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Fatty Acids Content from Several Microalgae Strains for Biodiesel Fuel

A.R. Babu[1], G. Amba Prasad Rao[2], T. Hari Prasad[3], Ananda Kumar Eriki[4]

[1]*Department of Mechanical Engineering, Sri Venkateswara College of Engineering & Technology, Autonomous, RVS Nagar, Chittoor, 517127, Andhra Pradesh, India*

[2]*Department of Mechanical Engineering, National Institute of Technology, Warangal, 506004, AP, India*

[3]*Department of Mechanical Engineering, Sree Vidyanikethan Engineering College, Autonomous, Rangampet, Tirupati, 517127, Andhra Pradesh, India*

[4]*School of Engineering, Science and Technology, Nilai University, Nilai, 71800, Negeri Sembilan, Malaysia.*

Abstract: Decreasing petroleum resources and increasing the fuel price have become the reasons to find alternative energy. Furthermore, microalgae are potential alternative energy that can be developed as biodiesel fuel because it grows fast and produces oil. Other advantages are to absorb carbon dioxide and save food security. The purpose of this research is to find microalgae strains containing high total fatty acids. Strains of microalgae were collected from marine. Several types of microalgae which are taken form marine are *nanochloropsis sp, chaetoceros sp and tetraselmis sp*. The measurement of fatty acid concentration was done by gas chromatography methods. The result indicates that the total of fatty acid in chaetoceros sp, in nanochloropsis sp, and in tetraselmis sp reaches 25.76%, 62.08% and 49.85%, respectively, from dry weight. Hence, it can be concluded that microalgae could become alternative energy resource.

Keywords: Microalgae; Alternative energy; Biodiesel; Fatty acid.

1 INTRODUCTION

Bio-fuel production is now the focal point of world attention due to rapidly escalating demand for crude oil, major security concerns over supply and the environmental damage associated with crude oil extraction, processing and consumption. In the global energy crisis context, biodiesel attracts increasing attention worldwide and has core advantages over mineral diesel in that it is renewable, biodegradable, clean-burning, non-toxic and carbon neutral with respect to carbon dioxide related climate change. Recently, microalgae have long been identified as a potential feedstock due to their many advantages for bio-diesel production. Natural fuels (e.g. petrol, diesel) are unrenewable energy and it diminishes every time. Some alternatives energy has been found for replacing the natural fuels like microalgae, corn, soybean, and canola. However the utilization of oil plant for biofuel can threat food security except microalgae. It has been used for two decades. Several microalgae contain oil 15 times compared with other plants. Beside that microalgae can grow in fresh water, saline, and waste water. Another positive side using microalgae is to decrease greenhouse effect. Microalgae can provide several different types of renewable biofuels which include methane, biodiesel (methyl esters) and biohydrogen [1]. The utilization of microalgae for biofuel is prospective because oil containing can be increased by 10% to 50% by adding carbon dioxide and other organic substances. The advantages of microalgae are oil contain is higher than other plants, can be grown in aquatic medium, can be cultivated in seawater or brackish water or non arable land, and do not compete for resources with conventional agriculture [2]. Microalgae are one of the photosynthetic organisms that change solar energy and carbon dioxide to carbohydrate and other organics substances like lipid. Several microalgae can accumulate lipid from photosynthetic more than 50% from body weight [3]. The type of lipid known with triglyceride (TGA) is the natural basic substances for biodiesel. Primary composed of TGA are saturated and unsaturated fatty acid that can be reserved for rebuild the cells [4]. TGA is the best substances to produce biodiesel that can be modulated by varring growth condition [5]. Microalgae oil is a potential energy source because it contains fatty acid that can be easly converted to methyl esther. Microalgae are the prime source of hydrocarbon substances formed million years ago [6]. Microalgae can be divided into many classes based on pigment contain, life cycle and cell structure [5]. Four classes of microalgae are Diatomae (Bacillariophycae), Chlorophyceae, Cyanophyceae, Chryzophyceae [7]. Microalgae have fast life cycle and growth. Big scale production is processed in bioreactor and open pond. Processing in bioreactor can

Corresponding author: arbabu.1973@gmail.com (A.R.Babu)

ICID: 1049212 (Index Copernicus)

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protect from contamination but it takes high cost construction [8]. pH maintained at 6.8 by passing saturated CO_2 at every 24 hrs interval. The cultures were maintained in an automated cul-ture laboratory with temperature of 25 ± 2℃ under a 16:8 h photoperiod with a light intensity of 3500 lux provided by cool white fluorescent tubes. The cultures were agitated at 126rpm in an orbitar shaker to avoid sticking [9].

2 METHODOLOGY

To find fatty acid in microalgae are culturing, fatty acid extraction, and measuring fatty acid concentration. Microalgae had been taken from Marine Culturing Development Centre such as *Nanochloropsis sp*, *Tetraselmis sp andChaetosaeros sp*. *Nanochloropsis sp* and *Tetraselmis sp* 25 ml was cultured in 100 ml combined marine water and Conway Walne medium. Comparation of marine water and Conway Walne medium was (4:1). Especially for *Chaetoseros sp* was cultured in Guilard medium and marine water with (4:1) comparation $Na_2SiO_39H_2O$ was special substances chemical that contain silicate. It was needed by *Chaetoseros sp* to build the cell walls. Adding the trace element $ZnCl_2$, $CuSO_4$, $CaCl_2$, $(NH_4)_6Mo_7O_{24}.4H_2O$ and vitamin B1, B12, Biotin was used in culture. Growing of microalgae was done by counting the numbers of live microalgae by using haemocytometer and microscope. Counting of microalgae was done from first day until stationed growth phase. Fatty acid extraction was done by filtering microalgae with filter paper and collected. Drying microalgae was done in 24 hours by using lamp. After that, drying microalgae sample 0.5 gram was put in to erlenmeyer and added 10 ml chloroform and 10 ml methanol. This solution was covered by using alumunium foil and put in to ultrasonic within 30 minute for distructing cell walls. After filtering the microalgae solution it was continued to destilating to find the total lipids. Methodology was to measure the fatty acid by using Gas Chromatography.

3 RESULTS AND DISCUSSION

The indicators of microalgae cultured had been growing well or not were qualified by colour and growth of microalgae (Figure 1, 2, 3, and 4).

Nanochloropsis sp and *Tetraselmis sp* looked green in liquid medium, while *Chaetoseros sp* was brown. It meant that requirement microalgae for growing well such growth medium, lamp lightening with 220 voltage (2000-8000 lux), aeration, and space for growing were fulfilled. Lack of nutrition and aeration in medium could decrease the growth of microalgae. The shortage of aeration would induce anaerobe reaction and decreased of pH contain. The shortage lightening could decrease of photosynthetic, and furthermore possibility to decrease of lipid synthesis. *Nanochloropsis sp* grew significantly especially in sixth day.

Figure 1: Nanochloropsis sp

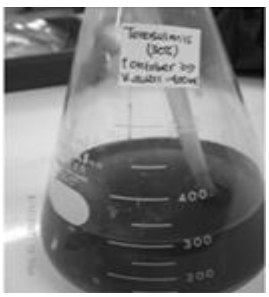

Figure 2: Tetraselmis sp

Figure 3: Chaetoseros sp

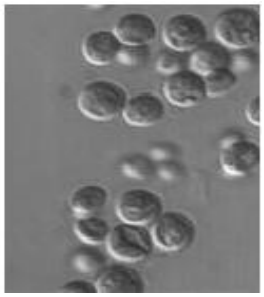

Figure 4 Chlorella sp

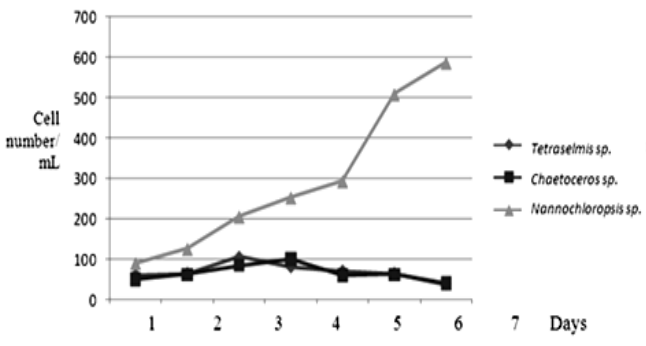

Figure 5: Marine Microalgae Growth Rate

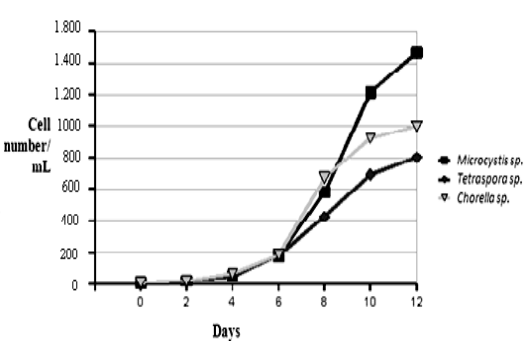

Figure 6: Fresh Water Microalgae Growth Rat0065

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In seventh day the growth was the highest. This was the best time to harvest and measure oil containing. Analysis result showed that fatty acid in *Nanochloropsis sp* reached 25.76% from dry weight. it was different with *Tetraselmis sp*, it did not growing well and after thirth day looked decreasing. *Tetraselmis sp* condition was suitable with another research which *Tetraselmis sp* growth rate was slightly lower 6. But decreasing of *Tetraselmis sp* number in this research must be continued to be investigated. Analysis of fatty acid in *Tetraselmis sp* reached 49.85%. *Chaetoseros sp* condition was similar to *Tetraselmis sp*, it showed decreasing in fourth day but the contain of fatty acid was the highest reached 62.08%. To culture *Chaetoseros sp* needed phosphate, if it was not supplied will reduce 45% to 55% of cells growth 7. Another research showed high level of nutrient could inhibit microalgae growth at the beginning but it sustained growth 14, as show in Figure 5. Based on the result it is needed research to know caused decreasing *Chaetoseros sp* growth. Research Science and Technology Center (BPPT) [10] explained that fresh water *Microsystis sp* growth reached 1.400 cell/ml in twelfth day showed the fastest growth than *Chlorella sp* 998 cell/ml and *Tetraspora sp* 802 cell/ml, as showed in Figure 6.

Oil containing of microalgae was dependent on growth. Table 1 showed that fatty acid contained in microalgae. Total fatty acid *Chlorella sp* was 47.55%, different from report which fatty acid content *Chlorella sp* 28-32% 4. *Chlorella vulgaris* under general growth condition up to 20% by weight of dry biomass 13. High fatty acid content was attributable to availability of the growth requirement.

To increase the fatty acid content in microalgae by adding high irradiances and de-privation of nitrogen or phosphorus because at this condition could induce photosynthesis [11]. Growth medium must be provided in organic element that constituted the microalgae cell. Essential element included Nitrogen (N), Phosphorus (P), Iron (Fe), and Silicon (Si). Starch is substance that is produced in photosynthesis, and can be converted into lipid. Increasing of oil in deprivation nitrogen and phosphorus is reasonable because the carbon, hydrogen, and oxygen are mayor components of the starch. BPPT reported that total fatty acid in *Mycrocistis sp* reached 32.21% and *Tetraspora sp* 24.46% [10].

From fatty acid percentage from biomass microalage (Table.1): microalgae from marine and freshwater contained fatty acid. Other fact showed that palmitic acid component in *Mycrocystic sp* reached 8.75%, *Tetraspora sp* 21.53%, *Chlorella* sp 14.49%, *Chaetoseros sp* 17.9%, *Nanochloropsis sp* 22.65% and *Tetraselmis sp* 15.24%. Percentage of linoleic acid was mayor component for each microalgae *Microcystic sp* reached 13.99%, *Chlorella sp* 22.13 and *Tetraselmis sp* 23.06%. Palmitic acid and linoleic had potential to be converted into methyl esther (biodiesel), they are: methyl palmitat and methyl lenoleat [12]. Docosahexaenoic acid (DHA) composed 22 carbon atoms and six double bonds had potensial to biodiesel [13]. Analysis of six microalgae in this research showed only *Chaetoseros sp* containing DHA reached 0.40%. Microalgae oils is different from most vegetable oil do to quite rich polyunsaturated fatty acid with four double bonds such as Eicosapentaenoic acid (EPA) and DHA [1], but in this research only *Chaetoseros sp* contained EPA reach 5.18%. Chaetoseros is one of microalgae in this research which contained g- Linoleic acid, Heneicosanoic acid, and cis 11, 14 Eicosedienoic acid C20:2, Arachidonic acid, DHA, and EPA.

Genetic is dominant factor in influencing for specific fatty acid production. Microalgae produce many different lipids, hydrocarbon and other complex oil depending on species. Another fact showed genetic will influence to produce fatty acid [14]. Fatty acid is the factor in building block for TGA and all other cellular lipid which synthesized in the chloroplast using a single set of enzyme of which acetyl Co Carboxylase is the key in regulating fatty acid synthesis rates [15].

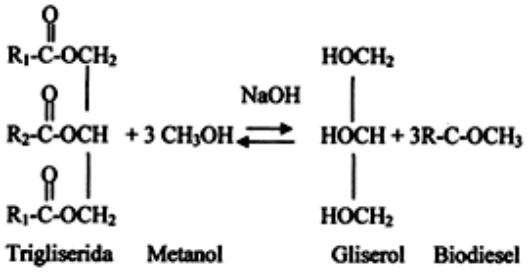

Figure 7: Reaction of transesterification

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Table 1: Fatty Acid Percentage from Biomass Microalgae

Fatty acid	Microcystis sp	Tetraspora sp	Chlorella sp	Chaetoseros sp	Nanochloropsis sp	Tetraselmis sp
Lauric acid C12:0	*0.15*	-	*0.04*	*0.22*	*0.19*	*0.04*
Myristic acid, C12:0	*0.15*	*0.18*	*0.56*	*3.71*	-	*0.58*
Pentadecanoic acid. C15:0	*0.07*	-	*0.53*	*0.67*	-	*0.55*
CIs-10-Pentadecanoic acid,	*0.08*	-	*0.13*	*0.10*	-	*0.13*
Palmitic acid, C16:0 & C16:1	*8.75 &1.01*	*21.53 & -*	*14.49 & 0.23*	*17.90 & 24.00*	*22.65 & -*	*15.24& 0.24*
Heptadecanoic acid. C17:0	*0.11*	-	*0.37*	-	-	*0.37*
Cis-10-heptadecanoic acid. C17.1	*2.02*	-	*0.59*	*0.99*	-	*0.62*
Stearic acid. C18:0	*1.23*	*0.68*	*6.49*	*0.19*	*0.72*	*6.90*
Eladeic acid. C18:1n9t	-	-	-	*1.39*	-	-
Oleic acid. C18:1n9c	*4.56*	*2.07*	*1.80*	*0.40*	*2.20*	*1.92*
Linoleic acid, C18:3n3	*13.99*	-	*22.13*	*0.51*	-	*23.06*
g-Linolenic acid. C18:3n6	-	-	-	*0.30*	-	-
Heneicosanoic acid. C21:0	-	-	-	*0.09*	-	-
Cis-11, 14-Eicoseclienoic acid C20:2	-	-	-	*0.73*	-	-
Arachidic acid, C20:0 & C20:4n6	*0.07&-*	-	*0.13 & -*	-	-	*0.14&-*
Tricosanoic acid. C23:0	*0.02*	-	*0.06*	*5.30 & -*	*- & -*	*0.06*
EPA	-	-	-	*5.18*	-	-
DHA	-	-	-	*0.40*	-	-
Total fatty acid	*32.21*	*24.46*	*47.55*	*62.08*	*25.76*	*49.85*

To get methyl esther from TGA or fatty acid is needed transesterification with catalyst. Transesterification requires 3 mol of alcohol for each molecul of tryglyceride in producing 1 mol of glycerol and 3 mol of methyl esther [16, 17, 18]. Transesterification needs methanol and sodium hydroxide as catalyst. The reaction of transesterification is showed in Figure 7 above.

4 CONCLUSION

Analysis indicated that total fatty acid of *Nanochloropsis* sp reach 25.76%, *Chaetoceros* sp 62.08% and *Tetraselmis sp* 49.85% from dry weight, based on this results to find an alternative energy resources. Palmitic acid was the component in microalgae of which in *Chaetoseros sp* reach 17.9%, *Nanochloropsis sp* 22.65% and *Tetraselmis sp* 15.24%. Percentage of linoleic acid was mayor component for each microalgae of which *Microcystic sp* reach 13.99%, *Chlorella sp* 22.13% and *Tetraselmis sp* 23.06%. *Chaetoseros sp* was one of microalgaes in this research that contain g- Linoleic acid, Heneicosanoic acid, and cis 11, 14 Eicosedienoic acid C20:2, and Arachidonic acid. Docosahexaenoic acid (DHA).

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