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ARTICLE

Exploration of fatty acid methyl esters (FAME) in cyanobacteria for a wide range of algae-based biofuels

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ABSTRACT

Due to high demand in the industry field, fuel supply has been reduced due to overconsumption. Algae served as the most promising biofuels; Cyanobacteria is preferred as a material for producing biodiesel based on the comparative advantage. Cyanobacteria can produce high lipids profiles that can be used to produce biofuel or biodiesel. The present investigation aimed to identify the application of Cyanobacteria that have proposed lipid standards for the algae biofuel industry. Three main objectives are involved in these experiments: to isolate and identify different strains of Cyanobacteria, convert the lipid from microalgae into biodiesel through transesterification, and estimate the proposed lipid standard of Cyanobacteria for the algae biofuel industry. The microalgae involve isolated using a plankton net. The water sample is brought into the lab for the identification of Cyanobacteria. After the Cyanobacteria are identified, the Cyanobacteria undergo mass cultivation to ensure the biomass is enough for lipid screening. After lipid content is identified, the lipid in the Cyanobacteria is extracted and further transesterification process. The GC analysis showed the variation of fatty acid in this cyanobacterium, a saturated, monounsaturated, and polyunsaturated fatty acid. The study also revealed that hexadecanoic acid, pentadecanoic acid and pentadecanoic acid was found in *Oscillatoria* sp. Lipid screening can determine the quantity of lipid present in the Cyanobacteria to estimate the lipid content for biofuel production.

1. Introduction

Nowadays, many countries have become dependent on another renewable source that can replace fossil fuels. According to (Bhuyar et al., 2019a), fossil sources are limited exhausted soon (Bhuyar et al., 2017). The United States, for example, currently imports a full two-thirds of its petroleum from only a few countries around the world. The energy demand is growing worldwide, especially in rapidly developing countries such as China and India (Ahmad et al., 2020; Bhuyar et al., 2019a). Furthermore, the

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continued combustion of fossil fuels has created severe environmental concerns over global warming due to the increased release of greenhouse gases (GHG) (Deepanraj et al., 2017; Bhuyar et al., 2020a). This situation leads to the production of biofuel. This biofuel has gained attention around the world. Biodiesel is one example of biofuel that is getting attention worldwide until biodiesel becomes an asset for a country, especially in growing their economies (Bhuyar et al., 2020d).

Interest in engineering cyanobacteria for biofuel production has increased recently (Bhuyar et al., 2019b), driven by photosynthesis to convert carbon dioxide into a desirable fuel directly. Additionally, cyanobacteria exhibit higher solar conversion efficiency and growth rate compared to plants and eukaryotic. *Synechocystis* sp. PCC6803 was the first cyanobacterium for which the complete genome was sequenced in 1996. So far, 126 genomic sequences of cyanobacteria strains are available. Well established genetic manipulation techniques have been applied for cyanobacteria. The techniques make cyanobacteria highly tractable platforms to build efficient biosynthetic pathways for biofuel production by genetic engineering (Bhuyar et al., 2020c; Bhuyar et al., 2020d). The term biofuel is solid, liquid, or gaseous fuels predominantly produced from bio-renewable feedstock. Biodiesel is produced through the chemical reactions transesterification and esterification by chemically reacting lipids with alcohol producing fatty acid esters (Bhuyar et al., 2019c; Khamee et al., 2020; Mejica et al., 2020a).

On the face of it, biofuels look like a great solution Bhuyar et al., 2020e;). Saengsawang et al. (2020) reported that biodiesel usage allows a balance between agriculture, environment, ecosystem and development. Cars are a significant source of atmospheric carbon dioxide, the main greenhouse gas that causes global warming (Khammee et al., 2020; Malek et al., 2020; Nithin et al., 2020; Ramli et al., 2020a). But since plants absorb carbon dioxide as they grow, crops grown for biofuels should suck up about as much carbon dioxide as comes out of the tailpipes of cars that burn these fuels. And unlike underground oil reserves, biofuels are a renewable resource since we can always grow more crops to fuel (Lawrence et al., 2011).

Unfortunately, it's not so simple. Growing the crops, making fertilizers and pesticides, and processing the plants into fuel consume much energy (Ramli et al., 2020a, b; Mejica et al., 2020b). It's so much energy that there is debate about whether ethanol from corn provides more energy than is required to grow and process it. Also, because much of the energy used in production comes from coal and natural gas, biofuels do not replace as much oil as they use (Saengsawang et al., 2020). For the future, many think of a better way of making biofuels from Cyanobacteria. If lipid from Cyanobacteria can be turned into biofuel, it could be more efficient than current biofuels and emit less carbon dioxide. Biofuel from Cyanobacteria is third generation renewable energy resource that does not compete with our food resources (Matado et al., 2012).

Biofuel can be produced from cyanobacteria (Bhuyar et al., 2020b). Cyanobacteria are photosynthetic microbes, which can absorb solar energy and fix carbon dioxide. Direct conversion of carbon dioxide to biofuels in photosynthetic cyanobacteria can significantly improve the efficiency of biofuel (Sundararaju et al., 2020). Production and other high-value chemicals by modifying amino acid metabolic pathways using protein engineering and metabolic engineering and building non-native biosynthetic pathways (Bhuyar et al., 2018). To isolate and identify the different strains of Cyanobacteria from Northern Thailand. To convert the lipid from cyanobacteria into biodiesel through transesterification. To estimate the proposed lipid standard of Cyanobacteria for the algae biofuel industry.

2. Materials and Methods

2.1. Isolation and identification

Cyanobacteria samples were isolated from water samples collected from a different place and the Chiang Mai province, Thailand. The water sample was collected from canal water nearby Nong Han, Maejo University, Thailand. The next day, a water sample was brought to the laboratory for further experiment to obtain the pure culture; 20 plates of Petri dishes of BG 11 media were prepared. BG 11 media Blue-green mediums explicitly used for the growth of freshwater algae and protozoa. Then 1 microlitre of water sample was spread on the 20 plate of BG 11 agar media. The Cyanobacteria grow on the BG 11 agar for 1 week before proceeding to the next experiment. Following that, 6 test tubes were filled with 10 mL of BG 11 Media. It was possible to see the growth of Cyanobacteria on the plate after one week. Inoculating the next inoculum on the agar plate with algae that grow well and have a blue-green colour. The Cyanobacteria were inoculated into each test tube that contains broth media of BG 11. Then the test tube left for 2 weeks to make sure the Cyanobacteria can grow well in the broth. This step is significant as a part of obtaining the pure culture of Cyanobacteria.

2.2. Identification of cyanobacteria strain

After the Cyanobacteria grow in the test tube for 2 weeks, the Cyanobacteria strain was identified using a fluorescence microscope and scanning electron microscope. At first, the morphology of the Cyanobacteria was identified by observation under the light microscope. The morphological of the Cyanobacteria examine based on their cell shape and colour. A few drops of culture with Cyanobacteria and BG11 medium were added on a microscopic slide, and a coverslip was carefully placed on a slide to obtain a clear view; the formation of an air bubble was avoided. Then the sample was observed at lower magnification (x40) and move sequentially up.

2.3. Mass cultivation of cyanobacteria

After the strain of the Cyanobacteria was identified, then Cyanobacteria was undergo mass cultivation. This step is essential to obtain more biomass of the Cyanobacteria for lipid screening and biodiesel production. Mass cultivation of Cyanobacteria usually involves BG 11 medium. BG 11 media are specific media that use to grow freshwater algae and protozoa. This BG 11 media contain $NaNO_3$, K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Citric acid,

Ammonium ferric citrate green, EDTANa_2 Na_2CO_3, and other trace metals. During mass cultivation, three conical flasks that contain 500 ml of BG 11 Media were prepared. Then the Cyanobacteria were inoculated into the conical flask to produce more Cyanobacteria. This conical flask was placed on the shaker to make sure the media and nutrients are mix well and promote the growth of Cyanobacteria.

Cultures were placed in a window for light and loosely covered to reduce evaporation. For artificial lighting, plant growth lights or wide spectrum bulbs were used. Cultures were placed at a distance of 12–18 inches from the bulbs. The temperature of the culture was maintained over 27 °C may be fatal for some algae species. After 2 weeks, each conical flask containing Cyanobacteria was split into two conical flasks with 250 ml each. Then another 250 ml of fresh BG 11 Media was added into the conical flask to make volume up to 500 ml again. Then the media left again on the shaker for another 2 weeks before further to next experiment. After the culture volume reaches 2000 ml, the growth rate of the cyanobacteria was measured. The growth rate of the cyanobacteria was constructed by measuring the concentration of the culture from day 1 until day 21 at interval 2 days using a spectrophotometer.

2.4: Harvesting

After 21 days of culture, the cyanobacteria at 2000 ml the culture was harvested using centrifugation. 50 ml of microalgae was transferred slowly into the 50 ml centrifuge tube and centrifuge at 5000 rpm for 5 minutes. After centrifuge, the supernatant that contains media was removed, and the pellet was kept. The pellet was washed with distilled water as apart to remove any left media. This step was repeated until all microalgae culture was finish harvested. All pellet was collected and placed in a centrifuge tube and then stored in a freezer at temperature -80 °C for 5 days. After the microalgae pellet was freeze, the sample was transferred into the freeze-dryer machine.

2.5. Lipid extraction

The lipid extraction protocol was carried out as described by Bhuyar et al. (2020a) the lipid extraction procedure. In this method, solvent chloroform and methanol was used. The cyanobacteria tissue was homogenized so that the cell membrane of cyanobacteria disrupt and broken. After that, the tissue was mixed with chloroform/methanol (2/1) to a final volume 20 times the tissue sample volume (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture was agitated for 15-20 min in an orbital shaker at room temperature. After that, the mixture was filtered to remove biomass residual and obtained only liquid parts that contain solvent and lipid. Then, the solvent was washed with distilled water and vortex for a few seconds. After vortex for some seconds, the mixture was centrifuged at low speed (2000 rpm) to separate the two phases. Then the upper phase was removed by siphoning. Then the lower phase that contains lipid and chloroform was collected, followed by the lower layer was washed a few times using distilled water to remove any residual biomass left in lipids. After that, the lower phase was evaporated under a fume hood to evaporated excess chloroform and obtained only a pure lipid.

2.6. Lipid profiling- GC-Flame Ionization Detector (GC-FID)

After the cyanobacterial lipid was obtained, the lipid was sent to GC-FID for lipid screening. The fatty acid or lipid composition was identified by using GC analysis. Using Flame Ionization Detector (FID) for fatty acid analysis done by Gas Chromatograph 2010 Plus (Shimadzu, Japan). Injector and Detector temperature was set at 225 °C and 250 °C, respectively. 0.5 ml of the sample was injected in a split mode (35:1) at a 184.9 ml/min flow rate with nitrogen as the carrier gas onto a FAMEs- RTX- column length 5. m, film thickness. µm, total run time 4 min). Peak areas were integrated using the GC solution software. The fatty acid methyl esters were identified using fatty acid standards (Sigma, Supleco, 37 FAMEs).

2.7. Transesterification

At first, dried cyanobacteria were added with hexane and methanol. Totally 8 ml of hexane and 4 ml of methanol was added into dried cyanobacteria and was mixed using a vortex. The mixture of dried microalgae and solvent was blended for 5 minutes. After that, the mixture was washed with hexane and distilled water. After that, the mixture was centrifuged at 4000 rpm for 15 minutes to separate the biomass and lipid. Then the upper layer that contains algae oil was collected and transfer into the glass vial. Then the algae oil was heated at 75 °C for 5 minutes in the water bath to evaporate water. After 5 minutes, methanol, sulphuric acid and hexane were added and blended for 35 minutes at 75 °C. After that, and the mixture was cooled to room temperature. Then crude ester layer was separated from glycerol using a separating funnel.

2.8. FAME's analysis by GC

GC analysis was performed for identifying the fatty acid composition. Fatty acid analysis was done by Gas Chromatography using column DB WAX. Injector and Detector temperature was set at 225 °C and 250 °C, respectively. A 0.5 ml sample was injected in a split mode (35:1) at a 184.9 ml/min flow rate with nitrogen as the carrier gas onto a FAMEs-RTX-2330 column (length 105.0m, Film thickness . µm, total run time 65 min). Peak areas were integrated using the GC solution software. The fatty acid methyl esters were identified using fatty acid standards (Sigma, Supleco, 37 FAMEs).

3. Results and Discussion

3.1. Isolation of cyanobacteria

In this study, the cyanobacteria sample was collected from Northern Thailand. The sampling was done at different locations near the irrigation canal from Nong Han, Maejo University, Thailand. The sample was collected from these three places and returned to the Faculty of Science, Maejo University, Thailand laboratory under the Faculty Industrial Of Science And Technology (FIST). After collecting the sample from the Canal, the sample was observed under a fluorescence microscope in IQS Laboratory. Most of the samples contain diatom, and only a few

samples from Canal were shown the presence of cyanobacteria. From observation of the selected indigenous cyanobacteria, isolates revealed their colony existence and were purified. Fig. 1 show the presence of cyanobacteria in the water sample after being isolated from Irrigation Canal. Fig. 1 shows some morphology and characteristic of cyanobacteria that were found from sample irrigation canal water. The morphology of the cyanobacteria in the water sample was long and thin, resembling a thread. Under a fluorescence microscope, cyanobacteria were observed in green colour.

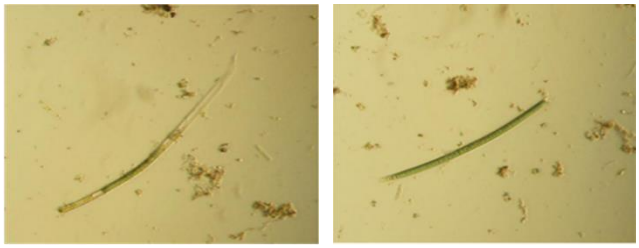


Fig. 1. *Cyanobacteria* sp. that are present in the sample irrigation canal water were observed under the fluorescence microscope.

3.2 Identification of cyanobacteria

After the sample was collected and confirm that cyanobacteria were present in the irrigation canal water, the species of cyanobacteria was determined. Usually, cyanobacteria species can also be identified for determination by scanning electron microscope (SEM) and fluorescence microscope. For this study, the cyanobacteria were observed under SEM and fluorescence microscope.

After observation under a fluorescence microscope, the result shows that the cyanobacteria morphology matched *Oscillatoria* sp. Under a fluorescence microscope, *Oscillatoria* sp. has an unbranched filament with sheaths not conspicuous or extending beyond the end of the filaments, heterocysts and akinetes absent. The filaments can move slowly back and forth (oscillate) under contractile glycoproteins reported by (Krambeck et al., 1981). Generally, *Oscillatoria* sp. has a body cell size 4.5 – 9 µm long and 4.4 – 4.7 µm wide reported by (Bhuyar et al., 2020a). This *Oscillatoria* sp. is being investigated for biofuel production. Previous research investigation reported that marine *Oscillatoria* sp. was suitable for biofuel production. Moreover, cyanobacteria and microalgae have simple growth requirements and efficiently use light, carbon dioxide, and other inorganic nutrients (Selvan et al., 2013).

The species of *Oscillatoria* sp., the sample, was observed under the scanning electron microscope. SEM is one method that can be used to confirm the morphology and species of cyanobacteria. SEM show a more detailed and precise structure of the cyanobacteria. Under SEM, it shows that *Oscillatoria* sp. has a long structure and horizontal line along with their structure. The captured image of *Oscillatoria* sp. under SEM was showed in Fig. 2.

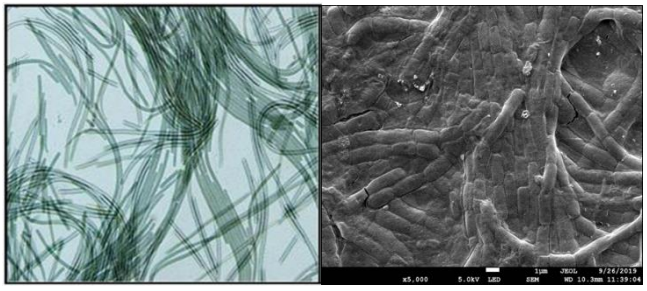


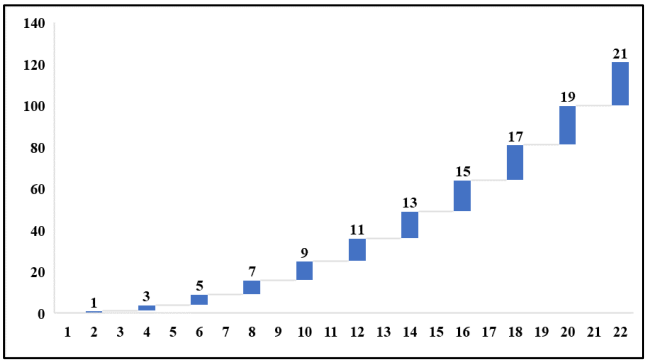
Fig. 2. Species of cyanobacteria *Oscillatoria* sp. under the fluorescence microscope

3.3 Mass cultivation

To obtain more biomass of *Oscillatoria* sp. for further analysis, the cyanobacteria were mass cultivated up to 2000 ml for 21 days. The growth curve established the relationship between age and biomass concentration. When the cyanobacteria increase in age, it undergoes stressful conditions, and more lipids accumulate in cyanobacteria (Hu et al., 2010). Generally, almost all cultures presented an exponential growth phase with the same duration and reaching the stationary phase at day 12 of culture.

Cyanobacteria growth culture was determined by measuring the concentration of the culture at Absorbance 665 nm at interval 2 days in 21 days using a spectrophotometer. After dry weight, 1.1583 g of dry biomass cyanobacteria were able to obtain. The various phases represent the reaction of the algae population to the changes of the environmental conditions and depend on the inoculums, the actual cultivation method, nutrient concentration, light intensity and temperature.

Fig. 3. A growth rate of *Oscillatoria* sp. from day 1 to day 21 at the absorbance of 665 nm.



The Fig.3, it shows that *Oscillatoria* sp. have a maximum growth rate at day 13. The *Oscillatoria* sp. start to grow slowly from day first until day 13. After day 13, *Oscillatoria* sp. grow start to decrease due to a few factors: nutrient depletion and toxin compounds released by other cyanobacteria.

3.4 Harvesting of cyanobacteria

After mass cultivation, the cyanobacteria biomass must be harvested so that the lipid can be extracted. Many methods can harvest the cyanobacteria biomass, including filtration, centrifugation, sedimentation, and flocculation. The method that wants to be used depends on the size and quantity of the biomass harvested. For example, large cyanobacteria usually use the filtration method since they cannot pass the small pore during filtration due to its large structure. In this case, the centrifugation method was used. The use of the centrifugation method as a biomass recovery is typically adequate for small scale. The main advantage of this method is that the cyanobacteria biomass cell separation was achieved more rapidly by increasing the gravitation field subjected to the cyanobacteria suspension. After the cyanobacteria were harvested, the pellet was dry in a dry freeze machine to transform the wet pellet into a dry pellet. After that, the dried sample was weighed using an analytical balance. The weight of dry cyanobacteria from 2000 ml of culture was 1.150 grams.

3.5 Lipid extraction of cyanobacteria

After the cyanobacteria biomass was obtained, the lipid content in the cyanobacteria was extracted for further lipid analysis. From lipid extraction, around 0.5 ml of cyanobacteria lipid was able to extract. Many methods can extract the lipid from cyanobacteria, including solvent extraction, mechanical pressing, and supercritical fluid extraction. Each method has its pro and cons. But for this study, cyanobacteria lipid was extracted using solvent extraction technique, which utilizes methanol and chloroform as a solvent.

3.6 Lipid proliferation

3.6.1 Fatty acid analysis by GC-FID

After the lipid was extracted, *Oscillatoria* sp. was analyzed using GC-FID for lipid profile. A 0.5 ml of *Oscillatoria* sp. was injected into the injector of GC-FID. Results obtained from the GC-FID the peak of the compound that present in this cyanobacteria. The retention time from GC-FID was compared to standard. Table 1 shows the lipid content that was detected in the *Oscillatoria* sp. No fatty acid was detected for the first peak since this peak belonged to solvent injected into the GC column and lipid. Then for other detected peaks, it represents the fatty acid present in *Oscillatoria* sp.

The triglycerides and biodiesel fuel properties are determined by the amounts of each fatty acid present in the molecule. That is why it is essential to know the carbon chain of fatty acids found in cyanobacteria *Oscillatoria* sp. From the GC-FID result, retention time indicates the carbon chain of fatty acids. The longer the carbon chain of fatty acid, the longer the retention time. The result shows that many fatty acids were detected at 37 minutes on above. It indicates that this *Oscillatoria* sp. has many fatty acids that have a longer carbon chain. Only one short fatty acid was detected in these cyanobacteria, which is at minute 17.148, which belongs to oleic acid. Cyanobacteria major fatty acid comprise of oleic acid, palmitic acid, hexadecanoic acid and palmitoleic acid.

Table 1 Lipid content in *Oscillatoria* sp. in GC-FID (ND= not determined)

Retention time (min)	Number of carbon	Name of fatty acid
5.494	ND	ND
17.14	C18:1	Oleic acid
37.290	C20:5	Eicosapentaenoic acid
45.684	C21:0	Heneicosanoic acid
46.111	C21:1	12-Heneicosanoic acid
47.142	C21:2	12,15-heneicosaidienoc
52.637	C22:0	Docosanoic acid
54.033	C22:1	Erucic acid

Apart from that, oleic acid, 12- heneicosanoic acid and erucic acid are three MUFA found in *Oscillatoria* sp. It shows that the lipid from this cyanobacterium can be converted into biodiesel due to the oxidative stability of MUFA. Bajpai and Tyagi (2006) have reported that the stability of fatty compounds was influenced by air, heat, traces of metal, peroxides, light, or structural features of the compounds themselves, mainly double bonds. The oxidation stability decreased with the increase of the contents of polyunsaturated methyl esters. A similar result also has been reported by (Knothe 2005, McCormick et al., 2007, Park et al., 2008).

Even though they are less stable than saturated fats, but is more stable than polyunsaturated fats. This intermediate status may give them certain advantages not shared by the saturated fatty acids (SFAs) or polyunsaturated fatty acids (PUFAs). Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odourless, colourless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number (C18:1). It has the formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$. This oleic acid is the perfect candidate fatty acid for biodiesel production since it was monounsaturated.

Furthermore, another monounsaturated fatty acid that was present in *Oscillatoria* sp. was 12-Heneicosanoic acid. This fatty acid has a 21 carbon chain and monounsaturated fatty acid. Another MUFA, erucic acid that has 22 carbon. Research of fatty acid composition in cyanobacteria by (Aharon Oren et al., 1985) found that the fatty acid composition of several filamentous cyanobacteria showed that many contain saturated and monounsaturated fatty acids only.

Apart from that, the result shows that most of the fatty acid from the cyanobacteria was saturated fatty acid and monounsaturated fatty acid. Only one polyunsaturated fatty acid was present. According to Aharon Oren et al. (1985), cyanobacteria synthesized monounsaturated fatty acids by desaturation their saturated counterparts. At the same time, Kenyon (1972) was found that cyanobacteria lack polyunsaturated fatty acids in their lipid, which was also reported by (Fattom and Shilo, 1985) that *Oscillatoria limnetica* able to synthesize monounsaturated fatty acids by desaturation of long-chain saturated fatty acids under aerobic as well as under anaerobic conditions.

3.7 GC-MS

After the fatty acid content in cyanobacteria was identified, the fatty acid from cyanobacteria was converted into biodiesel using the transesterification method. Two methods can be used for biodiesel production: in situ transesterification and two-step transesterification. In situ transesterification involves a single step by extracting and converting the lipid into biodiesel simultaneously. But for two steps, it involves a two-step of process. At first, the algae oil was extracted first, and second, the algae oil was converted into biodiesel. In this process, a two-step transesterification process was used. In this transesterification, biodiesel was produced by converting the fatty acid in the cyanobacteria into fatty acid methyl ester. Transesterification was a simple chemical reaction commonly used to make bio-oils less viscous, turning them into biodiesel. Oil from cyanobacteria are high in viscosity; because of that, it needs conversion to lower molecular weight constituents in the form of fatty acid alkyl esters.

Fatty acid methyl ester produced from the transesterification process was analyzed using gas chromatography-mass spectrometry (GC-MS). For FAME analysis, the fatty acid must be converted first to make the fatty acid volatile enough to be analyzed by GC-MS. During the transesterification process, 180 ml of cyanobacteria culture was harvest and dried. After that, dried biomass of cyanobacteria was added with methanol and hexane to extract the algae oil. Followed by the catalyst was added to catalyze the transesterification process to produce the fatty acid methyl ester. In this case, sulphuric acid was used as a catalyst. The transesterification process occurs, the mixture of the solvent and cyanobacteria was placed in the water bath at a temperature of 75 °C to disrupt the cell membrane and extract the lipid from cyanobacteria. This reaction is reversible, which means that excess methanol is needed to force the reaction to the right to the ester. Then using hexane to extract the FAMEs at the end of the reaction.

After that, two layers of the mixture were formed: the upper layer contains FAME and hexane, while the lower layer contained methanol. The upper layer was collected, and then the hexane was evaporated in the fume hood. The biodiesel produced was sent to GC-MS for the identification of fatty acid.

Table 2 List of FAME that can be obtained from cyanobacteria

Name of compound	Retention time	Percentage (%)	FAME name
n-Hexadecanoic acid	43.366	99	Methyl hexadecanoate
Pentadecanoic acid	43.366	90	Methyl pentadecanoate
Tetradecanoic acid	43.366	80	Methyl tetradecanoate

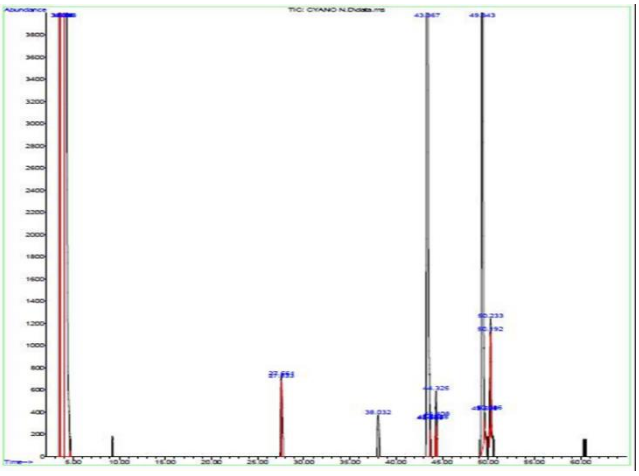


Fig. 4. List of FAME that can be obtained from cyanobacteria

Based on the obtained results (Fig. 4), cyanobacteria have a higher lipid profile of n-hexadecanoic acid, pentadecanoic acid and tetradecanoic acid. 99% of hexadecanoic acid was matched with the library, proving that these species have this fatty acid (Table 2). Hexadecanoic acid is called palmitic acid (IUPAC name) with 16 carbon chains. Palmitic acid is saturated fatty acid. Palmitic acid usually can be found in animals, plants and also in microorganisms. The chemical formula of palmitic acid was $CH_3(CH_2)_{14}COOH$. The name indicates that fatty acid is a significant component of the oil from palm trees (palm oil, palm kernel, and palm kernel oil). Other than that, it also can be found in meats, cheese, butter and dairy products.

Moreover, the second higher lipid profile is pentadecanoic acid. 90% of pentadecanoic was matched with the library, indicating that this *Oscillatoria* sp. has pentadecanoic acid. Another name for pentadecanoic acid is Pentadecylic acid. Pentadecanoic acid with 15 straight carbon chains with no heteroatom is referred to (Fig. 4). Pentadecanoic acid is also saturated fatty acid. This fatty acid is rare in nature. Pentadecanoic acid usually is found at the level of 1.2% in the milk fat from cows. The butterfat in cows milk is its primary dietary source, and it is used as a marker for butterfat consumption. Pentadecanoic acid also occurs in hydrogenated mutton fat. $CH_3(CH_2)_{13}COOH$ is its chemical formula.

Lastly, the third higher lipid profile is tetradecanoic acid. 80% of the tetradecanoic acid match with the library, which can be proven from this fatty acid, also presents at high quantity in *Oscillatoria* sp. Tetradecanoic acid is also known as Myristic acid. A myristate is a salt or ester of myristic acid. Myristic acid has 14 carbon chains and saturated fatty acids. Besides nutmeg, myristic acid is also found in palm kernel oil, coconut oil, butterfat and is a minor component of many other animal fats. It is also found in spermaceti, the crystallized fraction of oil from the sperm whale. $CH_3(CH_2)_{12}COOH$ is its chemical formula.

The three fatty acids, the hexadecanoic acid, can lead to biodiesel of higher fluidity, which is liquid at room temperature (25 °C). Hence, hexadecanoic acid in *Oscillatoria* sp. is the perfect

candidate that can be used for the production of biodiesel (Bhuyar et al., 2020a). Pentadecanoic acid is suitable for the production of biodiesel. Pentadecanoic acid is also common fatty acid used for that purpose (Bhuyar et al., 2020c). Tetradecanoic acid is the principal component of fatty acids, the most common fatty acid in biodiesel (Rasoul-Amini et al., 2014). In conclusion, the three fatty acids are suitable for the production of biodiesel.

Furthermore, obtaining a high quality of biodiesel depends not solely on the fatty acid used but also on biodiesel processing. Biodiesel quality must be analyzed using sophisticated equipment to ensure it meets any required specification during biodiesel processing and production, which is very important to ensure that the diesel engines are trouble-free. Usually, to produce quality biodiesel, it should have a complete reaction, remove glycerin, remove the catalyst, and free fatty acids.

4. Conclusion

In conclusion, one cyanobacteria species was able isolated and mass cultivated for further analysis. The species that are present in the sample are known as *Osillatoria* sp. From *Oscillatoria* sp. lipid, it was able converted into biodiesel by the transesterification process. Results revealed that *Oscillatoria* sp. produces a higher amount of n-hexadecanoic acid methyl ester (methyl hexadecanoate, IUPAC), pentadecanoic acid methyl ester (methyl pentadecanoate, IUPAC) and tetradecanoic acid methyl ester (methyl tetradecanoate, IUPAC). Methyl hexadecanoate, methyl pentadecanoate and methyl tetradecanoate have a high match with the library, 99%, 90% and 80%, respectively.

On the other hand, the lipid screening of cyanobacteria *Oscillatoria* sp. from GC-FID also shows a few types of fatty acid that can be converted into biodiesel. A fatty acid produced in this cyanobacteria was oleic acid, 12-heneicosaidienoc acid, docosanoic acid and erucic acid. Oleic acid and 12-heneicosaidecanoic acid are the most suitable fatty acids converted into biodiesel due to monounsaturated fatty acids from all of these lipids. Overall, it can be concluded that the lipid profile in cyanobacteria was able to be determined. A few data obtained can support that these *Oscillatoria* sp. cyanobacteria can be used as a biodiesel feedstock due to their higher lipid content.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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