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Oil Algae Extraction of Selected Microalgae Species Grown in Monoculture and Mixed Cultures for Biodiesel Production

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Abstract

The idea of using microalgae as a bio-source of biodiesel is being taken seriously because of the escalating price of fossil fuel. As the tropical country, which gets the sun a year round, Indonesia has big potential for cultivating microalgae in large scale, as biodiesel feedstock. This study examines the algal oil extraction of selected monoculture microalgae species of *Botryococcus braunii*, *Nannochloropsis sp.*, *Arthrospira platensis* and mixed cultures microalgae from South Coast of Yogyakarta, Indonesia. Among all species of microalgae studied, *Nannochloropsis sp.* was found to have the highest algal oil yield (0.0346 g dry algal oil/g dry microalgae) and theoretical calorific value (187.69 kcal/kg dry microalgae).

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Keywords: microalgae; monoculture; mixed cultures; algal oil extraction; biodiesel

1. Introduction

The world has been faced with an energy crisis associated with the lack of petroleum resources used as primary energy in the last few decades [1]. With the increasing concerns to reduce greenhouse gas emissions, considerable attention has been focused on the development of renewable-alternative fuels [2,3], such as biodiesel [16]. Besides, biodiesel is also non-toxic and degradable biofuel [4]. Nowadays, biodiesel feedstocks mostly derived from palm oil, soybean oil, jatropha oil, sunflower oil, rapeseed oil, used cooking oil, and Palm Fatty Acid Distillate. However, the sustainability production of this mode has

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been concerned because of requirement of large area of plantation. Recently, algae have received a lot of attention as a new biomass source for the production of biofuels [5]. The main characteristics which set algae apart from other biomass sources are that algae have a high biomass yield per unit of light and area and high oil or starch content, do not require agricultural land, fresh water is not essential and nutrients can be supplied by wastewater and CO_2 from combustion gas [6].

The cellular lipid content in microalgae reaches 75% in *Botryococcus braunii*, but it is associated with low productivity of biomass [5]. The microalgae *Nannochloropsis sp.* and *Arthrospira platensis* are two widely available microalgae strains in the commercial applications. They showed great potentials as future industrial biodiesel producers due to their high growth rate and their high oil contents. Also, many methods and techniques have studied such as using mixed culture of microorganism. When using a mixed culture, two or more preselected species of microorganism are synchronously cultivated within the same medium, where these microorganisms can mutually exploit complementary metabolic activities to survive, grow, and reproduce [7].

Algal oil extraction is another challenge that is most easily addressed from the engineering side. Extraction can be broadly categorized into three methods: mechanical, electrical and chemical methods. The mechanical press generally requires drying the algae, which is energy intensive, while the use of chemical solvents present safety and health issues [8]. In the electrical method, pulsed electric field is extraction technology using electrical current for breaking cell wall of microalgae [9]. However, the chemical solvent extraction is more widely used because it is easy in operation. The purpose of this study is to extract monoculture of selected microalgae species of *Botryococcus braunii, Nannochloropsis sp., Arthrospira platensis*, and mixed cultures microalgae from South Coast of Yogyakarta, Indonesia. The chemical solvent extraction, known as soxhlet extraction is chosen to separate algal oil from its biomass residue.

2. Experimental

Monoculture-grown microalgae used in this experiment were *Nannochloropsis sp.*, *Botryococcus Braunii*, and *Arthrospira platensis* cultivated in Situbondo, East Java, Indonesia. Mixed cultures-grown microalgae were isolated from South Coast of Yogyakarta, Glagah, Indonesia.

Stock of mixed cultures microalgae was rejuvenated in bold's basal medium (BBM). After 5 days cultivation, 800μL culture was collected in micro tube and added with 200 μL alcohol 70%. Afterward, the micro tube was shaken and left for 15 minutes to undergo fixation. Then, the mixture was observed under the light microscope for identification of microalgae species composing the Glagah Strain. The identification reference was according to Edmondson [10] and van Vuuren [11].

Samples from green microalgae were collected in the conical tube and then centrifuged at 3300 rpm for 15 minutes for dewatering. In soxhlet extraction, the ratio of 20 mL n-hexane:1 gram dry weight microalgae ($W_{m.a.-dry}$) are used. The extraction was undergone for 3 hours at 80^{o}C to achieve maximum extraction efficiency. After extraction, the vacuum evaporator was used to evaporate the *n*-hexane. The yield of dry oil extracted ($W_{oil-dry}$) from dry microalgae calculated using Eq. 1. Lipid samples extracted from microalgae were analyzed for their fatty acid profiles by GC-MS.

$$Yield = \frac{W_{oil-dry}}{W_{m.a.-dry}} x100\% \quad (1)$$