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*Research Article*

# Cultivating Microalgae *Botryococcus braunii* in Tofu Whey Medium

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**Abstract:** Tofu waste water is still being a significant issue in Indonesia owing to its level of BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) (Chemical Oxygen Demand). However, this waste also includes significant ammonia (230 mg/L) which is needed for microalgae development. Microalgae are photosynthetic microorganism which need nitrogen supply for their development. Among others, microalgae *Botryococcus braunii* is the one with large oil amount within their cells (25 – 75 %). This study was meant to examine the usage of tofu whey for culture medium for algae development. The experiment was done by adjustment of whey volume (5-20 %) in fresh medium and algal biomass was continually measured. The findings revealed that *Botryococcus braunii* obtained optimum growth in 10 % volume with biomass generated at 2.4 g/L and 0,8716 g/L of lipid production. At this circumstance, COD might be lowered up to 83.33 %.

**Keywords:** Biodiesel; *Botryococcus braunii*; whey; biomass; optical density

## 1. Introduction

Tofu is one of the traditional foods of Indonesia. Tofu wastewater consists of organic waste from the tofu business that cannot be released into the environment. If the wastewater is released directly into the environment without treatment, it will result in the accumulation of organic compounds in the water body, as well as the proliferation of harmful microbes (Sudaryati et al., 2007). Liquid waste consists of a viscous liquid that is separated from the so-called whey clumps. There is still biological stuff present in whey that might harm the environment. According to statistics from the Ministry of Agriculture, Indonesia's tofu business requires 450 thousand tons of soy per year, allowing it to produce up to 19.575 million tons of tofu whey every year, or 54,375 tons per day. Tofu whey contains 99.34 % water, 1.73 % protein, 0.63 % fat, 0.11 % ash, and 0.05 % nitrogen (Hartati, 2010; Nuraida et al., 1996).

Since the beginning of the new century, energy shortage has been a topic of considerable concern. Use of non-renewable natural resources is the primary cause of the world's energy resource shortage. It is projected that by 2030, the global energy demand would exceed 60 % of the present energy supply (Patil et al., 2008). British Petroleum (BP) also said in 2005 that 47.5% of Indonesia's energy requirements are fulfilled by fossil fuels. Currently, Indonesia's oil reserves account for 1 % of the world's total (reserves of Natural Resources, 2005). According to BP Migas, Indonesia has been a net importer of oil since July 2004, making it a complete importer of petroleum. Therefore, it requires renewable energy sources with a high economic value, often known as biofuels.

Biodiesel (methyl ester) is a potential biofuel that may be generated from vegetable oils, animal fats, and waste oil by trans-esterification (Ozgul and Türkay 1993; Pamuji et al., 2004; Gerpen 2004). Trans-esterification mimics hydrolysis in that the alcohol groups of the ester are replaced by another alcohol. In contrast to hydrolysis, the trans-esterification process utilizes alcohol instead of water.

One of the potential new energy sources is microalgae. Microalgae are photosynthetic microorganisms that can convert carbon dioxide and sunlight into biomass. The fat content of biomass is sufficient for its usage as biofuel. Microalgae have quick growth, great productivity, the ability to utilize fresh or salt water, minimal water consumption, cheap production costs, and do not compete with food (Guerrero, 2010).

*Botryococcus braunii*, a unicellular microalga, is included in the category of green algae. Algae species are capable of photosynthesizing. Proteins, carbohydrates, lipids, and nucleic acids constitute *Botryococcus braunii*. This microalgae contains between 25 and 75 % fat (Chisti, 2007). This lipid will be removed and transformed into biofuel.

*Botryococcus braunii* will be propagated using a medium based on its potential nutritional content in this investigation. This study aims to determine the effect of adding synthetic nutrients versus whey on the growth rate of Botryococcus braunii, the effect of adding synthetic nutrients versus whey on the concentration of *Botryococcus braunii* and lipid levels on biomass, the characteristics of whey out after being used as a medium for the growth of *Botryococcus braunii*, and the efficiency of using whey as a growth medium.

## 2. Method

### *2.1. Material*

*Botryococcus braunii* of stock culture BBPBAP seeds, whey, KNO 3, TSP, ZA, NaHCO3, FeCl3, tap water, filter paper, NaOH, and n-hexane are used as materials. A cultivation pond, vacuum pump, fluorescent light, spectrophotometers, cuvette, pipette, oven, measuring cups, Erlenmeyer flask, glass beaker, digital scales, ultrasound, filter paper, burette, stative and clamps, electric stove, and distillation apparatus are among the tools used, as shown in Figure 1.

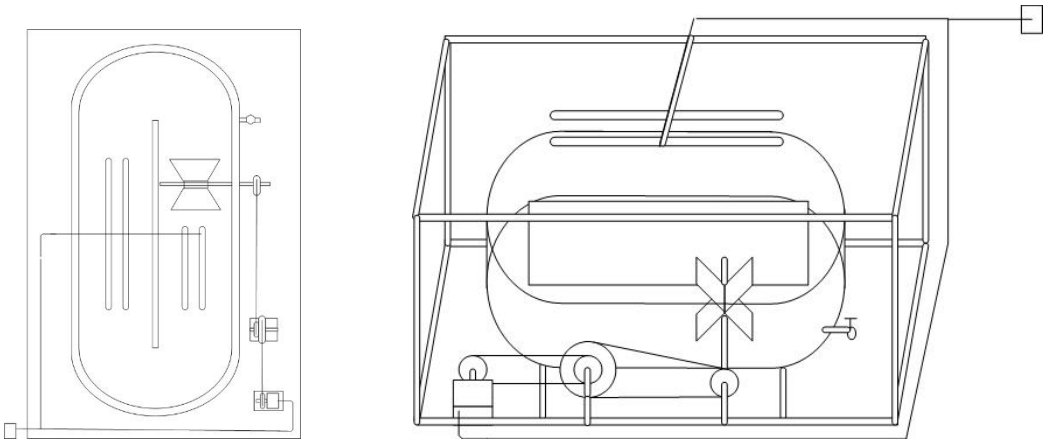

**Figure 1.** Mini scale of open pond for cultivation of *Botryococcus braunii* in tofu whey medium

### *2.2. Microalgae Cultivation*

The arrangement of cultivation according to the layout instrument. The container is filled with medium cultivation whey out with the addition of variable whey out of 0% (medium control), 5%, 10%, 15%, 20%, and 25% by volume of cultivation. *Botryococcus braunii* culture was completed without whey, 400 ppm $KNO_3$, 20 ppm TSP, 10 ppm ZA, 75 ppm $NaHCO_3$, and 1.3 grams of FeCl3 are to be added as synthetic nutrients to the media control (Chu-13). Around 10 % by volume of the culture cultivation *Botryococcus braunii* is added to the cultivation tub. Operation *Botryococcus braunii* is grown in a 16-liter culture at 30 degrees Celsius and pH 6-7. *Botryococcus braunii* culture tub samples collected on days 3, 5, 7, 9, and 11 for measurement of optical density (OD), the rate of biomass growth, and lipid contents.

### *2.3. Optical Density Measurements*

Using a spectrophotometer, analyze the optical density (DO). A spectrophotometer is an instrument used to measure the transmittance or absorbance of a sample as a function of wavelength. In this investigation, = 680 nm will be used. Connect the sp-300 spectrophotometer to a power source, switch on the sp-300 spectrophotometer, and wait for 5 to 10 minutes while pushing the % T button, setting the scale to infinity absorbance readings (transmittance = 0), and entering the pure solvent dis-tilled water in a cuvette. And pushing the 100 % T indicating absorbance = 0 (transmittance = 100 %) into the scale. Using a pipette culture, 10 ml samples are collected, and then the sample is transferred to a cuvette. The sample-containing cuvette is put into the measurement chamber. At = 680 nm, absorbance was measured. The % T shows on the display and is then recorded.

### *2.4. Lipid contents measurements*

The growth of *Botryococcus braunii* was measured by optical density (OD) at a wavelength (λ) of 680 nm (Sim, 2001). There is a clear correlation between dry biomass and optical density (OD). This association is determined by studies done in the control media. The link between optical density and dry biomass generated on connected medium. With conventional linearization, curves for additional variables may be generated. To extract lipids, dry biomass is crushed in a mortar containing a solution of n-hexane. Within 3 x 60 minutes, lipids extracted from the biomass combination are transferred gently to a solution of n-hexane solvent using ultrasonic.

*2.5 COD measurements*

Per sample, 10 ml of 0.01 N $H_2C_2O_4$, 5 ml of 4 N $H_2SO_4$, and 1 ml of solution KMnO4 were employed. Pour 10 ml of 0.01 N $H_2C_2O_4$ solution and 5 ml of 4 N $H_2SO_4$ into the Erlenmeyer flask to create a standardized KMnO4 solution. The mixture is then heated to a temperature between 70 and 80 °C. The combination is titrated with a solution of $KMnO_4$ until the red wine is produced, which cannot be removed by shaking. Consider titrant's requirements (b ml). Use the following formula to determine the normalcy of $KMnO_4$:

$$\text{N KMnO4} = \frac{(V \times N)H_2C_2O_4}{V\ KMnO_4}$$

The COD analysis is performed by placing up to 10 cc of waste in an Erlenmeyer flask and then analyzing it. Add 5 ml of 4 N H2SO4 to the Erlenmeyer and normalize the findings. 10 minutes were used to bring b ml of KMnO4 solution to a boil. Add 10 ml of 0.01 N H2C2O4 and keep the temperature between 70 and 80 °C. Titration using conventional KMnO4 solution to obtain TAT (a ml). Formula for calculating COD:

COD = [(a+b) x N KMnO4 standarisasi – (VxN) H2C2O4] x 8000

## 3. Result and Discussion

This study compared the optical density, biomass, and lipids generated by the medium control (varying whey addition of 0%) and the medium supplemented with tofu whey. The medium control employs a synthetic nutritional medium as its source of nutrients. In addition, the efficiency of whey usage will be examined by assessing the COD reduction content.

*3.1. Correlation Curve of Biomass and Optical Density*

A calibration curve was necessary to get the connection between the dry biomass (dry weight) and each optical density. Figure 2 depicts calibration curves for *Botryococcus braunii* derived from an examination of the growth rate in the control medium for *Botryococcus braunii*.

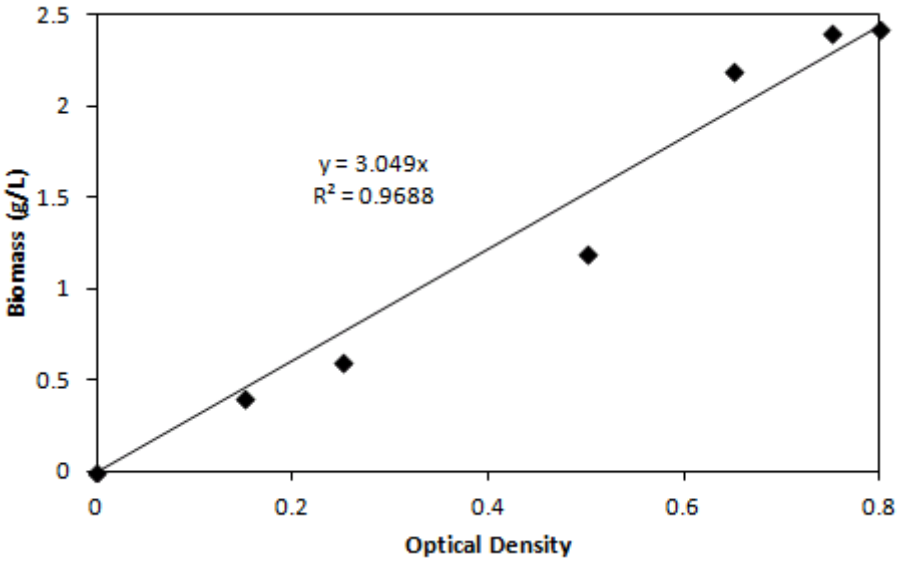

**Figure 2.** The correlation of biomass and optical density

The association between the optical density at a wavelength of 680 nm and the dry biomass of microalgae grown with synthetic fertilizers is seen in the graph above. Using the technique of auto-flocculation developed by Vandamme et al., (2012) the aforementioned study's findings are represented by the linear equation y = 3,087x-0.0066 ($R^2$ = 0.969). This demonstrates that the optical density (OD) is related to the dry biomass. Of linear equations is generated calibration curve will be used to quantify the quantity of dry biomass in *Botryococcus braunii* culture using whey.

*3.2. The effect of Whey Concentration to Growth Rate of Microalgae*

From studies done on five variables adding whey, the impact of adding whey on the growth rate of *Botryococcus braunii* during 11 days of mass culture can be determined. The link between optical density and cultivation time for each variable is shown in the Figure 3 below.

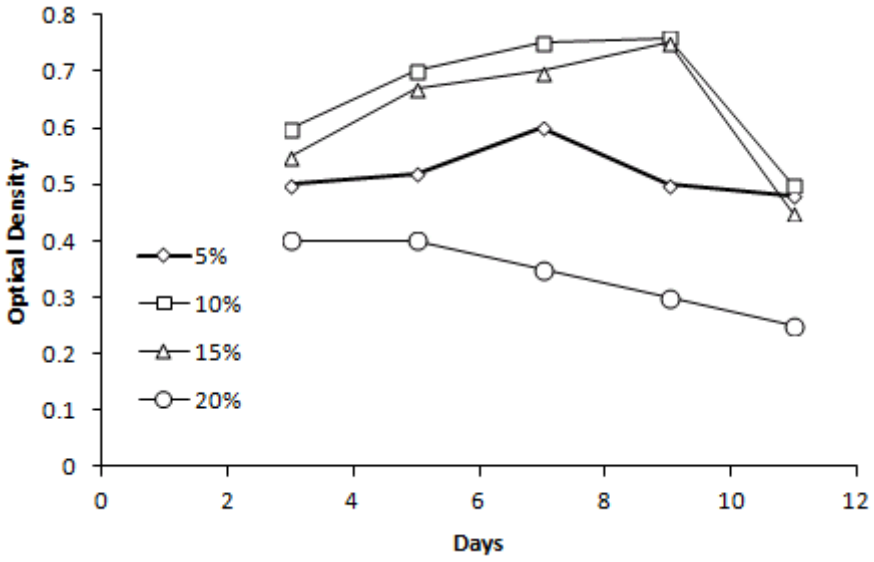


Figure 3. The growth of microalgae culture in various whey medium

From the graph, it can be observed that for variable 1, the *Botryococcus braunii* growth rate is lower when whey is added at 5% by volume of culture than when synthetic nutrients are added. This is owing to the absence of tofu whey needed by *Botryococcus braunii* in culture medium, hence reducing the capacity of cell division and resulting in a slower growth rate. The drop in optical density on day 9 is one evidence that a change has occurred. While the growth rate with the addition of synthetic nutrients dropped on day 11, the optical density decreased.

While in the second variable, whey was added at a rate of 10 % by volume of culture, and in variable 3, whey was added at a rate of 15 % by volume of cultivation, the growth rate of *Botryococcus braunii* was greater than with the addition of synthetic nutrients. In the second variable, the rise in optical density values from the first day of culture to the seventh day is greater than in the third variable. This suggests that *Botryococcus braunii* may use the two factors, nutritional content in whey, to achieve a higher growth rate than variable 3. While on day 9 the value of variable 3's optical density is 0.817% more than that of variable 2's, which is 0.802%.

In variables 4 and 5, the addition of whey at 20 and 25 % by volume of culture resulted in a reduced growth rate for *Botryococcus braunii* compared to the addition of synthetic nutrients. As with the initial variable, a sign is not ideal growth rate. *Botryococcus braunii* is distinguished by a decrease in optical density prior to the ninth day, i.e., on days 4 and 7 for variables 4 and 5 for variable 5.

This is because the nutritional content of whey is larger in variables 4 and 5 than in the other variables. In Suminto's research and Hirayama's (1996) study, it was shown that a medium with an excessive nutrient may limit the

development of microalgae, such as microalgae that need more time to adapt. Adaptation is the process of adjusting to the environment once whey was added to the growing medium. In addition, whey is aware that if surplus is not used correctly, it will build a heap of organic compounds that are poisonous to microalgae and may eventually limit their development. This is supported by study by Hastuti & Handajani (2011), which claims that if the whey is provided in excess in the tofu whey medium culture, there will be toxins that may limit development, owing to the presence of the toxic character of the direct cell metabolism will be disrupted.

*3.3. Effect of Whey concentration to Total Algae Biomass*

The nutritional content of whey is known to have a significant influence in the development of microalgae. Nutrients are chemicals essential for survival or the creation of the cell's organic components (cell growth). According to Amber (2009), the addition of nutrients to microalgae growing medium is the most significant factor in microalgal biomass production.

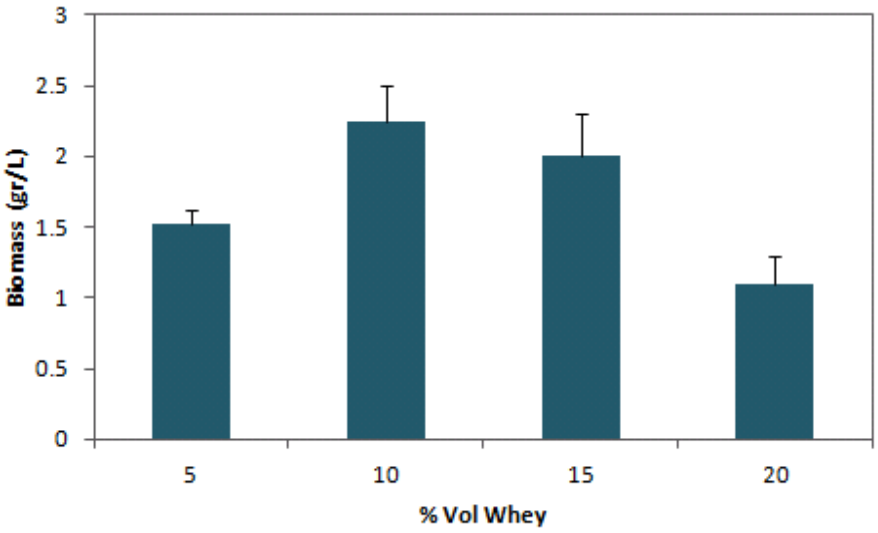


**Figure 4.** Total biomass after 11 days cultivation with variation of whey concentration

Based on the data obtained from the results of the study, the overall suspension of microalgae in the levels of tofu whey different show the same trend of growth in accordance with the culture of microalgae growth phase but with a different amount of dry biomass for each level of tofu whey. This refers to the amount of dry biomass is directly proportional to the optical density according to the calibration curve on the Figure 2.

From the Figure 4, the addition of whey out of level 5, 10, 15% by volume not give deleterious effects on *Botryococcus braunii* biomass that can be used by microalgae such as nutrients to increase the amount of biomass until at a certain time limit. From the research conducted, the average level of suspension of microalgae in tofu whey different achieve optimal growth on day 9.

The addition of tofu whey the levels of 20% by volume and 25% volume damaging effect on the biomass of microalgae. This is because microalgae are not able to digest nutrients in the tofu whey that the excess so that the lower the digestibility and the possibility of production of a toxic metabolite (Faradilla, 2011). Production of toxic metabolites that cause the rate of growth of microalgae is low (probably dead) so dry biomass produced lower. From the research conducted, the average level of suspension of microalgae in tofu whey this achieve optimal growth on the 7th day. Most microalgae biomass obtained in suspension microalgae cultivated in the levels of whey out of 10% by volume.

*3.4. The effect of Whey Concentration to Lipid content of algae biomass*

Figure 5 depicts the phenomena of a daily rise in the quantity of lipid generated by *Botryococcus braunii* throughout its development phase. In the first phase of culture, lipid production tends to be lower across all variables than it was when *Botryococcus braunii* began its third growth phase, indicating a drop-in growth rate (relative declining growth phase). When the growth phase of *Botryococcus braunii* reaches stationary phase and phase mortality, the quantity of lipid generated then drops (death phase). Casadevall et al. (1985) reported that the creation of lipids began in the early exponential phase of *Botryococcus braunii* development and ceased when the organism entered the late stationary phase, with the maximum level of lipid formation occurring during the exponential phase. While Widjaja et al. reported in

2009 that a decrease in nitrogen levels in the growing medium of microalgae might increase lipid levels, it can also slow development. This suggests that lipid production is optimal when the nitrogen level of the culture medium has dropped, which is in phase with a slower growth rate (relative declining growth phase).

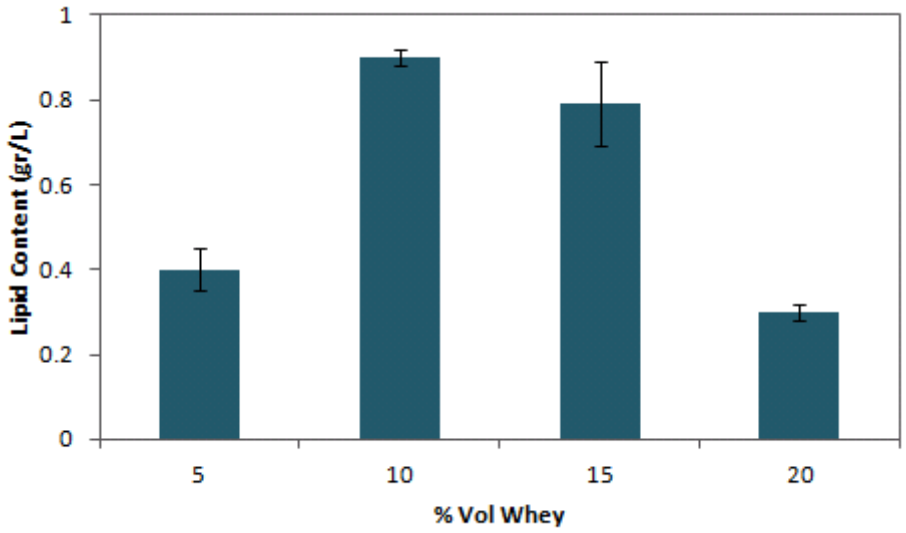


**Figure 5.** Lipid content produced by *Boyryococcus brunii*

Figure 6 depicts the correlation between daily lipid levels in *Botryococcus braunii* and all factors. From the figure above, we know that the greatest lipid levels are created by whey additions of 2 to 10 % by volume. Wang et al. (2010) predicted that the medium with a greater C/N ratio will also produce higher fat levels. This indicates that the higher the carbon concentration of the medium, the greater the lipid production. In contrast, the greater the nitrogen concentration, the lesser the lipid production.

Overall, the growth rate, the quantity of biomass, and the lipid content are most significant. *Botryococcus braunii* occurs under two conditions, namely the addition of 10% of the culture volume in tofu whey. This is because microalgae will enjoy healthy development provided their nutritional needs are addressed.

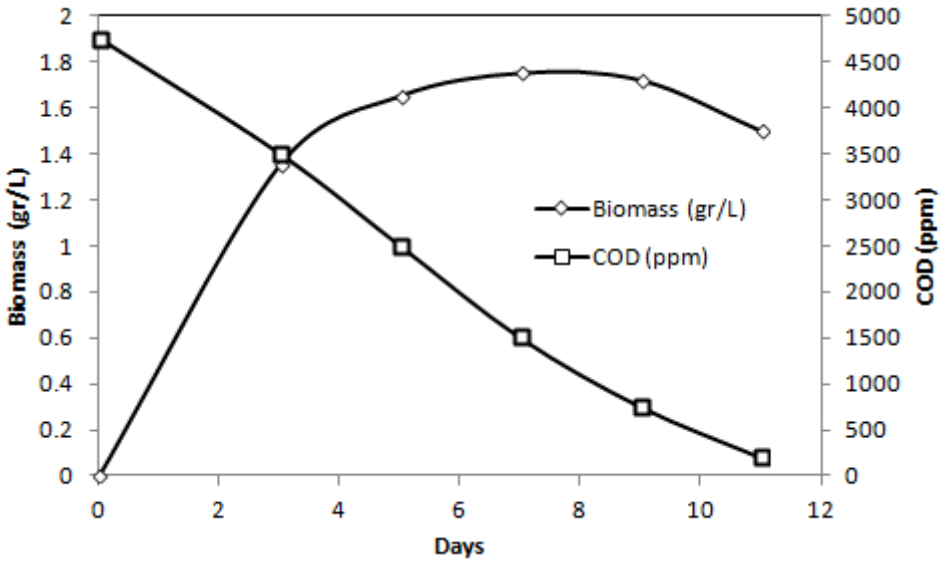


**Figure 6.** Biomass Production and COD changes during cultivation

## 4. Conclusion

*Botryococcus braunii* cultivation using tofu whey had the best results at a concentration of 10% by volume of the optical density (OD) optimum of 0.802 on the 9th day, with biomass of 2.4101 grams per liter and lipid content of 0.8716 gr/L. *Botryococcus braunii* cultivation with whey out at a concentration of 10% of the volume is superior than employing synthetic nutrients. COD reduction in the concentration of tofu whey addition was 83.33 % at the optimum (10 % by volume), whereas the largest COD reduction occurs at concentrations of whey addition ranging from 15% to 88.51 % removal efficiency.

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