Biomass generation and biodiesel production from macroalgae grown in the irrigation canal wastewater
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ABSTRACT

The wastewater concentration is commonly acceptable for macroalgae growth; this process consumes water and is applicable for bioremediation. This study evaluated biodiesel’s potential production from freshwater macroalga, *Nitella* sp., using batch experiment. Algae were collected from wastewater saturated from irrigation canals. Water quality and algae growth environment characteristics were monitored and analyzed. COD and BOD values were 18.67 ± 4.62 mg/L and 5.40 ± 0.30 mg/L, respectively. The chemical composition contents were high, demonstrating that water quality and sufficient nutrients could support algae growth. Oil extraction was estimated by the room temperature and heat extraction methods. The biodiesel in room temperature treatment was 0.0383 ± 0.014%, and in heat, extraction treatment was 0.0723 ± 0.029%. Results confirmed that the heat extraction treatment gave a high amount of oil and biodiesel yield. Gas chromatography/mass spectrometry (GC/MS) was used to analyze fatty acid methyl esters (FAME). Results revealed that 9-octadecane was a major portion of the substance. The obtained results confirmed that the wastewater contains many elements that can be utilized for dual-mode, like bioremediation and enhanced macroalgae growth for biodiesel production. Therefore, macroalgae grown in canal wastewater were highly feasible for use in sustainable biodiesel production.

Key words | biodiesel, irrigation wastewater, macroalgae, water quality

HIGHLIGHTS

- Irrigation wastewater-grown algae were utilized.
- Waste water contents can be utilized for dual mode bioremediation and energy generation.
- Wastewater-grown algae content rich in nitrogen, and organic contents.
- Reflux reaction gives enhanced biodiesel production.
- *Nitella* sp. is viable for sustainable biodiesel production.

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INTRODUCTION

Recently, increasing worldwide population, leading to low carbon dioxide emissions, decreases the resources from various energy sources (Mejica et al. 2021a, 2021b). The use of green energy from fossil fuels suggests that seeking potential renewable energy can replace depleting energy sources (Ramaraj et al. 2019). Ecofriendly energy such as biomass energy, bioenergy, solar energy, hydropower, and others have been alternative fuels since 1988 in Europe, clean energy that does not emit pollution (Saad et al. 2019). Biomass can be generated through various oil-containing crops; Thailand is a country that can grow oil-producing crops such as maya oil, stone, palm oil, and black soap (Sasujit et al. 2021). However, the main problem is that these food crop materials are still being used for public food consumption. Material uncertainty may arise, facing a food shortage problem when applied in large-scale biodiesel production. Also, there are other factors such as investment, and expenses may also count. The production process may be complex, such as oil palm, making it necessary to research other energy crops to produce oil to reduce the risk and uncertainty for biodiesel application. This will be the next source of alternative energy in a sustainable future (Nithin et al. 2020).

Traditionally, humankind has been using non-renewable sources of energy as the sole energy source for all purposes. They are mainly extracted from plant and animal remains (fossils). But the main issue of using them is the high amount of CO₂ release, which directly affects the environment, as in global warming (Bhuyar et al. 2019). Fossil feedstock can produce chemicals, fuels, pharmaceuticals and other commercial products. However, from the environmental and the economic perspective, researchers are finding alternative forms of energy that are sustainable and offer less harm to the environment. Biofuel from microalgae has emerged as a promising alternative energy solution (Deepanraj et al. 2017a; Carminati et al. 2018). Bioethanol, biodiesel, and biohydrogen can be used as transportation fuels to reduce the high depletion rate of non-renewable sources. Microalgae are quickly grown and are suited to extreme conditions like salinity and alkaline water (Deepanraj et al. 2017b; Vishwakarma et al. 2018).

Previously reported studies demonstrated that macroalgae could produce a large number of fatty acid methyl esters (Bhuyar et al. 2019). This is an oil used for biodiesel production since it has been found that single-celled algae provide 70% of the oil content (Saengsawang et al. 2020). The oil production is much higher than that of corn and soybeans, respectively (Bhola et al. 2014). In biodiesel production investigation, most of the time, freshwater microalgae or marine algae are used. Macroalgae are available worldwide and growing independently without proper growth conditions (Pham et al. 2018). They can get habituated to any surrounding environment. Macroalgae are capable of growing in wastewater, as it contains several nutrient elements that enhance the growth of the macroalgae. Irrigation canal water is free flowing and may be saturated due to the unavailability of continuous flow.
Saturated water contains many nutrients (Dahm et al. 1998; Rajhi et al. 2020).

Therefore, the purpose of this research investigation is to study the factors affecting efficiency of biodiesel production from large freshwater macroalgae, Nitella (Nitella sp.), a macroalgae that can be harvested on a large scale, which are abundantly available on the canal sidewall, and are found to easily grow in the wastewater. Dual productivity such as wastewater remediation and biofuel generation are the prospective novelty perspective of this work. This research investigation aimed to analyze the wastewater quality, which enhances algae growth. Secondly, the chemical composition of macroalgae, oil and biodiesel are studied. The research was enhanced for next-generation biodiesel production from macroalgae. The objectives of this study are (i) to study the method for biodiesel production from Nitella sp. by growth enhancement, supplementing with wastewater (ii) to study the composition of biodiesel obtained from Nitella sp. (iii) to study the efficiency of oil production oil and biodiesel from microalgae, (iv) finally, the wastewater bioremediation can be utilized.

MATERIALS AND METHODS

Sample collection and preparation

The macroalgae samples were collected from Chorlae Subdistrict, Mae Taeng District, Chiang Mai Province, Thailand, and the laboratory identified species. The morphological characterization of samples was studied. Macroalgae samples were washed with water to remove toxic impurities such as epiphytes, sand, gravel, shells, etc. Then again, samples were rinsed thoroughly with distilled water and dried in the incubator (hot air oven) at 50°C temperature for 24 hours and placed in a desiccant bowl for 30 minutes. The macroalgae were subjected to measurement of Chlorophyll a, Chlorophyll b and total carotene parameters. The macroalgae samples were observed under a microscope at the laboratory, Faculty of Biotechnology, Maejo University. The observed species were photographed as shown in Figure 1.

Wastewater analysis

Quantitative analysis of total solids

Total solids (TS), volatile solids (VS) and fixed solids (FS) characterization was done to estimate the quantity of elements present in the wastewater samples as described by Ching & Redzwan (2017). Samples were heated until evaporated at a high temperature to study various solid contents. Wastewater samples were kept in a desiccant bowl and heated at 550 °C for 20 minutes (for VS heat at 103–105 °C for 1 hour). The desiccant bowl was cooled and the crucibles weighed, followed by measuring the sample volume from 20 to 50 ml. Depending upon water impurity following the experiment, it could be repeated if the water sample was clean. The dried crucibles were put on the hot plate and the evaporated crucibles removed. Finally, the dried weight of the residue was recorded and calculated.

Analytical analysis

Several parameters were assessed to characterize the wastewater, such as total suspended solids (TSS), fixed suspended solids (FSS) and volatile suspended solids (VSS). The raw wastewater, the treated wastewater and the final algae-treated effluent generated were analyzed. Samples were taken in triplicate over seven consecutive days, and the following parameters were measured: total suspended solids (TSS, mg/L), total fixed solids (TFS, mg/L), suspended fixed solids (FSS, mg/L), and suspended volatile solids (VSS, mg/L) were calculated by subtraction. All analyses were performed following the Standard Methods for the Examination of Water and Wastewater (APHA 2017; Ramaraj et al. 2016).

Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) analysis is a measurement of a water sample’s oxygen-depletion capacity when contaminated with organic waste matter (Gupta et al. 2016). Precisely, it measures the equivalent amount of oxygen required to oxidize organic compounds in water. COD measurement is commonly performed according to the
standard methods (e.g. reflux digestion and \( \text{K}_2\text{Cr}_2\text{O}_7 \) titration, and sealed digestion and spectrometry as described in the International Standards ISO 6060-1989 and ISO 15705-2002. In the standard method, the excess \( \text{K}_2\text{Cr}_2\text{O}_7 \) was titrated against ferrous ammonium sulfate using ferroin as an indicator, and the open reflux procedure was performed for the oxidation of the sample. The closed reflux procedure was performed. The oxidation of the sample and \( \text{K}_2\text{Cr}_2\text{O}_7 \) used in the oxidized samples were determined by measuring the absorbance of the formed \( \text{Cr}^{3+} \). The amount of \( \text{K}_2\text{Cr}_2\text{O}_7 \) used is proportional to the oxidizable organic matter present in the sample.

**Biochemical oxygen demand (BOD)**

Biochemical oxygen demand (BOD) is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at a certain temperature over a specific period (Abou-Elela *et al.* 2010). The process started with dissolved oxygen analysis on the first day and after 5 days after incubation. Results obtained from COD were used for BOD analysis; the BOD was determined by substituting the water quality standard in surface water sources. The following formulae are used for the calculation of \( \text{BOD} = 0.8 \times \text{COD} \). After that range, BOD was determined by the additional amount of water required to put in the BOD bottle, and the BOD was calculated from the formula as follows:

\[
\text{BOD value (BOD, mg/dm}^3\) = \text{DO} \times \text{D5} \times \text{T}\]

where \( \text{DO} \) = dissolved oxygen at the tritium on the first day; \( \text{D5} \) = dissolved oxygen titrated on day 5 and; \( \text{T} \) = sample volume divided by the volume of the BOD bottle (300 mL).

**Chemical composition of algae**

**Pigments extractions**

The collected seaweed was washed with clean water, pan dried and then weighed to a weight of 2 gm; the weight was recorded. The algae pulp was crushed and covered with protective foil. The method of chlorophyll-a quantification was as followed by previous research (Gregor & Maršílek 2004). The optical density was measured at 650 nm wavelength and 96% methanol used as the blank. The readings were recorded, and the amount of chlorophyll was calculated in units of mg/mL from the formula (Wannapokin *et al.* 2018).

\[
\begin{align*}
\text{Ca} &= (15.65A_{666} - 7.340A_{653}) \\
\text{Cb} &= (27.05653 - 11.21A_{666}) \\
\text{Cc} &= ((1000A_{470}) - (2.860\text{Cb}) - (129.2\text{Cb})/245) \\
\end{align*}
\]

when:

\[
\begin{align*}
\text{Ca} &= \text{Chlorophyll-a} \\
\text{Cb} &= \text{Chlorophyll-b} \\
\text{Cc} &= \text{Total carotene}.
\end{align*}
\]

Organic carbon was analyzed by the ammonium ferrous sulfate titration method. Determine the amount of organic carbon (OC)

\[
\text{OC(\%)} = ((\text{BS}) \times \text{N (Ammonium ferrous sulfate)} \times 3 \times 100) \times 1,000 \times \text{weight of seaweed samples}
\]

where,

\[
\begin{align*}
\text{B} &= \text{Ammonium ferrous sulfate volume applied to titrate with blank (ml)}; \\
\text{S} &= \text{Ammonium ferrous sulfate volume applied to titrate to seaweed samples (ml)}; \text{and} \ 
\text{N} &= \text{Ammonium ferrous sulfate concentration (N)}.
\end{align*}
\]

**Biodiesel production**

Two temperature conditions were used to extract oil from algae and produce biodiesel: extraction at room temperature and heat treatment (soxhlet) extraction, hexane as a solvent. An 8 gm of ground algae samples were taken in a round bottom flask, then 150 ml of hexane added and mixed well. The reflux reaction was carried out for the oil extraction process. NaOH catalyst was used to speed up the recovery. Glycerin was separated, and the amount of oil was recorded.

**GC mass spectrometry (GC-MS)**

A high-speed gas chromatographic method has been developed to determine biodiesel’s FAME distribution, including the analysis of fatty acid methyl esters. According to the adapted methodology, the lipid fraction was esterified to obtain methyl esters of fatty acids, and finally distilled by the soxhlet device (Yuvarani *et al.* 2017). The crude extract was analyzed by a gas-chromatography mass spectrometer.
(GC-MS), GC-MSD Toolkit 6890/5973, equipped with a 5 MS capillary column (30 m × 0.25 mm). Analysis times are typically on the order of 4–5 min depending upon the composition. Nitrogen was used as the carrier gas at a constant flow rate of 20 mL/min. The column oven temperature was programmed from 50 to 130 °C (at the rate of 40 °C/min) and held at 130 °C for 5 min, then raised to 260 °C at 2.5 °C/min and maintained at this temperature for 15 min (Bhuyar et al. 2020).

RESULTS AND DISCUSSION

Water quality and elemental analysis

Results obtained as shown in Table 1 by the characterization were total solids (TS) 89.56 ± 5.09 mL, volatile solids (VS) 22.67 ± 1.76 mL, and fixed solids (FS) 66.89 ± 1.76 mL. The results obtained for total suspended solids (TSS) were 10.33 ± 0.50 mL, volatile suspended solids (VSS) 3.00 ± 0.50 mL and anhydrous fixed solids (FSS) 7.33 ± 1.61 mL. Results revealed that algae were growing well due to the essential elements being available in the wastewater (Ramaraj et al. 2016).

Water testing analysis (DO, BOD, COD)

BOD analysis is generally a measure of the amount of oxygen that has been depleted over days in a controlled temperature cabinet at 20 °C. The amount of waste that is diluted is directly equivalent to the amount of oxygen available. This theory supports a higher amount of oxygen content, supports fastidious microorganisms’ growth, enhances microorganisms’ growth, enhances microorganisms’ growth, and enhances microorganisms’ growth. Wastewater contains toxic substances and toxic substances, but toxic substances and toxic substances and toxic substances and enough supplements for the growth of microorganisms such as nitrogen, phosphorus, etc. The organic matter produced from various organisms degraded into carbon dioxide, which was analyzed.

BOD from the water oxidation rate analysis experiment showed dissolved oxygen content of about (DO) 2.17 ± 0.05 mL, and the impurities of water using chemical measured (COD) is 18.67 ± 4.62 mL, as shown in Table 2. This result explains that the oxidation level is higher. It was found that the BOD was not minimum, to the standard values of 20 mL. The BOD values reveal that water quality is not minimum, and wastewater is highly polluted. Environmental conditions affect microflora in the wastewater. The amount of oxygen used by microorganisms was equal to (BOD) 5.40 ± 0.30 mg/L. In this experiment, the wastewater collected for analysis becomes stagnant, reducing the exposure to oxygen. Therefore, it generally benefits the algal growth, as providing a lower value of oxygen solubilized in water benefits the algal growth, as it provides a high number of nutrients. The growth of algae was not toxic to the environment as algae use them for nutrition purposes.

Chemical components of algae

The chemical composition of algae was studied with a series of experiments. The results observed are chlorophyll-a 329.00 ± 4.07 μg/gfw, chlorophyll-b 184.98 ± 2.57 μg/gfw and carotene content was observed as 59.56 ± 1.86 μg/gfw. The carbon content was obtained as 26.14 ± 0.38% and nitrogen 3.97 ± 0.09%, as shown in Table 3. Wastewater contains an injectable substance that causes chlorophyll

Table 1 | Total solid substances content from the wastewater

| Substances                | Average (mg/L) |
|---------------------------|----------------|
| Total solids (TS)         | 89.56 ± 5.09   |
| Volatile solids (VS)      | 22.67 ± 1.76   |
| Fixed S solids (FS)       | 66.89 ± 1.76   |
| Suspended solids (TSS)    | 10.33 ± 5.09   |
| Volatile suspended solids (VSS) | 3.00 ± 0.50   |
| Fixed suspended solids (FSS) | 7.33 ± 1.61   |

Table 2 | Wastewater content analysis

| Results obtained             | Average (mg/L) |
|------------------------------|----------------|
| Dissolved oxygen (DO)        | 2.17 ± 0.03    |
| Chemical oxygen demand (COD) | 18.67 ± 4.62   |
| Biochemical oxygen demand (BOD) | 5.40 ± 0.30   |

Table 3 | Chemical composition of Nitella sp. macroalgae

| Composition         | Content          |
|---------------------|------------------|
| Chlorophyll-a (μg/gfw) | 329.00 ± 4.07   |
| Chlorophyll-b (μg/gfw)  | 184.98 ± 2.57   |
| Carotene (μg/gfw)     | 59.56 ± 1.86    |
| Inithree Yecarbon (%) | 26.14 ± 0.38    |
| Nitrogen (%)          | 3.97 ± 0.09     |

Corrected Proof
depletion. The chlorine was primarily used in wastewater treatment, which affects glyphosate, which will cause chlorophyll depletion. Reduced chlorophyll content leads to decreased transmission of photosynthesis. The chlorophyll content depends on the algae growth area, and CO concentration depends upon the atmosphere of water and energy from the sun (Tsai et al. 2015). In this experiment, the chlorophyll value was high because of the plant’s structure and behavior. Most autotrophic plants produce their food by fixing atmospheric CO₂, water, and solar energy.

**Biodiesel production**

Results obtained (Table 4) from two different treatments were observed: extortion at room temperature (RT) showed oil content at a room temperature of 3.36 ± 0.50% and biodiesel production was about 0.0383 ± 0.014%, and thermal extraction showed the oil content at about 7.86 ± 1.70% with an increased number of biodiesel production recorded as 0.0723 ± 0.029%. The obtained results indicate that providing a higher temperature in reflux reaction gives enhanced biodiesel production. Higher temperatures increase the amount of oil production and lead to higher biodiesel production (Jayakumar et al. 2021). Oil yield from various macroalgae is illustrated in Table 5. This present study has demonstrated that the oil yield from *Nitella* sp. is higher than that of the reviewed macroalgae species. This species could be utilized for further enhancement of biodiesel production.

| Oil content and biodiesel content after reflux reaction | Value analysis | Average (%) |
|---------------------------------------------------------|---------------|-------------|
| Oil extraction (RT)                                     | 3.36 ± 0.50   |
| Oil extraction (soxhlet)                               | 7.86 ± 1.70   |
| Biodiesel (RT)                                          | 0.0383 ± 0.014|
| Biodiesel (soxhlet)                                    | 0.0729 ± 0.029|

| Oil yield from various macroalgae | Macroalgae     | Oil yield (g/L) | References                      |
|----------------------------------|----------------|----------------|--------------------------------|
| Nitella sp.                       | 7.86           | This study      |
| Rhizoclonium sp.                  | 6.44           | Saengsawang et al. (2020) |
| Chana vulgaris                    | 3.60           | Siddiqua et al. (2015) |
| Chana sp.                         | 1.06           | Trifa et al. (2013) |

**Fatty acid methyl ester (FAME) analysis**

FAME analysis of the treatment 1 (Table 6) condition biodiesel showed the organic compounds and fatty acids contain 69.95% organic compound. The most common esters were 9-octadecane 36.03% followed by heneicosane 6.07%. Treatment 2 (Table 7) analysis of organic compounds showed fatty acids comprise 92.98% of various organic compounds. The most common esters (FAMEs) were 9-octadecane 36.08%, followed by eicosane 19.45% and remaining other substances 7.02%.

| GC spectra of FAME extracted at room temperature | % Area | Retention time% (min) | Quality |
|--------------------------------------------------|--------|-----------------------|---------|
| Tetradecamethyl                                   | 1.02   | 24.63                 | 90      |
| EicosaMethylcyclodecasiloxane                     | 0.22   | 41.9                  | 80      |
| Hexadecanamide                                    | 3.13   | 45.19                 | 94      |
| N-Docosane                                        | 4.4    | 45.18                 | 97      |
| Docosane                                          | 3.61   | 45.25                 | 98      |
| 9-Octadecane                                     | 36.03  | 46.54                 | 98      |
| Pentacosane                                       | 5.98   | 47.18                 | 99      |
| Eicosane                                          | 2.21   | 47.18                 | 97      |
| Heneicosane                                       | 6.07   | 47.74                 | 96      |
| n-Eicosane                                        | 5.36   | 48.35                 | 96      |
| Octadecane                                        | 1.92   | 49.10                 | 96      |
| Others                                           | 30.05  | –                     | –       |
| Total                                            | 69.95  | –                     | –       |

| GC spectra of FAME extracted by soxhlet apparatus | % Area | Retention time% (min) | Quality |
|---------------------------------------------------|--------|-----------------------|---------|
| Hexadecanamide                                    | 3.13   | 45.24                 | 97      |
| N-Docosane                                        | 4.32   | 45.24                 | 97      |
| Docosane                                          | 7.88   | 47.73                 | 90      |
| 9-Octadecane                                     | 36.08  | 46.50                 | 97      |
| Pentacosane                                       | 4.68   | 47.18                 | 99      |
| Eicosane                                          | 19.45  | 48.34                 | 95      |
| n-Eicosane                                        | 5.18   | 47.52                 | 98      |
| Octadecane                                        | 12.26  | 47.17                 | 95      |
| Others                                           | 7.02   | –                     | –       |
| Total                                            | 92.98  | –                     | –       |

**CONCLUSION**

Studies and experiments on biodiesel production from large freshwater *Nitella* were investigated. The wastewater...
analysis showed COD of 18.67 ± 4.62 mg/mL, and BOD was 5.40 ± 0.30 mg/mL. The chemical composition of algae was characterized as pigments, nitrogen, and organic carbon was studied. It was concluded that Nitella sp. could grow well in the canal wastewater as it has a high content of nitrogen and organic carbon, which promotes algal growth. Results revealed that the wastewater was of good quality and contained numerous nutrients. Higher microalgae growth showed sufficient oil extraction from algae. There is a higher oil yield from soxhlet extraction than the treatment extracted at room temperature and resulted in higher biodiesel content as well where at room temperature, treatment extracted at room temperature and resulted in quality and contained numerous nutrients. Higher microalgal content of nitrogen and organic carbon, which promotes algae could grow well in the canal wastewater as it has a high content.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.
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