The Effect of Various Agitation Speeds on Lipid Production from Fungal BR 2.2 Isolate

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Abstract. Biodiesel is an alternative energy that can be sourced from oleaginous microbes to form of single-cell oil. BR 2.2 isolate is classified as oleaginous organisms because of their ability to accumulate lipids for 28.44% of the total dry biomass. The speed of agitation in the lipid production process is one of the major factors for the effectiveness of the production process and reduced production costs. This study was conducted to determine the effect of the agitation speed and the incubation time on biomass production and lipid profile of BR 2.2 isolate by submerged fermentation. The stages of biomass and lipid production of BR 2.2 isolate were carried out at agitation speeds of 0 rpm, 50 rpm, 100 rpm, 150 rpm, and 200 rpm. The product was harvested in each incubation time of 0 h, 48 h, 96 h, and 144 h. The highest production yield occurred at 100 rpm with a total biomass of 2.78 g/L and a total lipid of 0.63 g/L for 144 h of incubation. Based on the research conducted, it can be concluded that the agitation speed and incubation time affect the production of total biomass and lipids in the production medium.

Keywords: oleaginous microorganism · BR 2.2 isolate · agitation speed · incubation time · lipid production

1 Introduction

The twentieth century has faced a lot of challenging issues, one of which is the high usage of fossil fuels. Fossil fuels are mostly used in the sectors of industry and transportation. The fossil fuel demand for meeting the needs of industry has been significantly growing in the latest years [1]. To tackle this issue, biodiesel is widely believed as one of the most effective alternative energies. Biodiesel works by utilizing oleaginous microorganisms to form of single-cell oil [2]. These microorganisms have the ability to accumulate lipids from the total biomass of 20–80% [3]. One example from this microorganism category is fungal BR 2.2 that has a specific character. This fungal soil isolated from the Batu Raden Botanical Gardens, Central Java, Indonesia is an oleaginous microorganism with the ability to accumulate lipids up to 28.44% [4]. With this ability, it is necessary to optimize that it can be seen the optimum lipid production of BR 2.2 isolate.

In regards lipid production process, the influence of agitation speed is one of the major factors for the effectiveness of the production process and reduced production costs. The effect of agitation speed increases oxygen supply in the growth medium

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[5]. This will support an increase in biomass formation which will also be followed by an increase in the amount of lipid formed [6]. Therefore, this study was conducted to determine the effect of appropriate agitation speed to produce optimum lipids in fungal isolates of BR 2.2.

2 Material and Methods

2.1 BR 2.2 Isolate as an Oleaginous Microorganism

BR 2.2 isolate is an filamentous fungal soil which isolated by Rizki and Ilmi in Batu Raden Botanical Gardens, Central Java, Indonesia. This isolate categorized as oleaginous organism with the ability to accumulate lipid up to 28,44%. High lipid production occurred in the screening medium that contained of C/N ratio 20,21% at room temperature [4].

2.2 Inoculum Preparation

Inoculum preparation was carried out by sub culturing BR 2.2 isolate culture stocks. The sub culturing method adapted streak plate on PDA medium. Then, the cultured BR 2.2 was incubated for 14 days or until it generated spores at a ± 30 °C room temperature. One done, the spores were harvested by adding a sterile 10 ml TritonX-100 (0.01%) solution to cover the entire colony surface. The surface of the colony was scraped slowly using a ose, so that the spores or hyphae were suspended in the TritonX-100 [7]. Finally, the number of spores in suspension was estimated using a haemocytometer and spectrophotometer (λ 550 nm) [8, 9].

2.3 Lipid Production

The lipid production process is carried out by the submerged fermentation method using a 250 ml glass bottle. The production medium used has a composition (g/L) of KH_2PO_4 2.5; $ZnSO_4 - 7H_2O$ 0.01; $CuSO_4 - 5H_2O$ 0.001; $MnSO_4$ 0.01; $MgSO_4 - 7H_2O$ 0.5; $FeSO_4 - 7H_2O$ 0.02; $CaCl_2$ 0.1; yeast extract 5; KNO_3 1; and glucose 30 [10]. 1.82×10^6 CFU/ml were inoculated into 50 mL of production medium which had been sterilized by autoclave (121 °C, 15 min) and incubated at 28 °C with a pH of 5.5. The experiment was carried out using different agitation speeds: 0 rpm, 50 rpm, 100 rpm, 150 rpm, and 200 rpm. In each agitation treatment, the product was harvested at incubation times of 0 h, 48 h, 96 h, and 144 h.

2.4 Dry Biomass and Pellet Diameter Estimation

The pellets formed were separated from the production medium using filter paper; while the remaining medium was accommodated in an erlenmeyer. Pellets were washed twice with sterile distilled water. The pellet size was measured with a digital caliper. The pellets that have been measured were then dried in an oven for 24–48 h at 60 °C. The dry biomass was then weighed to a constant degree with an analytical balance as the total biomass [11].

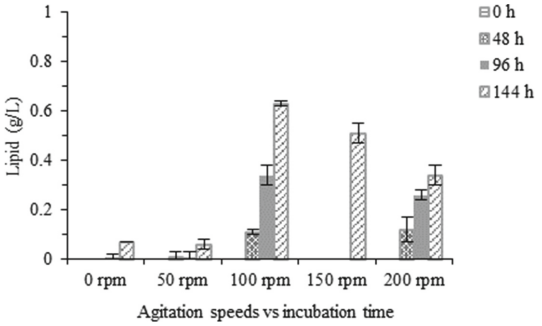

Fig. 1. Lipid production

2.5 Lipid Extraction and Lipid Content Estimation

The dry biomass was crushed together with acid sand using a mortar and pastel in a ratio of 2:1 (w/w). Chloroform: methanol (2:1) was added and incubated for 20 min with an agitation speed of 200 rpm. It was then centrifuged at 4000 rpm for 10 min. The solvent was transferred to a vial and evaporated at 60 °C for 24 h. The lipid remaining in the vial was eventually weighed using an analytical balance as total lipid [12].

2.6 Estimated Glucose Consumption and Nitrogen Consumption

Nutrient consumption in the form of glucose and nitrogen was estimated by calculating the difference between the initial glucose and nitrogen with the remaining glucose and nitrogen in the production medium. The remaining glucose content in the fermentation medium was analyzed using the DNS method [13]; meanwhile the remaining nitrogen content was analyzed using the Kjeldahl method [14].

3 Result and Discussion

The results showed that the increase in agitation speed and incubation time determined pellet size, total biomass, lipids and nutrient consumption of BR 2.2 isolate. The highest lipid production occurred at an agitation speed of 100 rpm with an incubation time of 144 h where the amount of lipid was 0.63g $\pm$ 0.01 g/L (see Fig. 1). These results were obtained from a total biomass of 2.78 $\pm$ 0.03 g/L (see Fig. 2). The details are presented in the following figures.

Lipid production tended to increase during the addition of incubation time to 144 h. However, fluctuating results were found due to the effect of differences in agitation speed. At agitation speeds of 0 rpm, 50 rpm, and 150 rpm, the total biomass formation and lipid production were not optimal. The difference in oxygen concentration in the growth medium will determine cell growth [15]. The cell biomass formed can be followed by an increase in the amount of lipids [16]. As a result, glucose consumption (see Fig. 4) and nitrogen consumption (see Fig. 5) increases with increasing incubation time [6].

The stable agitation speed of 100 rpm during the production process resulted in a more optimum lipid production compared than other treatments. However, it is possible that the

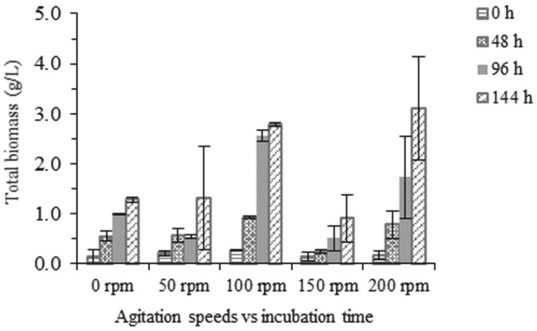

Fig. 2. Total biomass

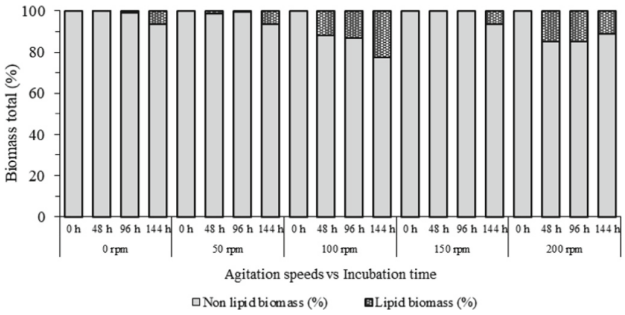

Fig. 3. The effect of various agitation speeds and incubation time on percentage of lipid biomass and non lipid biomass BR 2.2 isolate

actual optimum production occurred at an agitation speed of 200 rpm with a total biomass production of 3.11 ± 1.03 g/L (see Fig. 2) and a high glucose consumption of 87.19 ± 1.5% g/L (see Fig. 5). According to the theory, high glucose consumption is directly proportional to lipid production [6]. This is in line with the research result demonstrating that the instability of the agitation speed caused a decrease in the percentage of total lipid production (see Fig. 3). However, this assumption was validated when the instability occurred. The treatment of 200 rpm isolate BR 2.2 could still produce lipids that were classified as high known as 10.85% (see Fig. 3).

The effect caused by the agitation movement was the emergence of frictional forces that occurred between the surface of the bottle, the growth medium, and the surface of the pellet. The highest pellet size occurred under the influence of the agitation speed of 0 rpm, which was 1.28 ± 1,08 cm. At the higher agitation speed, the pellet size was only in the range of 0.20 ± 0.03 cm 0.33 ± 0.03 cm (Fig. 4). This result is supported by some explanation that friction caused by agitation causes delays in hyphae elongation and makes pellets rounded [17, 18].

In addition to the prior result, the effect of agitation speed of 150 rpm showed overall low yields in terms of pellet size, total biomass, lipids, and nutrient consumption. The size of the orbit of the agitator used in this tool was smaller than the size of the orbit of the agitator in other treatments. Consequently, a greater frictional force causes cell

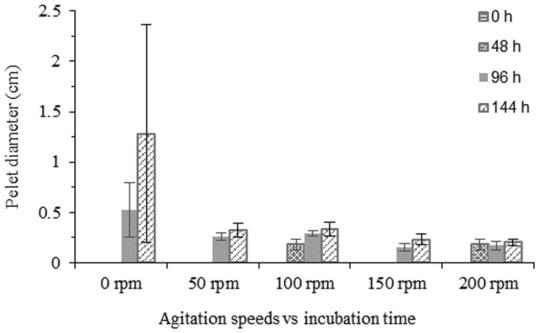

Fig. 4. Effect of various agitation speeds and inkubation time on pellet diameter BR 2.2 isolate

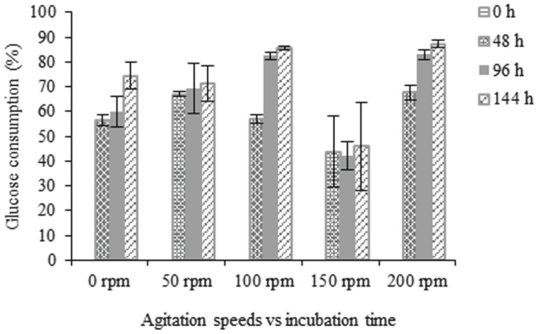

Fig. 5. The effect of various agitation speeds and incubation time on glucose consumption of BR 2.2 isolate

damage during the production process. This causes inhibited cell growth characterized by low biomass formation [19]. As a result, the lipid produced was also very low. Even when the product was harvested with an incubation time of 48 h and 96 h, no lipids were produced. The friction factor also affected the nutrient consumption which was low compared to other treatments.

In this study, nitrogen consumption was considered unusual because it did not exceed 50%. It can be said that nitrogen consumption was relatively low. (see Fig. 6) shows that at a speed of 100 rpm, the highest nitrogen consumption only reached 22.27 ± 5.42%. On the other hand, at a speed of 200 rpm, it was only 42.57 ± 11.75%. In this study, it was not known for certain the cause of the low nitrogen consumption with limited nitrogen in the medium. Therefore, further research is needed to determine the cause of low nitrogen consumption by isolate BR 2.2 in producing lipids.

4 Conclusions

The yield of total biomass and lipids increased with increasing incubation time but showed fluctuating production yields with increasing agitation speed. Meanwhile, the consumption of glucose and nitrogen increased with the increase in agitation speed

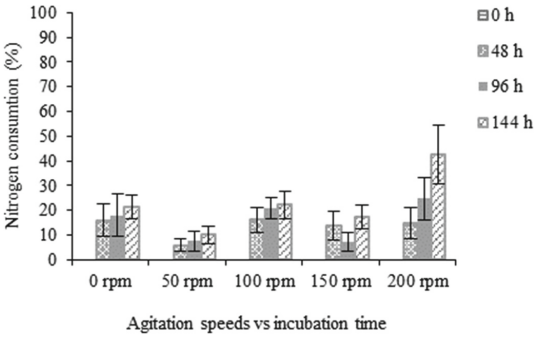

Fig. 6. The effect of various agitation speeds and incubation time on nitrogen consumption of BR 2.2 isolate

and incubation time. High biomass and lipid productivity can be achieved by agitation treatment of 200 rpm at an incubation time of 144 h with a stable agitation effect.

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