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Research Article

Lipid And Biomass of Scenedesmus Abundans

Enhancement of Lipid and Biomass Production in Microalgae Scenedesmus abundans by Microwave Irradiation

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Abstract: Due to the rising depletion of fossil fuels and increased energy demands, human society is looking for clean sustainable energy. Commercially produced algal biodiesel is limited by the expense and difficulty of oil extraction and subsequent biodiesel conversion technologies. Microalgae with high oil content are only alternatives 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 abundans* to increase the production of triacylglycerol, which is the main source of biodiesel. Microalgal cultures were exposed to varied microwave irradiation over different time periods. Under microwave irradiation, 20-25 mins reaction time seems suitable for the complete in situ transesterification reaction. Microwave heating transesterification has been shown to be more effective for adequate biodiesel yield compared to the conventional transesterification process. Maximum increase of 2.22-fold in biomass, and 2.5-fold in triacylglycerol was observed for microwave irradiation of 25 mins and 20 mins intervals respectively. The percentage of some monounsaturated fatty acids increased in gas chromatographic examination of neutral lipid fractions from total lipids of microwave irradiated samples, is considered as one of the preferable properties of biodiesel. According to our study findings, *Scenedesmus abundans* qualifies as the most efficient feedstock for biodiesel production, and microwave-assisted in situ transesterification reduces the requirement for a large amount of solvents, longer reaction times, and high reaction temperatures and pressures.

Keywords: *Scenedesmus Abundans*, Biodiesel, Microalgae, Triacylglycerol, Microwave Radiation.

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1. INTRODUCTION

The demand for energy sources is growing every day as a result of increased industrialisation and population. Over the last century, there has been a great deal of pressure on energy production, which has resulted in the overexploitation of nonrenewable resources[1]. Machine-driven energy can't be accomplished effectively without petroleum, normal gas, coal, hydro power and atomic energy; and they turned into the fundamental regular hotspots for energy[2]. There were various sources of energy in the past, but they are rapidly disappearing, resulting in a conflict between food and fuel production[3]. Microalgae can be collected numerous times throughout the year, and they can grow in water with varying quantities of nutrients and a wide range of light and temperature[4]. Their biomass doubles every 24 hours, and they develop quicker than trees[5]. The requirement of petroleum and its by-products are increasing day by day because of the increment in population and industrialization[6]. The world is entering a time of declining non-renewable energy assets, prominently known as 'peak oil', while energy concern is expanding[7]. The world's oil production is expected to decline in the middle of one and ten decades. Because of this approaching energy emergency, both government and private industries are looking at options of energy[8]. In recent years, biodiesel 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 also for heating purposes[9]. 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 [10]. 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[11]. It also has some disadvantages like the quantity of biodiesel produced was very low, hence biodiesel production from microalgae came into existence[12]. 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[13]. 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, *Nannochloropsis sp. contains* 31 – 68% of lipid and *Schizochytrium sp. contains* 50 – 77% of lipid[14-17]. Microalgae produce and store neutral lipids as unsaturated fats, phospholipids, glycolipids, and it can be utilized as feedstocks for biodiesel production[18]. Microalgae oil was employed in this work to produce biodiesel via a transesterification reaction aided by microwave irradiation, speeds up the reaction rate[19]. In our study, the growth, oil content and biodiesel production from microalgae *Scenedesmus abundans* were examined and an attempt was made to increase the TAG production through microwave irradiation[20]. Our study, is done for strain improvement using microwave radiation in *Scenedesmus abundans* to increase the production of triacylglycerols.

2. MATERIALS AND METHODS

2.1 Culturing and harvesting of Scenedesmus abundans

The strain of *Scenedesmus abundans* was originally obtained from the culture collection of NCIM-Pune, India (Accession No. 2897). The *Scenedesmus abundans* cells were cultured in sterilized seawater enriched with FOG medium which contains: KNO3 2000 mg/L, K2HPO4 200 mg/L, MgSO4.7H2O 200 mg/L, CaCl2.2H2O 100 mg/L, Fe-EDTA solution 5 ml/L (Fe-EDTA solution: 745 mg Na2-EDTA and 557 mg FeSO4.7H2O in 100 ml distilled water), Trace metal solution 1 ml/L (Trace metal solution: H3BO3 2.86 g/L, MnCl2.4H2O 1.81 g/L, ZnSO4.7H2O 0.222 g/L, Na2 MoO4-2H2O 0.39 g/L, CuSO4.5H2O 0.08 g/L,) per litre at 25°C for 4 days under fluorescent light illumination, with the aeration of 2ppm. 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[21,22].

2.2 Microwave irradiation

Scenedesmus abundans, 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[23].

2.3 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 the 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[24].

2.4 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°C, 1 min hold; ramp at 10°C min^{-1} until 180°C with 1 min hold; ramp at 10°C min^{-1} until 240°C, with a 2 min hold[25].

3. RESULTS AND DISCUSSION

In the current experiments, we have attempted to compare the growth of algal species with and without the exposure of microwaves, under the same conditions, in order to choose the best procedure giving high efficiency[26]. Our hypothesis is, that microwave irradiation should show a much higher efficiency than the wild strain, Figure 1 indicates the efficiency represented by the number of colonies using hemocytometer. We observed a huge difference in the growth efficiency of the organism with the exposure of microwaves[27].

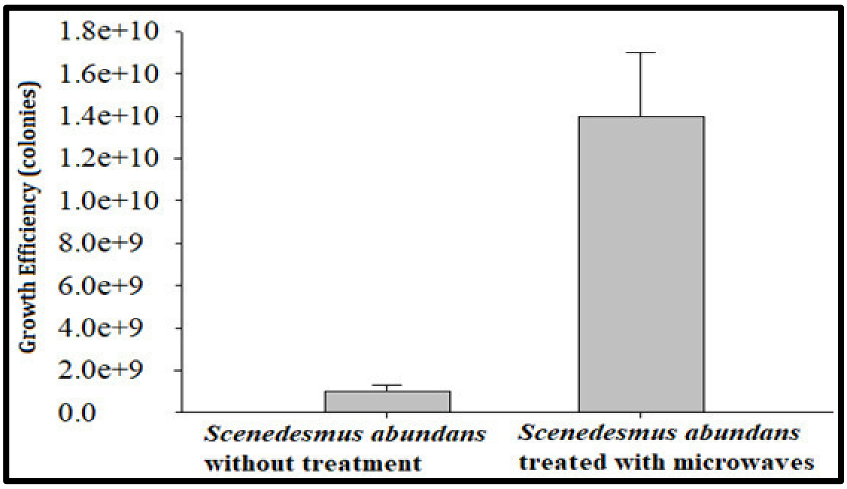

Fig 1: Growth efficiency of *Scenedesmus abundans* with and without exposure of microwaves.

3.1 Total lipid extraction

Microwave treatment for *Scenedesmus abundans* showed the highest increase in biomass yield of 2.22-fold for the sample treated for a period of 25mins, whereas the samples treated for 10 and 5 minutes showed an increase in biomass yield of 1.2-fold and 1.48-fold, respectively[28-30]. 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 2. This study shows an improvement of biomass yield over exposure to microwave irradiation for long duration. Earlier studies by Rokicka *et. al.,* in 2020 used ultrasonic and microwave pretreatment of microalgae biomass was used to enhance the lipid extraction for which they achieved 8.8% using *Nannochloropsis oculate*[31]. Our approach and choice of organism has proven a significant raise in biomass yield for the production of lipids[32].

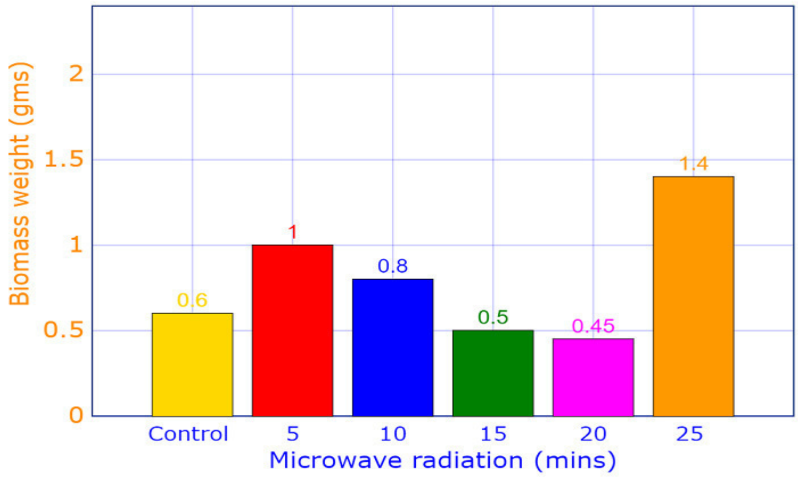

Fig 2: Biomass yield for different microwave exposure times

According to the study, microwaves selectively extracted lipids from the biological matrix of algae, whereas the waves that produce during the irradiation might damage the cell walls and alter the structure of the cells[33,34]. Moreover, it is possible that microwaves enhanced the extraction of undesired substances due to the dominance of disruptive mechanism. On the other hand, microwaves caused a local superheating of the lipid compounds to selectively extract them. However, higher temperatures generated by microwaves may cause the extracted products to oxidize[35]. Total lipid content enhanced with 1.6, 1.93 and 2.4-fold for microwave treatment of 20, 15 and 5mins, respectively[36]. The total lipid variation with different exposure time was given in Figure 3.

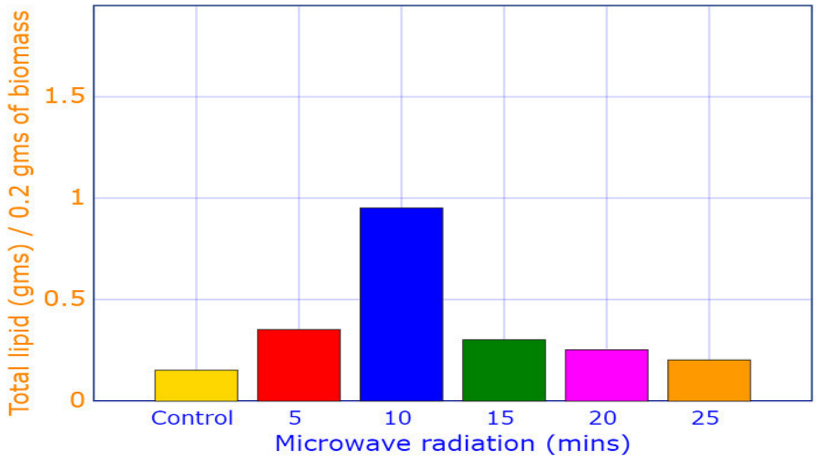

Fig 3: Total lipid variation with different exposure time

Significant TAG enhancement of 2.5-fold and 2.17-fold was found in the samples with microwave exposure time of 20 and 15minutes using excel graphs, respectively, whereas other exposure times showed insignificant increase in TAG[37]. TAG yields for different microwave irradiation are shown in Figure 4. The advantage of this technique includes substantial reduction in consumption of energy, time and cost in order to produce bio-oil from biomass materials. Large biomass particle size can be used directly in microwave heating, thus saving grinding as well as moisture removal cost[38].

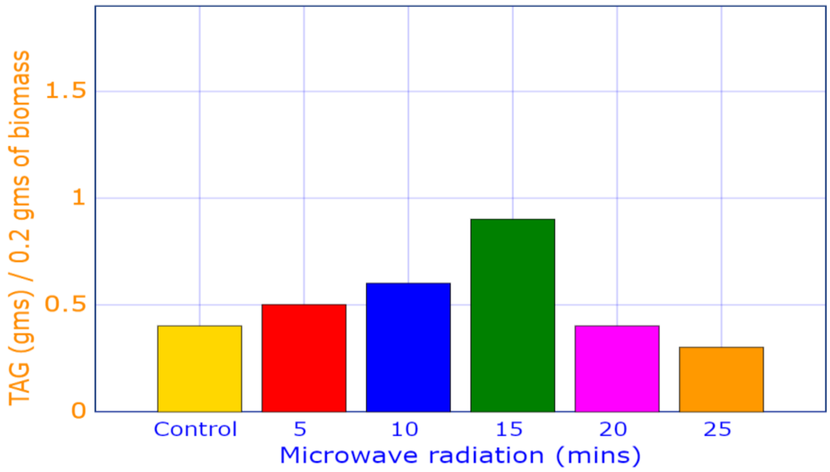

Fig 4: TAG yields for different microwave irradiation

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[39]. 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[40].

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

Table 1 Impact of various microwave intervals from microalgal species for fatty acid profile with microwave irradiation.

3.2 *Gas chromatography analysis of lipids*

The Gas chromatography 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[41,42]. The fatty acid analysis of the neutral lipid fraction for different microwave irradiation times was given in table 1. Gas Chromatography is used to identify the chemical ingredients in the biodiesel. It was found that there are different major esters in the Algal Oil Methyl esters, identified using TRACE software installed in Gas chromatography[43].

4. CONCLUSION

In this study an attempt was made to enhance the production of TAG in *Scenedesmus abundans* 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 abundans genome* that led to an increase in the yield of TAG which can be further investigated by whole genome sequencing.

5. ACKNOWLEDGEMENT

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6. AUTHORS CONTRIBUTION STATEMENT

Bhanu Rajarajeswari Kapavarap and Sree Rama Chandra Karthik Kotikalapudi taken responsibility in the conception and design of the study. Krishna Keerthika Oruganti and Sreedhar Bodiga collected the data and performed the analysis. Vijaya Lakshmi Bodiga involved in data analysis and interpretation of results. Suryanarayana Veeravilli and Sudhakar Poda checked the references and made critical revision of the article. Praveen Kumar Vemuri has given final approval of the version to be published.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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