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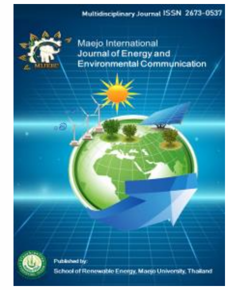

ARTICLE

Towards a sustainable approach for the development of biodiesel microalgae, *Closterium* sp.

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ABSTRACT

As fossil fuels are the principal source for the automobile and energy sectors, global warming and a rapid decrease in their availability are seen. Alternative fuels that are sustainable, renewable, and eco-friendly are widely investigated in order to maintain an aesthetic environment and combat fossil fuel depletion. Biofuels have the ability to both reduce pollution and provide energy. This study focuses on the extraction of oil from freshwater microalgae, *Closterium* sp. algae using the Soxhlet extraction process for biodiesel production. Oils are extracted from dry microalgae biomass and used in biodiesel production using solvent (hexane and acetone) extraction. With the help of solvents and catalysts, the extracted oil undergoes transesterification, which transforms it to biodiesel. *Closterium* sp. oil extraction using hexane and acetone yielded 7.8 and 5.6 g, respectively, as well as biodiesel was achieved 6.4 and 4.1 g. In the near future, this would be a revolutionary approach to produce cost-effective biodiesel from microalgae. Moreover, in this research article, *Closterium sp.* biotechnology for biodiesel production developments and prospects are discussed.

1. Introduction

Sustainable environmental requirements and rising energy demands, as well as the depletion of conventional energy resources and environmental deterioration as a result of abrupt climate change, have prompted scientists to look for renewable sources of green and clean energy for long-term development (Vu et al., 2018; Nguyen et al., 2020; Nong et al., 2020a, b, c). Bioenergy is a great alternative because, with the right conversion technology, it may be used to meet a variety of energy needs. Microalgal cultivation has sparked much attention in recent years due to its applications in CO_2 sequestration, biofuels, food, feed, and bio-molecule production (Bhuyar et al., 2020a). Although estimates of the number of algal species range from 350,000 to 1,000,000, only about 30,000 have been researched and analyzed. Microalgae are a varied group of organisms found in a variety of natural settings (Tipnee et al., 2015; Tsai et al., 2015; 2016; 2017). Many of the microalgae investigated are photosynthetic, but only a handful are known to grow in a mixotrophic or heterotrophic environment (Ramaraj et al., 2013). Light (photosynthetic and mixotrophic), carbon, macronutrients such as nitrogen, phosphorus, magnesium, and silicates, and many micronutrients (species dependent) are required for efficient microalgal production (Bhuyar et al., 2019). Apart from foods and feeds, another commodities business where algae have attracted a lot of attention is fuel production (Ramaraj et al., 2014a,b). The need to limit fossil fuel use is getting more critical as global energy demand continues to climb. The creation

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of renewable energy is currently receiving more awareness (Van Tran et al., 2020).

Algae could be used to make a range of sustainable fuels. Algal biomass might be burned directly or turned into ‘green crude,' syngas, heat, power, and liquid fuels by liquefaction, gasification, or pyrolysis. Algae may also create substantial amounts of lipids and carbohydrates, which can make biodiesel or bioethanol (Ramaraj et al., 2015a,b; Khammee et al., 2021). There has also been some investigation into the use of algae for the creation of biological hydrogen. Furthermore, algal biomass could be used as a substrate for methane production by anaerobic digestion (Ramaraj et al., 2016a,b,c,d; Wannapokin et al., 2018).

Despite the attention from both academics and businesspeople, algal fuels are not available to the public since it is impossible to mass-produce an algae-based commodity product that has little value. Around 50 companies have been set up to use algae as a biofuel, but none of them are currently manufacturing products commercially at competitive prices (Unpaprom et al., 2017). The aquatic species program of the Solar Energy Research Institute contributed a lot of the early research into the development of algae-based fuel. Although the technological feasibility of algal biofuels has been established, the method looks to be economically unviable at now (Nithin et al., 2020). The fake cheap cost of energy from fossil fuels is largely the result of the fact that they are able to draw on a huge historical energy storage store created by microalgae over thousands of years, and so overlooks the costs of production and CO_2 emissions (Dussadee et al., 2017;). The ability to create algal fuel with positive net energy is essential. This time around, a significant amount of effort must be invested in lowering process energy input.

The conversion of food crops such as wheat, corn, maize, and sugarcane into an energy source is related with first-generation biofuels (Saengsawang et al., 2020). Non-food wastes and lignocellulosic biomass generated as agriculture and forestry by-products are used to make second-generation biofuels (Ramaraj, and Unpaprom, 2016; Manmai et al., 2020; 2021). Separation of xylose and lignin from crystalline cellulose is required in the conversion of lignocellulosic biomass to biofuels. Second-generation biofuels made from lignocellulosic biomass are expensive and necessitate a lengthy pre-treatment process that uses a lot of energy, particularly during scarification and steam explosion (Trejo et al. 2020).

Nomenclature and Abbreviation

FAME	Fatty acid methyl ester
BB Medium	Bold’s Basal growth medium
NaOH	Sodium hydroxide
CO_2	Carbon dioxide

Furthermore, the high cost of enzymes required in hydrolysis is the most significant barrier to the commercialization of second-generation biofuels. Microalgal lipids/oils, a third-generation biofuels feedstock, are gaining traction as a renewable fuel source (Govindan et al. 2020). After extracting lipids from microalgal biomass using organic solvents, algae-based biodiesel synthesis has been effectively established in laboratory circumstances (Unpaprom et al. 2015).Biodiesel is a diesel fuel made from methyl esters of long-chain fatty acids sourced from plant or animal oils. The chemical composition of the oil, particularly the chain length of the fatty acids, is determined by the oil's origin. Chemical transesterification is used to make biodiesel from oil, in which the glycerol to which long-chain fatty acids are esterified in the source oil is substituted by another alcohol. Methanol is commonly used, though not always. Biodiesel can be used in normal diesel engines, although it's more common to mix it with regular diesel (Ramaraj et al., 2016a). Long-chain fatty acids are esterified to methanol in these compounds. As a result, biodiesels are frequently used as FAMEs.

The conversion of lipids from algae through indirect transesterification in two phases is typical for biodiesel synthesis from algae. The dewatering of algae and drying of algal biomass are usually the first steps, followed by the extraction of lipids, which are subsequently transesterify for biodiesel production. Mechanical, physical, chemical, and biological approaches can all be used to extract lipids. Direct transesterification is a one-step method that is 15-20 % more efficient than indirect trans-esterification. It is based on the catalytic conversion of lipids in algal biomass to FAMEs or biodiesel. Therefore, the purpose of this study was to verify the suitability of freshwater green microalgae *Closterium* sp. for biodiesel production.

2. Materials and Methods

2.1. Sample collection, identification, and isolation

Freshwater microalgae were used in this experimental study. The algae were utilized from fishpond, Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. Algae samples collected from all ponds were examined under a microscope to determine their species.

Figure 1 Freshwater microalgae, *Closterium* sp.

The sample was evaluated at magnifications of 10, 40, and 100x. The physical characteristics of the species were used to identify by phycologist Dr. Yuwalee Unpaprom. The microalgal strain used in the present study was isolated (i.e., *Closterium* sp. Figure 1).

2.2. Cultivation

Isolated *Closterium* sp. was grown in 125-mL stoppered Erlenmeyer flasks containing 50 mL Bold's Basal growth medium (BB Medium) at room temperature. Then culture was transferred to open cement pond, and the algal production procedure was adopted from Ramaraj et al. (2016a,c).

2.3. Biomass estimation, oil extraction and transesterification

Twenty-five liters *Closterium* sp. sample was collected from the cement pond and dried in solar dryer. The biomass was collected after dried and weighted. The dried algal biomass was 25 grams. A mixer grinder was used to grind dried algal biomass into powder, which was then stored in dry, airtight glass jars. Oil was extracted from this algae powder using solvent extraction by soxhlet apparatus. Algal oil was extracted by using hexane (40 ml) and acetone (ml) separately. The solvent solutions were mixed with the dried ground algae to extract oil. Oil extraction from the microalgal *Closterium* sp. was done using Bligh and Dyer method (Bligh and Dyer, 1959). Using a rotary evaporator, the extracted oil was evaporated in a vacuum to release the solvent solutions. Mixing of catalyst and methanol procedure was adopted from Hossain et al. (2008), and 0.25 g NaOH was combined with 24 mL methanol and well agitated for 20 minutes. Overall biodiesel production procedure was displayed in Figure 2.

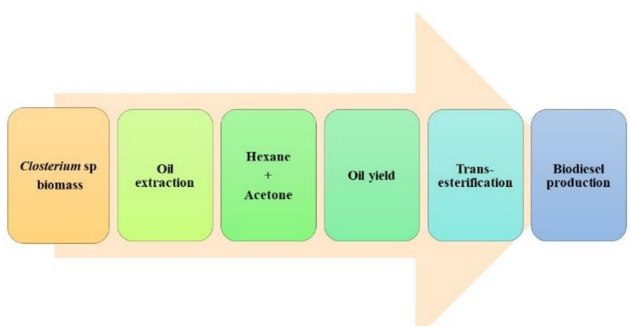

Figure 2 Closterium sp. biodiesel production procedure

2.4. Biodiesel production and purification

In a conical flask, the catalyst and methanol combination were put into the algal oil. The solution was maintained for 16 hours after shaking. to accurately separate the biodiesel and sediment layers. The biodiesel was properly separated from sedimentation using a flask separator. Sediment quantity (glycerine, pigments, etc.) was determined. The biodiesel was cleaned with 5% water until it was clean. The biodiesel was dried in a dryer and then stored under a running fan for 12 hours.

3. Results and Discussion

3.1. Closterium sp. characteristics

Closterium is filamentous algae. It is a common desmid that may be found in most freshwater environments. It lacks the isthmus described above, but it does have a small spherical vacuole at the extreme ends of each semi-cell in which changing amounts of microscopic particles are visible in constant agitation. Closterium cells are elongated or crescent-shaped and lack spines. Some are very narrow and needle-like, while others are much wider and have curved ends. The cell's ends usually are tapered, and they might be pointed or rounded. A single axial, ridged chloroplast with at least one pyrenoid is present in each semi cell (Wang and Ki, 2020).

3.1. Closterium biomass and oil extraction

Algae are significant as a biomass source. In addition, algae will someday be competitive as a source for biofuel. Before commercialization, algae biofuel production usually requires 5–15 years of steady operational development (Embong et al., 2021). Furthermore, algal biomass has been identified as a biofuel resource because of its efficient solar energy conversion into chemical energy. *Closterium* sp. biomass contains many biochemical components, including lipids/oils, making it a viable biodiesel feedstock. Therefore, oil extraction process is essential in this study.

Extraction of oil from algae can be done in three ways: (1) expeller/press, (2) solvent extraction with hexane, and (3) supercritical fluid extraction. Using a press to extract a substantial percentage (70–75%) of the oils from algae is a simple technique. Chemicals can be used to extract algal oil. Hexane is the most used solvent extraction chemical since it is reasonably affordable. Traditional solvent separation methods are significantly less efficient than supercritical fluid extraction. Supercritical fluids are selective, resulting in high purity and concentrations of product. This can extract nearly all the oils on its own. CO_2 is liquefied under pressure and heated to the point where it has the qualities of both a liquid and a gas in the supercritical fluid carbon dioxide extraction. The oil is subsequently extracted using this liquefied fluid as the solvent. In this study, hexane and acetone solvents were used for *Closterium* sp. oil extraction.

Microalgae lipid and fatty acid concentration varies depending on cultivation conditions. Saturated and monounsaturated fatty acids are found in algae oil. The fatty acids in the algal oil were found to be oleic (18:1), palmitic (16:0), stearic (18:0), iso-17:0, and linoleic (18:2). A large amount of saturated and mono-unsaturated fatty acids in these algae is regarded as ideal in terms of fuel quality, as fuel polymerization during burning would be much less than fuel obtained from polyunsaturated fatty acids. In this study, *Closterium* sp. oil extraction using hexane and acetone were 7.8 and 5.6 g.

3.3. Biodiesel from Closterium sp. oil

Hexane and acetone extractions of *Closterium* sp. oil gave 7.8 and 5.6 g, respectively along with biodiesel was achieved 6.4 and 4.1 g, respectively (Figure 3). Biodiesel is made up of mono-alkyl esters of vegetable oils or animal fats. Biodiesel is manufactured by transesterifying the source oil or fat to achieve a viscosity similar to petrodiesel (Nithin et al., 2020; Ma'arof et al., 2021). The chemical process of turning oil to its corresponding fatty ester is known as transesterification (biodiesel). Biodiesel is a biofuel made up of methyl esters derived by the transesterification process from organic oils, either plant or animal (Unpaprom et al. 2015). The process of biodiesel transesterification is simple. First, an overabundance of methanol is used to force the reaction to favor. The methanol that isn't used is collected and reused later (Unpaprom et al. 2015). The energy density of biodiesel is comparable to that of petroleum diesel. The heating value of petroleum diesel is 42.7 MJ/kg. The value of biodiesel varies depending on the biomass source. Biodiesel made from agricultural oils like rapeseed or soybean typically yields 39.5 MJ/kg, while biodiesel made from algal biomass yields 41 MJ/kg. Despite having the lowest energy density, seed-based biodiesels have enough energy density to be a viable alternative to petroleum diesel (Hossain et al., 2008; Abd Malek et al., 2020).

Biodiesel is often made from unicellular green algae found in the water. This type of algae is a photosynthetic eukaryote, which means it grows quickly and has a high population density. Under optimum conditions, green algae can double its biomass in less than 24 hours (Unpaprom et al. 2015). Furthermore, the lipid content of green algae can be exceptionally high, frequently exceeding 50%. This high-yielding, high-density biomass is well-suited to intensive farming and could be an excellent biodiesel source (Khazaai et al., 2021).

Seed crops have a far lower annual production and oil content than algae. Soybeans produce around 450 liters of oil per acre. Canola produces 1200 gallons per acre, while palm produces 6000. Take algae, for example, which can yield 90,000 liters per hectare. It's feasible that a tenth of the acreage currently utilized for soybean production could be converted to grow algae, thereby meeting the US's need for liquid fuel.

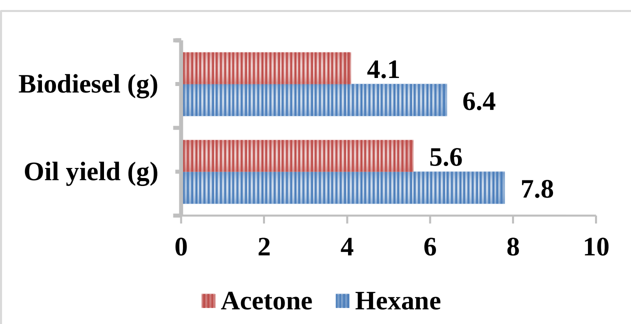

Figure 3 *Closterium* sp. biodiesel production procedure

A microalgal biomass generation stage, which requires light, carbon dioxide, water, and inorganic nutrients, is part of creating microalgal oils. Nitrates, phosphates, iron, and a few trace elements are among the latter (Unpaprom et al. 2015). Carbon, primarily produced from carbon dioxide, makes up around half of the dry weight of microalgal biomass. As a result, creating 100 tons of algal biomass fixes about 183 tons of CO_2. During daylight hours, this CO_2 must be continuously delivered. CO_2 is frequently supplied at a low or free cost. Many microalgae grow best at temperatures between 293 and 303 K. A temperature outside of this range could cause the cells to die or be damaged in other ways. Most current research on oil extraction is focused on microalgae to produce biodiesel from algal oil (Bhuyar et al., 2020b). The biodiesel from algal oil is not significantly different from biodiesel produced from vegetable oils (Ramaraj and Dussadee, 2015; Unpaprom et al. 2015). In the future, the method of preparing and characterizing *Closterium* sp. physical qualities, as well as fuel density, viscosity, heating value, spray pattern, engine performance, and exhaust gas emission, will be evaluated.

4. Conclusion

Microalgae may be a superior feedstock for biofuel production than terrestrial vascular plants. Microalgae can grow swiftly and thrive in a wide range of salinities and chemical compositions. Algal cells produce a lot of neutral lipids and oils, as well as other biochemical products, which can be sold to help pay for biofuel production. Microalgae biodiesel has received a lot of attention as a clean renewable fuel for diesel engines due to positive properties such as high productivity, rapid growth rate, and ability to convert CO_2 to fuel. This study looks into the use of microalgae biodiesel derived from *Closterium* sp. The current study proved the viability of an algae-based biodiesel manufacturing system. The findings of this study demonstrate that increasing *Closterium* sp growth in open ponding systems for biodiesel production is possible, although further research is needed in the future.

Declaration of Competing Interest

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

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