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ORIGINAL ARTICLE

Batch culture and repeated-batch culture of *Cunninghamella bainieri* 2A1 for lipid production as a comparative study

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Gamma-linolenic acid;
Harvesting time;
Harvesting volume

Abstract This research was performed based on a comparative study on fungal lipid production by a locally isolated strain *Cunninghamella bainieri* 2A1 in batch culture and repeated-batch culture using a nitrogen-limited medium. Lipid production in the batch culture was conducted to study the effect of different agitation rates on the simultaneous consumption of ammonium tartrate and glucose sources. Lipid production in the repeated-batch culture was studied by considering the effect of harvesting time and harvesting volume of the culture broth on the lipid accumulation. The batch cultivation was carried out in a 500 ml Erlenmeyer flask containing 200 ml of the fresh nitrogen-limited medium. Microbial culture was incubated at 30 °C under different agitation rates of 120, 180 and 250 rpm for 120 h. The repeated-batch culture was performed at three harvesting times of 12, 24 and 48 h using four harvesting cultures of 60%, 70%, 80% and 90%. Experimental results revealed that nitrogen source (ammonium tartrate) was fully utilized by *C. bainieri* 2A1 within 24 h in all agitation rates tested. It was also observed that a high amount of glucose in culture medium was consumed by *C. bainieri* 2A1 at 250 rpm agitation speed during the batch fermentation. Similar results showed that the highest lipid concentration of 2.96 g/L was obtained at an agitation rate of 250 rpm at 120 h cultivation time with the maximum lipid productivity of 7.0×10^{-2} mg/ml/h. On the other hand, experimental results showed that the highest lipid

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concentration produced in the repeated-batch culture was 3.30 g/L at the first cycle of 48 h harvesting time using 70% harvesting volume, while 0.23 g/L gamma-linolenic acid (GLA) was produced at the last cycle of 48 h harvesting time using 80% harvesting volume.

1. Introduction

In recent years, the production of polyunsaturated fatty acids (PUFAs) such as GLA, arachidonic acids and eicosapentaenoic acids by oleaginous microorganisms has received great interest from researchers. Among these fatty acids, GLA has extensively been used in biomedical products, nutritionals and health supplements (Zikou et al., 2013). Many research studies have been carried out over the last decades to develop lipid production. These attempts have aimed at improving the economic production of microbial lipids rather than plant and animal derived oils. In this view, microbial oils are superior to plant oils and animal fats due to less time required for their circulation in environment, higher possibility for large scale production and higher sustainability under climate changes (Li et al., 2008).

Previous studies have revealed that a high amount of lipid could be accumulated by the fungal species of *Cunninghamella* depending on the fermentation methods and culture conditions (Fakas et al., 2007, 2009; Somashekar et al., 2003). Similar studies have shown that a high lipid accumulation is attained by *Cunninghamella bainieri* 2A1 in the submerged batch culture (Taha et al., 2010). It is well known that *C. bainieri* 2A1 is capable of producing up to 30% lipid (g/g biomass) which contains 10–15% GLA. In this regard, nutritional intake of GLA and other PUFAs have been used in clinical treatment of human diseases such as blood cholesterol, acute and chronic inflammations, and atopic eczema, hypertension, Crohn's disease, rheumatoid arthritis and asthma (Shuib et al., 2014; Vadivelan and Venkateswaran, 2014).

The production of lipid by oleaginous fungi is highly dependent on medium composition. It has been observed that lipid production by *C. bainieri* 2A1 is related to the stress conditions created by the deficiency of nitrogen in the medium. On the other hand, it has been found that lipid synthesis by this strain is affected by carbon and nitrogen concentration in the culture medium (Taha et al., 2010). However, little is known about the effect of agitation rate on simultaneous consumption of nitrogen and glucose of the culture medium in relation to lipid production by *C. bainieri* 2A1. Agitation rate is an important factor which affects microbial growth, especially in shear sensitive microorganisms. Higher agitation rates result in better oxygen supply, which in turn favors cell growth. Hence, optimization of agitation rates is essential to provide high oxygen supply conditions for the mycelia and to increase their metabolic activities throughout the fermentation process (Abd-Aziz et al., 2008; Sun et al., 2012).

Fungal lipid fermentation could be performed as repeated-batch culture. The repeated-batch culture is a fermentation mode which offers many advantages over the microbial batch culture including the better depletion of medium in the bioreactor at the end of cultivation, the reuse of microbial cells for subsequent fermentation runs, higher cell concentration in the culture and less time required for process operation. Moreover, the repeated-batch culture is expected to increase cell productivity ensuring a high cell growth rate (Huang et al., 2008; Radmann et al., 2007). It has been noted that the repeated-batch culture is affected by operating factors. In this view, it has been observed that the repeated-batch culture is influenced by harvesting times and harvesting volumes of the culture broth (Jin et al., 2011; Masuda et al., 2011).

A number of studies have already been performed to study lipid accumulation by various fungal strains in the batch fermentation (Bellou et al., 2014; Fakas et al., 2009; Gao et al., 2013; Papanikolaou et al., 2004; Zikou et al., 2013). However, much less work has been performed to study fungal lipid synthesis in the repeated-batch cultivation. Current research was performed to investigate lipid production by *C. bainieri* 2A1 in the batch culture and the repeated-batch culture as a comparative study using a nitrogen-limited medium.

Furthermore, a detailed study on the use of different agitation rates was carried out to investigate the effects of agitation intensities on the depletion of glucose and ammonium tartrate as carbon source and nitrogen source, respectively in the culture medium for the enhancement of lipid production. On the other hand, the effect of two pivotal factors, namely harvesting time and harvesting volume of the culture medium on lipid production by *C. bainieri* 2A1 in the repeated-batch culture was studied.

2. Materials and methods

2.1. Microorganism and culture medium

C. bainieri 2A1 was obtained from *School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia*. Stock culture was maintained on potato dextrose agar (PDA) at 4 °C. Inoculum was prepared from the spore suspension containing 10^6 spores/ml harvested from 7-day-old PDA plates. The nitrogen-limited medium employed by Kendrick and Ratledge (1992) was modified and then utilized in this study with the compositions as follows (in g/L): glucose, 30; ammonium tartrate ($C_4H_{12}N_2O_6$), 1.0; KH_2PO_4, 7.0; Na_2HPO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 1.5; $CaCl_2{\cdot}2H_2O$, 0.1; $FeCl_3{\cdot}6H_2O$, 0.008; $ZnSO_4{\cdot}7H_2O$, 0.0001; $CuSO_4{\cdot}5H_2O$, 0.001; $Co(NO_3)_2{\cdot}6H_2O$, 0.0001 and $MnSO_4{\cdot}5H_2O$, 0.0001. The initial pH of the culture medium was adjusted to 6.0 using 1.0 M HCl or 1.0 M NaOH. Seed culture was prepared by transferring 20 ml spore suspension into 180 ml of the growth medium. Seed culture was then incubated at 30 °C and 250 rpm agitation rate for 48 h.

2.2. Batch and repeated-batch cultivation

The batch cultivation was carried out by an addition of 10% (v/v) of seed culture (20 ml) into 180 ml fresh medium in four