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Biodiesel production using *Chlorella pyrenoidosa* algae: A review

Sindhu G, Punya H. A[1]., Lingayya Hiremath[2], Sumathra Manokaran[3] and A.H. Manjunatha Reddy[4*]

Department of Biotechnology, RV College of Engineering, Bangalore 560059, Karnataka, India[1,2,3,4]

[*]Author for correspondence

Abstract: As the demand for biofuels increases globally, micro-algae offer a viable biomass in rural communities these materials could be converted to biodiesel. A one-step process was applied to directly converting wet oil-bearing micro-algae biomass of *Chlorella pyrenoidosa* containing about 90% of water into biodiesel. In order to investigate the effects of water content on biodiesel production, distilled water was added to dried micro-algae biomass to form wet biomass used to produce biodiesel. The results showed that at lower temperature of 90∘C, water had a negative effect on biodiesel production. The biodiesel yield decreased from 91.4% to 10.3% as water content increased from 0% to 90%. Higher temperature could compensate the negative effect. When temperature reached 150∘C, there was no negative effect, and biodiesel yield was over 100%. Based on the above research, wet micro-algae biomass was directly applied to biodiesel production, and the optimal conditions were investigated. Under the optimal conditions of 100 mg dry weight equivalent wet micro-algae biomass, 4 ml methanol, 8 ml n-hexane, 0.5 M H SO , 120∘C, and 180 min reaction time, the biodiesel yield reached. As high as 92.5% and the FAME content was 93.2%. The results suggested that biodiesel could be effectively produced directly from wet micro-algae biomass and this effort may offer the benefits of energy requirements for biodiesel production.

Keywords: Micro-algae biomass, *Chlorella pyrenoidosa*, Biodiesel yield.

I. INTRODUCTION

1. An everlasting consumption of fossil fuels and effect of greenhouse-gas emissions on global climate change, the world is compelled to focus on finding alternative fuels to the existing fossil fuels. Biodiesel, as an alternative fuel, is considered as one of the most promising alternatives. A large number of studies have shown that biodiesel is environment-friendly, renewable, and biodegradable. Moreover, compared to traditional fossil fuels, biodiesel has lower CO, CO2, and hydrocarbon emissions. Micro-algae, which embody lots of unparalleled advantages as non food resources, are viewed to be a promising feedstock of the third-generation biodiesel.

Biodiesel can be used to immediately replace conventional diesel in the transportation market and has many environmental benefits over other fuels, such as, helping in the reduction of the carbon footprint. It has the potential to substitute a portion of the oil consumed by automobiles because of the pre-existing diesel distribution infrastructure and vehicle fleet. Compression-ignition diesel engines in the transportation sector can operate on biodiesel with little or no costly alterations. One specific source for biodiesel production that holds much potential is micro-algae.

Biodiesel offers various advantages such as prolonged engine life, safety, biodegradability, higher flash point, lower exhaust emissions, comparable performance and engine durability, non- flammable and non-toxic, and reduces tailpipe emissions, visible smoke and noxious fumes, and odours. Short-term problems of using biodiesel occur due to cold weather, plugging and gumming of filter lines and injectors, and engine knocking. Long-term effects include the coking of injectors and carbon deposits on the piston and head of the engine and failure of the proper functioning of the engine lubricating oil due to polymerisation. Most of them can be overcome by timely maintenance.

Micro-algae-based biodiesel is a potentially renewable resource for displacement of liquid transport fuels derived from petroleum. Micro-algae are easy to culture and require less space for cultivation. They convert carbon dioxide to potential bio fuels, valuable bioactive compounds such as carbohydrates, proteins, lipids and pigments. They contribute around 40 to 50 per cent of the oxygen in the atmosphere and consume carbon dioxide to grow photo-autographically and can be converted into several different types of renewable bio fuels such as Green Diesel, Jet Fuel, Methane Biogas, Ethanol,

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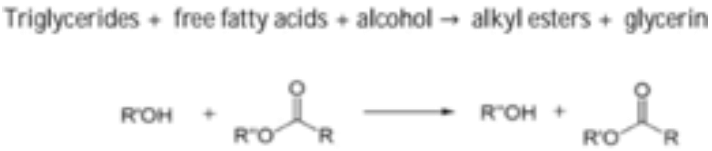
and Butanol. Green Algae (Chlorophyceae), Diatoms (Bacillariophyceae) and Golden Algae (Chrysophyceae) are the three most important classes of algae. Green Algae, belonging to the genera Chlamydomonas, Chlorella, Haematococcus and Dunaliella are widely utilised. As aquatic relatives of plants, micro-algae flourish in aerated, liquid cultures where the cells have sufficient access to light, carbon dioxide, and other nutrients.

Triglycerides, a major neutral lipid can be used for the production of biodiesel from algae. The chain lengths of fatty acid vary from C-10 to C>20 depending on the species. Neutral lipids basically consist of hydrocarbons and triacylglycerols (TAGs). TAGs can be used as a feedstock for the production of biodiesel.

Production of biodiesel has been done primarily in four ways - direct use and blending, micro emulsions, thermal cracking (Pyrolysis) and transesterification. Most commonly, production from micro-algae occurs by base catalysed transesterification with alcohol. Excess alcohol is added to drive the equilibrium toward the product‘s (biodiesel) side. The simplified transesterification reaction is



Reaction 1.1 Generalized Transesterification Reaction

II MATERIAL AND METHODS:

Strain and Culture Conditions: The green micro alga Chlorella pyrenoidosa was purchased from the algae strain laboratory of Aquatic Organism Research Institute of Chinese Academy of Science. It was maintained in 700 ml of media in 1 L baffled flasks, illuminated by Cool White fluorescent lighting (40 μmol/ (m2 s)), and aerated with air from the bottom of the vessel with an aeration flow rate of 200 ml/min (i.e., 0.25 vvm, volume gas per volume media per min). A modified BG11 medium was used as the basic medium which contains 100 mg NaNO3, 74.9 mg MgSO4·7H2O, 30.5 mg KH2PO4, 27.18 mg CaCl2, 20 mg Na2CO3, 8.9 mg C6H5O7Na3·2H2O, 6 mg ferric ammonium citrate, 1.04 mg Na2·EDTA, 2.86 mg boric acid, 0.222 mg ZnSO4·7H2O, 0.079 mg CuSO4·5H2O, 1.81 mg MnCl2·4H2O, 0.39 mg Na2MoO4, and 0.049 mg Co (NO3)2·6H2O per litre. Glucose (10 g/L) was used for heterotrophic cultivation of micro-algae cells. The details of culture of heterotrophic cells were reported in our previous research. Once the culture reached stationary growth phase, cells were harvested by centrifugation, frozen immediately, and then lyophilised prior to lipid analysis and subsequent in situ transesterification. As for biodiesel production directly from wet micro-algae biomass, the micro-algae biomass was harvested and stored immediately in a refrigerator at −80∘C.

Lipids Content: Total lipids in Chlorella pyrenoidosa cells were extracted according to a modified method. Lyophilised-dry micro-algae powder (200 mg) was triturated in liquid nitrogen for cell fragmentation then was blended with 3 ml chloroform/methanol (2 : 1), shaken for 20 min and centrifuged (4,500 g) for 10 min. The supernatant was collected in a pre weighed vial. The process was repeated twice. All the supernatants of were collected together, evaporated, and dried to constant weight at 45∘C. The dried vials were weighed to establish the total lipids.

In Situ Transesterification: A method of in situ transesterification of micro-algae biomass was introduced to produce biodiesel. In order to investigate the effects of water content on biodiesel production, according to the method of Wahlen et al. Different volumes of distilled water ranging from 0 to 9 ml were added back to lyophilise dried *Chlorella pyrenoidosa* biomass to form wet micro-algae biomass with different water content from 0 to 90%. The in situ transesterification reaction took place in a 20 ml cylindrical tetrafluoroethylene reaction vessel covered with a steel shell to make the reactor air-proof and be safe from high temperature. When using dried biomass added with distilled water to produce biodiesel, 1 g micro-algae powder with different volumes (0–9 ml) of distilled water and three different temperatures of 90, 120, and 150∘C were used. The optimal reaction conditions (4 ml methanol, 6 ml, n-hexane, 0.5 M

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H2SO4, 120 min) reported in our previous study were applied to this system. When directly using wet biomass to produce biodiesel, 100 mg dry weight equivalent wet micro-algae biomass was used. Three different temperatures (90, 120, and 150∘C), five different reaction times (90, 120, 180,240 and 300 min), five levels of methanol volume (1, 2, 4, 6, 8 ml), and five levels of n-hexane volume (2, 4, 6, 8, 10 ml) were used for the experimental trials to establish optimal conditions.

After the reaction stopped, 2.0 ml distilled water was added to the mixture. Samples were then cooled down to room temperature, centrifuged at 8,228 g for 10 min. Three layers formed which were composed of water phase (lower layer) containing water and alcohol, solid phase (middle layer) containing micro-algae residue, and organic phase (upper layer) containing solvent and biodiesel, respectively. The organic layer was collected and evaporated at 45∘C to constant weight for analysis. The biodiesel yield from micro-algae biomass was calculated by,

Biodiesel yield (%) = (Biodiesel mass (g)/algae mass (g) × oil content (%)) × 100%.

Analysis of Biodiesel Composition: The composition of biodiesel produced from in situ transesterification of micro-algae biomass was analysed by GC-linked mass spectrometry (GC-MS) equipped with a DB-5MS column (30 m × 0.25 mm ID DF = 0.25 μm) and with a flow rate of 1.0 mL/min.

III. RESULTS AND DISCUSSION:

Effect of Water Content and Temperature on Micro-algae Biodiesel Production: Before directly using wet micro-algae biomass as feedstock to produce biodiesel, the effects of water content and temperature on biodiesel production from dried micro-algae biomass with distilled water were studied. The results were shown in Figure 1. From the results obtained (Figure 1), it was observed that biodiesel yield decreased while water content increased from 0 to 90% at lower temperature of 90∘C. The biodiesel yield was 91.4% when water content was 0%, and when water content was increased to 90%, the biodiesel yield was only 10.3% (Figure 1).This was in accordance with the previous study that water could dramatically impede biodiesel production. However, at higher temperature of 120∘C, lower water content from 0% to 30% seemed to have no effects on biodiesel yield.

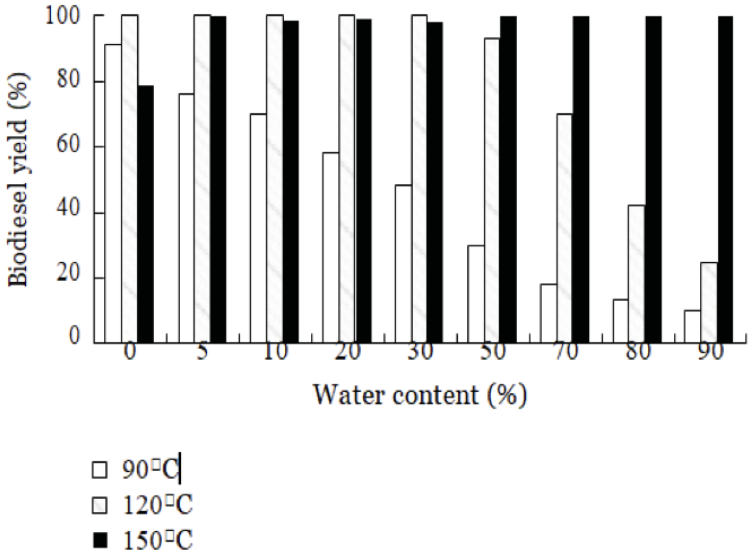

Figure 1

Figure 1: Effects of water content and temperature on biodiesel yield by in situ transesterification of dried *Chlorella pyrenoidosa* biomass with distilled water. Reaction conditions: 1 g dried micro-algae biomass (oil content 47%) with 4 mL methanol, 6 mL n-hexane and 0.5 M sulphuric acid for 120 min. Water content ranged from 0 to 90%. Temperature: 90, 120, and 150∘C.

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The biodiesel yields were all over 100%. This was probably because other molecules (e.g., phospholipids) were also converted into biodiesel. Further, increasing the water content from 50% to 90%, the biodiesel yields began to decrease, which was only 24.8% under 90% water content (Figure 1). The FAME contents of the biodiesel from dried micro-algae biomass with different water content under different temperatures are shown in Table 1. As shown in Table 1, the FAME contents were all over 87% under different water content at temperature of 120∘C. These results suggested that higher temperature could partially compensate the negative effect of water on transesterification. This is probably because high temperature could speed up the reaction (micro-algae biomass, methanol, n-hexane, and catalyst) and make transesterification reaction take place more effectively. Available water requires more energy to cause the reaction to effectively occur. Wahlen et al, found that when using 100 mg micro-algae biomass with 400 mg distilled water (water content 80%) to produce biodiesel, the FAME yield was 54% of the expected under the reaction conditions of 5 mL methanol, 1.8% (v/v) H2SO4, 80∘C, and 20 min of reaction time. In our study, when 9 mL distilled water was added back to 1 g micro-algae biomass (water content 90%), biodiesel yield was over 100% (Figure 1) and FAME content was 89.81% (Table 1) at temperature of 150∘C, more biodiesel produced than expected. As shown in Figure 1, the biodiesel yields were all over 98% under the water content ranging from 5% to 90% at temperature of 150∘C, and the FAME content was almost all over 88% (Table 1). It was reasonable that available excess water compensated for the effects higher temperature in the reaction mixture.

Effects of Temperature and Reaction Time on Biodiesel Pro- duct-ion from Wet Biomass: Based on the above experimental trials, the wet micro-algae biomass was directly applied to the biodiesel production. The results of effects of temperature

Table 1: FAME contents of the biodiesel from dried *Chlorella pyrenoidosa* biomass with different water content under different temperature through in situ transesterification. Reaction conditions: 1 g micro-algae biomass (oil content 47%) with 4 mL methanol, 6 mL n-hexane, and 0.5 M sulphuric acid for 120 min.

Water content (%) (g/g)	FAME content (%)		
	90°C	120°C	150°C
0	93.24	91.08	90.89
5	90.38	90.48	89.12
10	88.72	89.57	88.72
20	89.46	90.38	92.17
30	91.75	88.58	91.58
50	86.47	89.76	87.31
70	89.13	87.48	88.68
80	88.91	89.69	90.26
90	90.25	87.73	89.81

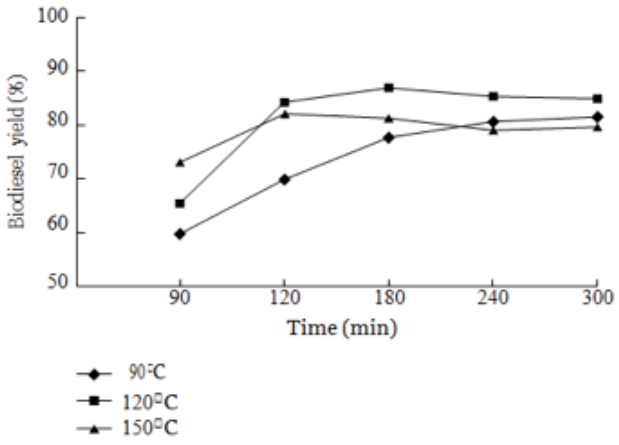

Figure 2

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Figure 2: Effects of reaction time and temperature on biodiesel yield by in situ transesterification of wet *Chlorella pyrenoidosa* biomass. Reaction conditions: 100 mg dry weight equivalent wet biomass (oil content 47%, water content 90%) with 4mL methanol, 6mL n- hexane, and 0.5 M sulphuric acid. Reaction time: 90, 120, 180, 240 and 300 min. Temperature: 90, 120, and 150∘C.And reaction time on biodiesel production directly from wet micro-algae biomass are shown in Figure 2.

It suggested that for the samples investigated at 90∘C, asymptotic biodiesel conversion values were not reached within the time bound- arises of this study. When in situ transesterification process was carried out at 120∘C, the highest biodiesel yield of 86.8% was obtained within 180 min (Figure 2), the FAME content was about 92% (Table 2). The biodiesel yield was slightly decreased to 85.3% and 84.7% when the reaction time increased to 240 and 300 min, respectively (Figure 2). The FAME contents of biodiesel produced at reaction times of 240 and 300 min under 120∘C were all about 90% (Table 2). These results suggest that longer reaction time could not efficiently produce more biodiesel. As reported by Patel et al , extended reaction time may result in overheating of the reaction mixture, greater losses of solvent, by- product formation, and energy losses. As shown in Figure 2.

Optimizing conditions of biodiesel production from wet microalgae biomass: Methanol always played an important role in transesterification because during the transesterification s reaction, methanol was considered not only as reactant, converting the lipids to fatty acid alkyl esters, but also as solvent, extracting the lipids from the biomass. Previous studies have demonstrated that methanol was optimal for

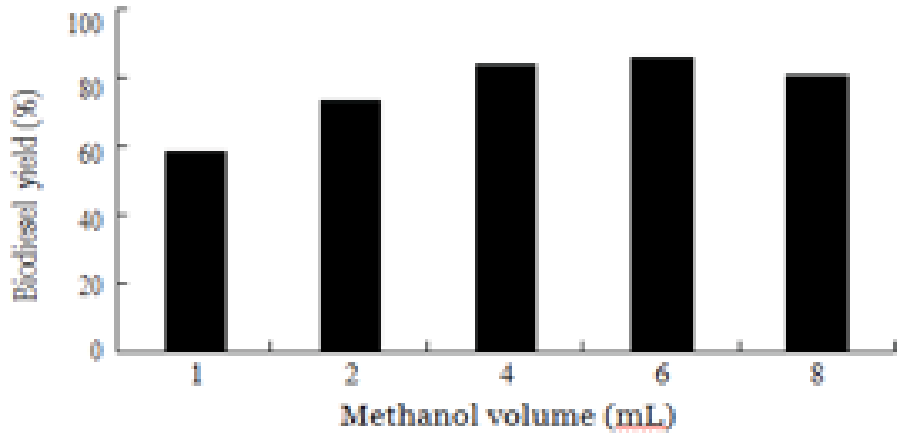

Figure 3

Figure 3: Effects of methanol volume on the biodiesel yield by in situ transesterification of wet *Chlorella pyrenoidosa* biomass. Reaction conditions: 100 mg dry weight equivalent wet biomass (oil content 47%, water content 90%) with 6 mL n-hexane and 0.5 M sulphuric acid at 120∘ C for 120 min. Methanol volume: 1, 2, 4, 6, and 8 mL.

Biodiesel production and the volume of methanol had a dramatic effect on biodiesel yield. To identify the optimal methanol volume, wet micro-algae biomass (100 mg dry weight equivalent) of *Chlorella pyrenoidosa* were reacted with 6 mL n-hexane, 0.5 M sulphuric acid and different volumes of methanol (1, 2, 4, 6, and 8mL) at 120∘C for 120min. The results showed that the biodiesel yield was increased from 59.2% to 83.9% with the methanol volume increasing from 1 mL to 4 mL (Figure 3). The maximum biodiesel yield of 86.6% was obtained at 6 mL of methanol. Further, increasing methanol volume to 8 mL, the biodiesel yield was decreased to 81.3%.

Wahlen et al determined that to reach the maximum biodiesel productivity, 2.5mL methanol was needed for 100 mg dried micro-algae biomass. Patel et al suggested that during transesterification, more methanol was needed to shift the reversible reaction forward (as observed) perhaps due to the increased contact area between methanol and lipid, resulting in higher yield of FAME. In the present research, we found that the volume of methanol necessary for maximal direct conversion of algal lipids to biodiesel was higher than that reported by Wahlen et al. More methanol was needed for

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optimal direct conversion of lipids from wet microalgae biomass, which is probably because the water within cells may dilute the methanol and make the contact of lipids with methanol much harder, although the maximum biodiesel yield was reached at level of 6 mL methanol, which was only 2.7% higher than that at level, of 4 mL methanol. From the practical point of view, it was beneficial to keep the methanol levels as low as possible to reduce the downstream separation costs. Therefore, 4 mL methanol was selected for the following study.

Biodiesel yield from wet microalgae under optimal conditions: The optimal conditions (4mL methanol, 8mL n-hexane, 0.5 M H2SO4 with temperature of 120∘C, and 180 min reaction time) were applied to in situ transesterification of wet micro-algae biomass for biodiesel production. The biodiesel yield could reach as high as 92.5% and FAME content was 93.2%.

IV. CONCLUSION

Water could generate a significant negative effect on in situ biodiesel production when dried microalgae biomass suspended in distilled water was used as feedstock at low temperature. However, this negative effect could be compensated by higher temperature. High biodiesel yield and high quality biodiesel could be obtained through in situ transesterification directly from wet microalgae biomass. This effort may offer the benefits of energy requirements for biodiesel production by eliminating the needs for drying and extraction of microalgae biomass. The in situ transesterification of wet microalgae biomass had the potential to provide an energy efficient and economical route to microalgae biodiesel production.

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