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Freshwater Microalgae (*Chlorella* sp.): Pure Culture and Crude Oil Extraction for Biodiesel

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Abstract: *Chlorella* sp. was cultivated with Conwy medium, BG-11 medium and MN medium at microalgae laboratory. The pure strain with different media and pH were observed within one month. There are various methods for extracting the oil from freshwater microalgae, such as physical factors, mechanical pressing and solvent extraction etc. Solvent extraction method was used for extracting of crude oil from algae biomass by using n-hexane. In this study, crude oil extract of *Chlorella* sp. was identified and interpreted by Fourier Trans-form Infra-Red spectroscopy (FT-IR) to employ as a fast and reliable analytical technique for the quantification of various fatty acids esters and refined oil spectra in the produced biodiesel. This research has been focused on growing pure algae with high oil content and then extracting the potential of biodiesel from it.

Keywords: Microalgae, *Chlorella* sp., Solvent extraction, n-hexane, Oil extract, Fourier Trans-form Infra-Red spectroscopy (FT-IR)

1. INTRODUCTION

The term “algae” encompasses many different groups of living organisms. Algae have been commonly regarded as simple plants. Others, such as cyanobacteria, are more closely related to higher plants or animals in character. Algae have been part of the pattern of life on earth science primeval period and range from small single-celled organisms’ too much larger multicellular organisms that are related to plants, but without a vascular system, roots, stems leaves or embryos (fruits and seeds). They are mainly aquatic and are the primary producers in the aquatic food chain [1].

Depending on the species and cultivation conditions, algae can contain extremely high percentages of lipids or carbohydrates that are easily converted into a whole range of biofuels including biodiesel or bioethanol. Furthermore, the remaining biomass, mostly protein and carbohydrate, may be processed into many other products such as: foods, chemicals, medicines, vaccines, minerals, animal feed, fertilizers, pigments, salad dressings, ice cream, puddings, laxatives and skin creams (Edwards 2008) [2].

The use of fossil fuels is not sustainable and known petroleum reserves are estimated to be depleted in less than 50 years at the present rate of consumption (Sheehan et al., 1998) [3].

Algae technology has enormous potential, not only for algae based-biofuel, but also for food, feed, renewable chemicals and many other products that are critical for a more sustainable society. Algae have recently received a lot of attention as a new biomass source for the production of renewable energy. Some of the main characteristics which set algae apart from other biomass sources are that algae (can) have a high biomass yield per unit of light and area, can have a high oil or starch content, do not require agricultural land, fresh water is not essential and nutrients can be supplied by wastewater and CO2 by combustion gas [4].

pH is another important growth factor for algae. Different algal species have different favorite pH ranges and outside the optimal range the growth is affected resulting in slower specific growth rates. Both *Chlorella* and *Botryococcus* are growing very well at around pH6. *Scenedesmus* prefer alkaline medium between pH 9 to 10. In addition, algae can also change the pH of the medium during cultivation. When using CO_2 as carbon source, rapid growth of algae can cause the pH to rise due to photosynthetic uptake of inorganic C [5].

Lipids are one of the main components of microalgae; depending on the species and growth conditions 2–60 percent of total cell dry matter (Wijffels, 2006), as membrane components, storage products, metabolites and storages of energy. Among the biofuels, biodiesel represents an alternative to petroleum-based diesel fuels. Chemically speaking, biodiesel is a mixture of mono-alkyl esters of fatty acids, most often obtained from extracted plant oils and/or collected animal fats (Usta et al., 2005)[6]. These hydrocarbons are mainly accumulated on the outside of the cell, making extraction easier than when the cell wall has to be passed to reach the organics inside the cell (Wijffels, 2006)[7].

Most microalgae biomass contains three main components such as (1) lipids, (2) proteins, and (3) carbohydrates and/or hydrocarbons. Microalgae produce and store lipids in the form of fatty acids, phospholipids, glycolipids and it can be used as feedstock for biodiesel production by trans-esterification reaction in the presence of acid or base with methanol [8].

The aim of this paper is to study the mass cultivation, oil extraction and potential of freshwater microalgae for biodiesel production.

2. MATERIALS AND METHODS

2.1 Sample Collection

The freshwater microalgae (*Chlorella* sp.) sample was brought from Sal Taw drainage, in front of Mandalay Technological University, Mandalay, Myanmar. The strain was treated with enrichment culture for the favor of the rapid increase in number of a desired species of the microalgae. And then, they were kept in algae laboratory.

2.2 Preparation of Microalgae Culture

15ml of microalgae sample was taken from enrichment culture in each of four centrifuge tubes. These tubes were centrifuged at 5000 rpm for 5 minutes. After removing the supernatants, the cells will be suspended in distilled water in each tube. Centrifugation and washing were repeated for three

times to expel most of the microorganisms presented in algal sample. Washed microalgae were allowed to streak through loop on nutrient agar plates under axenic condition and to keep for at least 2 weeks to grow microalgae. Repeated streak-plating was carried out to peak up single colony from earlier streaked plates and to make free from bacteria.

From last streak plates, the single colonies were picked up by loop and allowed to grow in glass bottles for inoculum. Before putting in the 50 ml test tubes, the single cell growth and purity of single specie was confirmed after observing under microscope. Then, the pure culture of microalgae strain was maintained in 50ml test tubes, and placed onto the lighted shelf. And then, the test tube 10ml was transferred into the glass bottle 50ml by adding 40ml distilled water and nutrient with the ratio of 1ml/1L at room temperature and were cultured 15 days for inoculum.

After that, 50ml algae strain was cultured 20 days, with 200ml distilled water and nutrient. The cell density of strain was counted with Haemocytometer (x10) one day interval for one month. After one month culturing, they were turned over to 220 liter aquarium for two months with blower. The optical densities of microalgae were measured by using UV Spectrophotometer one day interval during the culture period (2 months).

2.3 Harvesting and Drying Process

The freshwater microalgae biomass was harvested by sedimentation. It was dewatered by filtration with a filter. And then, the wet microalgae biomass was collected from the filter with a spatula and taken in beaker. The biomass was dried at room temperature for 5days.The total biomass was weighted and stored at room temperature for extracting microalgae oil.

2.4 Pretreatment of Microalgae Biomass for Extraction Crude Oil

Before extraction, dried algal biomass was kept in freezer at 4°C overnight. And then, freezing biomass was grinded by mortar and pestle for disrupting the cell wall and enhance the efficiency of the lipid extraction process (Figure 1). Disrupting the cell wall for easier recovery of the intercellular lipids, resulted data was rapidly and increased efficiencies in lipid extraction.

Figure1. Pretreatment of Biomass by Mortar and Pestle

2.5 Solvent Extraction Method

Solvent Extraction Method was selected by using n-hexane as a solvent. Soxhlet extractor was prepared in the laboratory. 5 gm of dry biomass was wrapped in thimble and placed in the extractor. Three hundred milliliter of n- hexane solvent was used for extraction. The extraction was done by many cycles for 10 hours. The solvent-oil mixture was separated by simple distillation for recovering of n-hexane solvent. Simple distillation was operated at 80°C because the boiling point of n-hexane was (50-70) °C.

2.6 Simple Distillation Method

After extracting, the solvent-crude oil mixture was placed into pre-weighed flask for evaporation. In the laboratory setup, the solvent and crude oil mixture was exposed to a vacuum and then heated at 80°C by using simple distillation because the boiling point of n- hexane was (50-70) °C. After distillation, the flask was weighed again and compared with first weight. In this way, the weight of the crude oil content was calculated. And then, the obtained microalgae oil was kept in tube at room temperature. The percentage of crude oil extraction was calculated by the following formula:

$$\%\ \text{of total crude oil recovered} = \frac{\text{Weight of Crude Oil Extract}}{\text{Weight of Microalgae Biomass}} \times 100$$

2.7 Quantitative Analysis of Microalgae Crude Oil (FT-IR) Analysis

Fourier Transform Infra-Red spectroscopy (FT-IR) is an analytical technique that collects spectra based on the temporal coherence measurements from an infrared source. FT-IR identified the functional group present in the molecules.

3. RESULTS AND DISCUSSIONS

For obtaining the best growth rate of *Chlorella sp*. with BG-11 medium was tested in optimum culture condition such as nutrient, pH, temperature and so on. For optimum pH value, *Chlorella* sp. culture with BG11 medium was tested by adjusting three different pH values in 500ml glucose bottles (Figure 2). And also, *Chlorella* sp. was tested with three different media Conwy medium, BG-11 medium and MN medium which are shown in figure 3. And then, the *Chlorella* sp. was cultured with BG11 medium at room temperature for a month period. The resulted data of microalgae cells were counted by haemocytometer one day interval was showed in Figure 4.

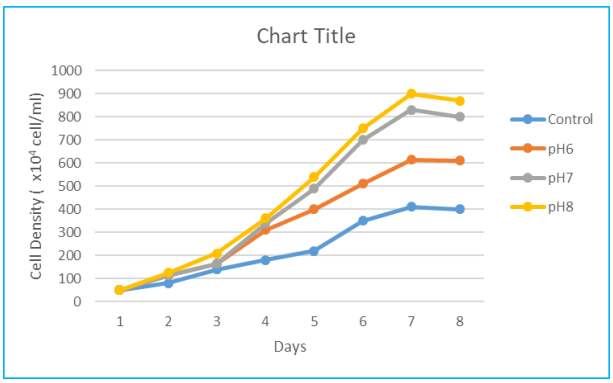

Figure 2. Growth Curve of *Chlorella sp*. Culture with Three Different pH Values by Measuring Haemocytometer

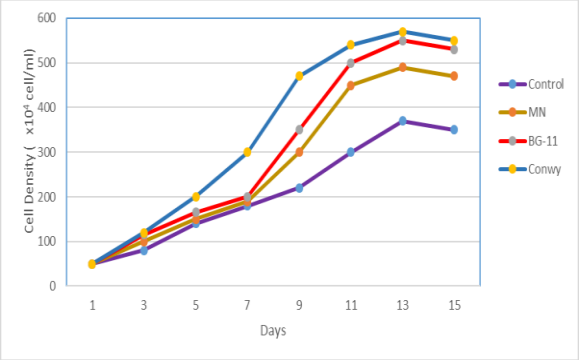

Figure 3. Growth Curve of *Chlorella sp*. Culture with Three Different Media by Measuring Haemocytometer

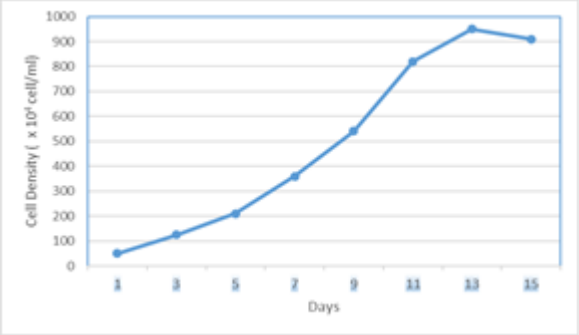

Figure 4. Growth curve of *Chlorella* sp. Culture with BG11 Medium by Measuring Haemocytometer

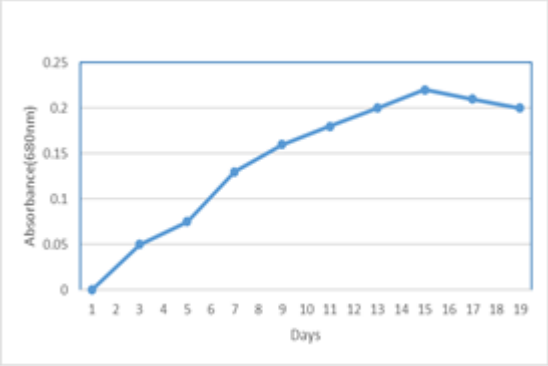

Figure 5. Growth Curve of *Chlorella sp*. Mass Culture with BG11 Medium by Measuring with UV Spectrophotometer (680nm).

Figure 6. Mass Culture of *Chlorella* sp. (200L)

It has been observed their growth conditions within one month. The resulted data of microalgae cells was counted by UV spectrophotometer (680nm) one day interval during culture period was showed in Figure 5. For mass culture of *Chlorella* sp. was cultured with BG11 medium for two months at outdoor condition as shown in Figure 6.

A. Biomass Processing

Microalgae biomass was harvested by sedimentation, dewatering and filtration. The harvested biomass was dried at room temperature and weighted. The result of 30gm was harvested from the maximum growth rate of *Chlorella* sp. (220 liters) (Figure 7).

Figure 7. Dried Biomass Harvested from the Cultivation of *Chlorella* sp.

B. Extraction of crude oil

From extraction oil of microalgae (*Chlorella* sp.) 5gm of microalgae dry weight, by soxhlet extraction method using n-hexane as solvent, the extract microalgae was approximately 1% (w/w) of dried *Chlorella* sp. biomass. The soxhlet extraction with n-hexane solvent was able to extract (18-20) % of crude oil present in the algae. The extract microalgae oil was showed in Figure 8.

Figure 8. Crude Oil Extract of *Chlorella* sp.

C. Quantitative Analysis of Microalgae Crude Oil (FT-IR) Analysis

Fatty acid ester content of extracted microalgae crude oil was analyzed by IR spectrum to identify microalgae crude oil used as feedstock for biodiesel production. It was used under the following condition: 4000-400cm^{-1}. Methoxy compounds, including methyl carboxylic esters, exhibit a weak band in the 2860- 2800cm^{-1}. Region of the infrared spectrum, which is diagnostic for the methoxyl group. The band occurs at a lower frequency than that of the main C-H absorption [9]. IR spectra of crude oil of *Chlorella* sp. strain was shown in figure 9. According to reference [9],[10] in figure 9, the characteristic absorption bands for the vibrations of C-H band, around 2923.25 cm^{-1} and 2853.81 cm^{-1} corresponding to the asymmetric and symmetric vibration modes of methyl groups respectively, was detected.

Rather subtle differences can be observed between the spectra, since the product of the transesterification process (FAME) is chemically similar to its precursor (the refined oil). In the

region from 1800-1700 cm^{-1}, it can be observed peaks that can be attributed to the stretching of C=O, typical of esters, and thus are common in both FAME and refined oil spectra [11].

The C=O absorption band of saturated aliphatic esters (except formats) is in the 1750-1735cm^{-1} region. The C=O absorption bands of formates, α, β-unsaturated, and benzoate esters are in the region of 1730-1715cm^{-1}. The C=O stretch, 1771cm^{-1} : this is higher frequency than that from a normal ester C=O stretch 1740 cm^{-1}[9].

So, the intense C=O stretching band of aliphatic esters , benzoate esters, typical of esters and refined oil spectra appeared around 1740.83cm^{-1} and 1715.76cm^{-1} in figure 9.

There is a sharp peak for OCH_3 between 1500cm^{-1} and 900cm^{-1}, known as "fingerprint" region. The peak at 1459.21cm^{-1} correspond to the asymmetric stretching of OCH_3 present in the biodiesel spectrum in figure 9.

The properties of biodiesel are largely determined by the structure of its component fatty acid esters (Knothe, 2005)[12].

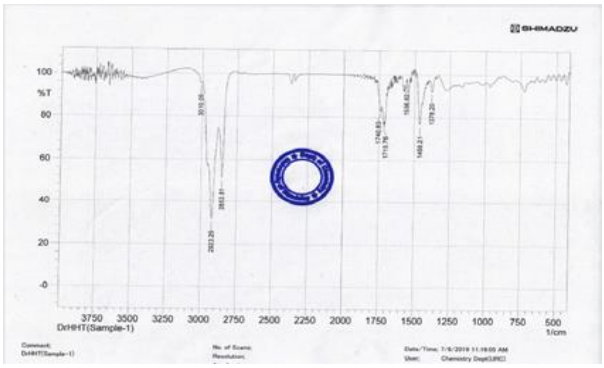

Figure 9. IR Spectra of Crude Oil Extract from *Chlorella* sp.

4. CONCLUSIONS

In this research, *Chlorella* sp. was successfully cultivated and grown with optimum culture condition such as nutrient (BG11medium), pH8, room temperature and so on. The microalgae strain gave more crude oil content amount and high biomass production according to their results. N-hexane solvent has been used as an efficient extraction agent of algae crude oil from *Chlorella* sp.

According to this results, the chemical typical structure of esters and refined oil spectra were identified and interpreted using FTIR. This led to the identification of functional groups in the molecules of the biodiesel with the aid of structure correlation chart. Therefore, microalgae *Chlorella* sp. can be considered as a potential crude oil for biodiesel production.

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