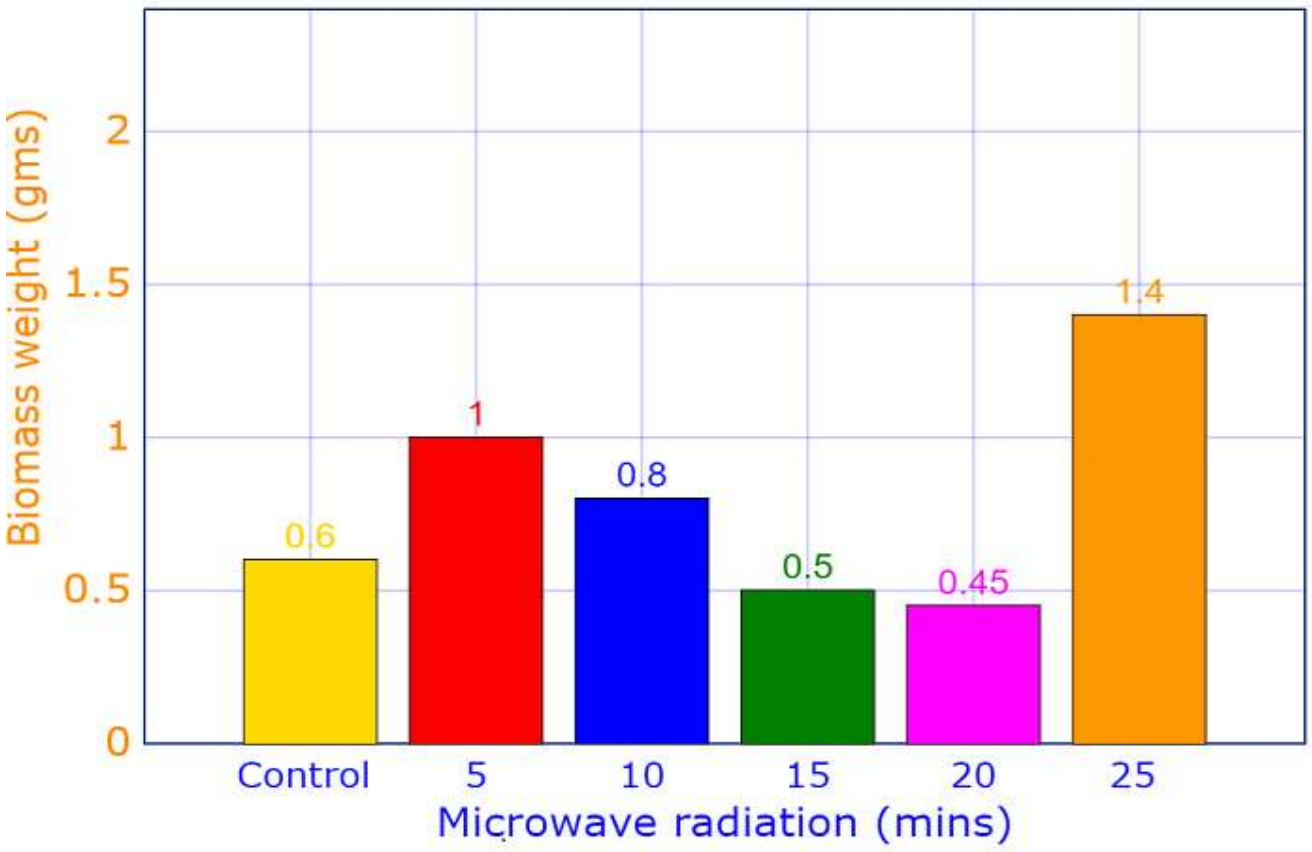

Figure 1:- Biomass yield for different microwave exposure times.

Total lipid content enhanced with 1.6, 1.93 and 2.4-fold for microwave treatment of 20, 15 and 5mins, respectively. The total lipid variation with different exposure time was given in Figure 2.

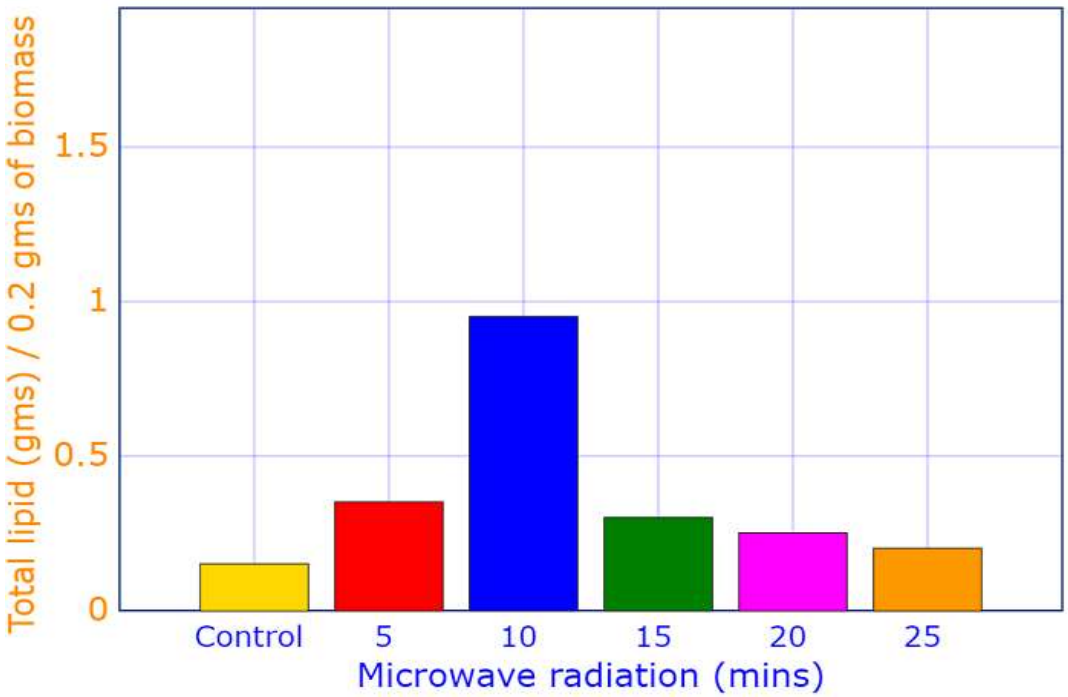

Figure 2:- Total lipid variation with different exposure time.

Significant TAG enhancement of 2.5-fold and 2.17-fold was found in the samples with microwave exposure time of 20 and 15minutes, respectively, whereas other exposure times showed insignificant increase in TAG. TAG yields for different microwave irradiation are shown in Figure 3.

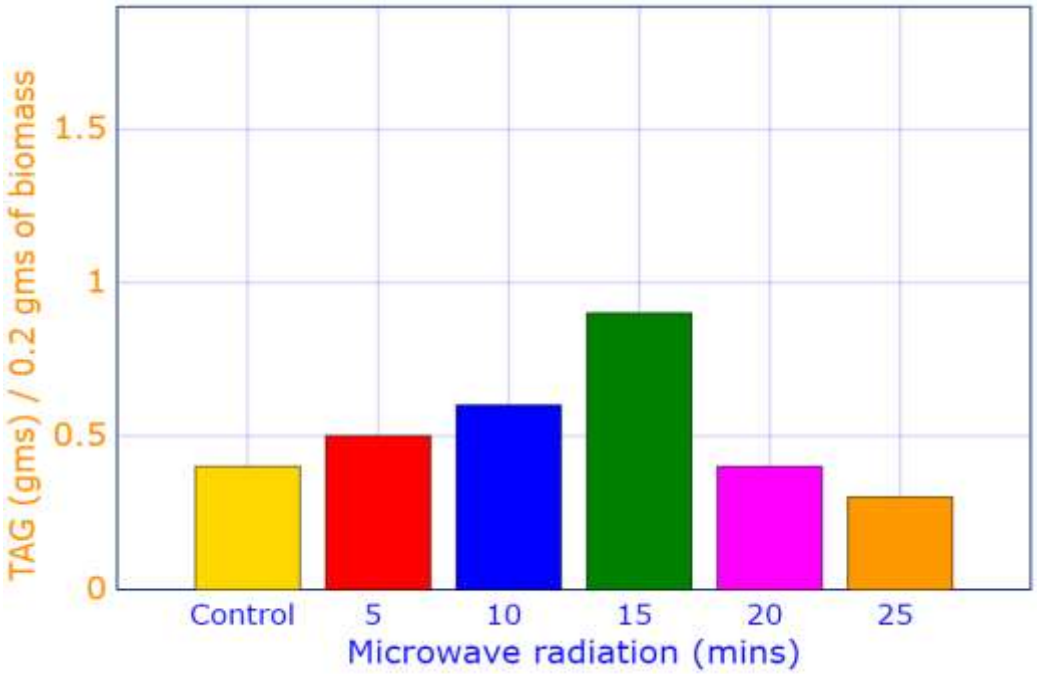

Figure 3:- TAG yields for different microwave irradiation.

The exposure time of 25mins lead to significant increase in biomass but it did not show any effect on total lipid and TAG yield whereas exposure time of 20mins and 15mins reported an increase in total lipid as well as in TAG yield. Microwave irradiation for 5 mins shown drastic increase in total lipid over 15 and 20 mins but that did not contribute to the increase in TAG yield as an increase in total lipid might be because of other two lipid fractions i.e

glycolipids and phospholipids. The GC analysis of the neutral lipid fractions showed an increase in monounsaturated fatty acids like myristoleic acid and Cis 10 pentadecanoic acid, which is considered as one of the preferred properties for biodiesel. The fatty acid analysis of the neutral lipid fraction for different microwave irradiation times was given in table 1.

Table 1:- Fatty acid analysis of the neutral lipid fraction for different microwave irradiation times.

Fatty acid	Control	5 min	10 min	15 min	20 min	25 min
Myristoleic acid methyl ester (14:1)	0.6	2.1	2.7	1.5	4.2	-
Petadecanoic acid methyl ester (15:0)	2.1	3.7	4.3	3.0	5.1	3.9
Cis 10 pentadecanoic acid (15:1)	0.8	0.8	3.9	1.2	-	1.8
Palmitic acid (16:0)	0.8	1.0	4.9	1.7	0.8	2.6
Palmitoleic acid (16:1)	13.9	10.7	6.8	12.8	11.8	10.2
Heptadecanoic acid methyl ester (17:0)	1.7	5.7	2.0	6.2	1.4	5.1
Cis 10 heptadecanoic acid (17:1)	0.8	2.4	0.8	3.0	3.0	4.4
Oleic acid (18:1n9c)	0.2	5.3	9.4	12.1	6.4	11.8
Lenoelaidic acid (18:2n6t)	5.3	5.7	6.7	6.3	4.6	4.3
Linoleic acid (18:2n6c)	0.2	0.5	0.6	-	0.5	-
Arachidic acid (20:0)	4.2	3.3	5.6	5.3	2.6	-
Cis 11 eicosenoic acid (20:1)	0.3	-	-	0.4	-	0.5
Linolenic acid (18:3n3)	12.2	10.2	12.4	18.4	4.5	9.2
Behenic acid (22:0)	2.0	-	1.4	-	-	0.9

Conclusion:-

In this study an attempt was made to enhance the production of TAG in Scenedesmus obliquus using microwave irradiation was made. In these processes significant increase in TAG was observed under microwave exposure of 20mins and 15mins with 2.5-fold and 2.17-fold rise in TAG yield respectively. The highest biomass yield was observed for 25mins but showed an insignificant increase in TAG. The effect of microwave irradiation on Scenedesmus obliquus genome that led to an increase in the yield of TAG which can be further investigated by whole genome sequencing.

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