Sains Malaysiana 50(7)(2021): 1947-1957
http://doi.org/10.17576/jsm-2021-5007-10

Production of Docosahexaenoic Acid, DHA using Different Modes of Cultivation by *Aurantiochytrium* sp. SW1

(Penghasilan Asid Dokosaheksaenoik, DHA menggunakan Mod Pengkulturan Berbeza oleh *Aurantiochytrium* sp. SW1)

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ABSTRACT

Thraustochytrids, such as members of the genus Aurantiochytrium*, are rich in docosahexaenoic acid (DHA, C22:6n-3) and represent a promising source of omega-3 fatty acids which plays a vital role in the enhancement of human health, particularly for neurological and visual functions. Different modes of cultivation (batch, fed-batch and repeated-batch) by* Aurantiochytrium *sp. SW1 were studied for effective docosahexaenoic acid (DHA) production. In this study, three different modes of fermentation were carried out in 1 L shake flasks with a working volume of 500 mL, incubated at 30 ºC and 200 rpm. Batch cultivation significantly exceeds the rest of cultivation modes, achieving maximal lipid and DHA concentrations of 11.22 g/L and 5.87 g/L, respectively, and DHA productivity of 0.061 g/L/h. Lipid and DHA concentration of the repeated-batch process decreased through the cycles for all three different types of replacement ratio (80, 90 and 95%). The average decrease percentage of DHA concentration for cycle one and cycle two were 21.76 and 32.52%, respectively. However, the fatty acid composition of lipids obtained in the cycles remained consistent with 16:0 and DHA being the most abundant fatty acids indicating that this mode of fermentation is highly useable for industrial applications.*

Keywords: Aurantiochytrium *sp. SW1; docosahexaenoic acid; fed-batch; repeated-batch*

ABSTRAK

Thraustochytrid, seperti dalam kumpulan genus Aurantiochytrium *adalah kaya dengan asid dokosaheksaenoik (DHA, C22:6n-3) dan merupakan sumber asid lemak omega-3 yang memainkan peranan penting dalam memelihara kesihatan manusia terutamanya dalam fungsi neuron dan penglihatan. Penghasilan DHA oleh* Aurantiochytrium *sp. SW1 dalam mod pengkulturan berbeza (kultur kelompok, kultur suap-kelompok dan kultur kelompok ulangan) yang lebih berkesan diteliti. Semua mod pengkulturan ini dijalankan dalam kelalang goncangan 1 L, dengan 500 mL medium penghasilan pada 30* ºC dan 200 rpm. *Mod pengkulturan kultur kelompok adalah lebih signifikan berbanding mod pengkulturan lain apabila menghasilkan kepekatan lipid dan DHA tertinggi dengan nilai masing-masing 11.22 dan 5.87 g/L, serta produktiviti DHA bersamaan 0.061 g/L/jam. Dalam kultur kelompok ulangan (isipadu tertentu medium dituai dan baki kultur ditambah dengan sejumlah medium baru), kepekatan lipid dan DHA didapati berkurangan daripada kitaran 1 sehingga memasuki kitaran 2 bagi ketiga-tiga medium nisbah gantian (80, 90 dan 95%). Peratus pengurangan bagi kepekatan DHA dalam kitaran 1 dan 2, masing-masing adalah 21.76 dan 32.52%. Walau bagaimanapun, ketekalan komposisi asid lemak pada nisbah 16:0 dan DHA merupakan asid lemak yang paling banyak diperoleh dalam dua kitaran pengkulturan, menunjukkan bahawa mod penapaian bagi kultur kelompok ulangan berpotensi tinggi bagi kegunaan industri.*

Kata kunci: Asid dokosaheksaenoik; Aurantiochytrium *sp. SW1; kultur kelompok-ulangan; kultur suap-kelompok*

INTRODUCTION

Docosahexaenoic acid (DHA) is an ω-3 polyunsaturated fatty acid which is known to be essential for physiological functions

and human health. It is naturally found in breast milk and has been clinically proven to be a crucial building block for brain and eye development in infants especially during the last trimester (Fan et al. 2007). Thus, it is particularly vital for the development and maintenance of optimal cognitive and visual functions (Makrides & Bhatia 2016). It also has beneficial effects in lowering the incidence of certain cardiovascular diseases (Federova et al. 2011), hypertension, arthritis, sclerosis, and thrombosis (Furlan et al. 2017). The main commercial source for DHA rich oils is fish oil which is the prime ingredient for growth of the global aquaculture industry. Fish oil market is expected to garner $2.63 billion by 2020 due to largely used as a feed ingredient in numerous animal and human nutritional application, which include aquaculture feed, animal nutritions and pet feed, pharmaceuticals, supplements and functional food (Barajas-solono et al. 2016).

However, the high cost in the extraction and purification processes and environmental concerns such as chemical pollution, declining fish stock, seasonal variations in the composition of fish oil, typical unpleasant odor and taste associated with using marine resources have prompted searches for alternative sources of such oils (Qu et al. 2013). Therefore, industrial utilization of oleaginous microbes particularly microalgae capable of producing DHA has received much attention as a potential alternative of DHA due to high demand in fish oil market (Ren et al. 2014). Among the heterotrophic marine dinoflagellates, *Crypthecodinium cohnii* has been identified as a prolific producer of DHA and actively produced by Martek Biosciences Corporation (Ratledge 2005). Optimisation of various growth and cultivation parameters for enhanced DHA production by oleaginous thraustochytrids have been extensively studied. However, development of suitable modes of fermentation is also crucial for effective and efficient DHA production. As the batch fermentation are the simplest fermentation modes that could be employed, most published studies are related to this process.

We have previously isolated *Aurantiochytrium* sp. SW1, a heterotrophic thraustochytrid, which is commonly found in marine environments that could produce substantial amounts of lipids, up to 55% of the dry cell weight (CDW) where DHA (ω-3, C22:6) comprises as much as 50% of the total fatty acid (TFA) content (Manikan et al. 2015). Although optimization of cultivation media for enhanced DHA production for this thraustochytrid has been extensively studied in batch fermentation (Manikan et al. 2015, 2014; Nazir et al. 2018), this mode of fermentation has limitations especially in providing different specific nutritional and physiochemical requirements for the growth and DHA production phase, commonly resulting in low biomass concentration at the end of the growth phase. This could be addressed through the fed batch cultivation system that allows specific nutrients to be fed through out the process. Currently, the impact of other modes of fermentation such as fed-batch and repeated-batch for effective DHA production of *Aurantiochytrium* sp. SW1 has not been thoroughly assessed. Generally, fed-batch fermentation process is cost-effective as additional nutrients are provided to the reactor while cells and products remained in the reactor until the end of fermentation (Lee et al. 1999). Therefore, the optimal fed batch cultivation strategy for *Aurantiochytrium* sp. SW1 has not yet been established, it is crucial to further develop this cultivation system for this species.

Repeated-batch process, on the other hand, is known to enhance the productivity of microbial fermentation as it saves the time for cleaning, sterilization, seed culture, and inoculation processes between each batch fermentation cycle (Bae et al. 2004; Shakeri et al. 2007). It has been proven with the results obtained by Giulia (2016), where higher total of triacylglycerides (TAGs) productivity in culturing of *Nannochloropsis* sp. achieved compared to a batch process. Barajas-solono et al. (2016) also found that by applied a semi-continous culture or repeated-batch process for cultivation of *Aurantiochytrium* sp. strain NYH-2 was a 30% improved the biomass and fatty acids production. This mode of fermentation involves harvesting of a certain percentage of the culture of up to 95% (v/v) and replaced by the same volume of fresh media at the end of batch fermentation. Therefore, the remaining cultures serve as the seed cultures for the subsequent immediate fermentation runs. This mode of fermentation had been applied in various fermentation processes for producing different biochemical product, such as acetic acid (Ito et al. 1991), erythritol (Koh et al. 2003), amino acid (Hermann 2003) and citric acid (Moeller et al. 2010). In this study, experiments were focused on the effects of batch, fed-batch and repeated batch fermentations on DHA and lipid production by *Aurantiochytrium* sp. SW1. Consistencies of fatty acids compositions of the lipids from each cycles of the repeated-batch cultivation were also determined. The biomass, lipid and DHA yield and productivity in response to the different modes of fermentation were also investigated.

Materials and Methods

Microorganism

Aurantiochytrium sp. SW1 (GenBank: KF500513) was provided by Microbial Physiology Laboratory, Department of Biological Sciences and Biotechnology, Universiti Kebangsaan Malaysia. This microorganism was maintained on seawater nutrient agar (SNA) as slant cultures which contained 28 g/L nutrient agar and 17.5 g/L artificial seawater accounting for 50% salinity.

Culture condition

Seed cultures were prepared by inoculating 200 mL of a seeding broth with a strip of SNA slant agar containing approximately ten colonies of 48 h old microalga cells in 1 L Erlenmeyer flasks. Seed cultures were then incubated at 30 °C for 48 h under an agitation rate of 200 rpm. The medium used in seed cultures contained 100 g/L fructose, 2 g/L yeast extract, 8 g/L monosodium glutamate (MSG) and 16.45 g/L sea salt (47% salinity) (Manikan et al. 2014, 2015). A 10% (v/v) inoculum was the applied for subsequent initiation of the fermentations. All three different modes of cultivation (batch, fed-batch and repeated-batch) were carried out in 1 L Erlenmeyer flasks with 200 mL working volume. Medium used for the cultivation contained 100 g/L fructose, 2 g/L yeast extract, 8 g/L monosodium glutamate (MSG) and 47% sea salt. For batch fermentation, the cultures were incubated for 120 h at 30 °C with agitation speed at 200 rpm. The cultures were performed in duplicate with identical seed culture.

Fed-batch cultivation was carried out by employing two different feeding strategies. In feeding strategy 1, cultivation was carried out with an initial concentration of fructose of 35 g/L. Fructose was fed into the cultures at 24 h intervals to achieve the final concentration of 100 g/L. For feeding strategy 2, cultivation was carried out with an initial concentration of fructose of 100 g/L and fructose was fed into the cultures at 48 h intervals. Both cultivation with different feeding strategies were carried out for 120 h.

Meanwhile, repeated-batch cultivations were initiated with the culture conditions as employed in the batch cultivation. The cultures were harvested at various harvesting volumes (95%, 90%, and 80% of total culture volume) at 96 h of cultivation. The subsequent cycle of the cultivation was then initiated by transferring fresh medium (95%, 90%, and 80% of total culture volume) to the flask containing the remaining cultures. This step was repeated until 2 cycles were completed.

Determination of dry cell weight

Cultures (30 mL) were harvested by centrifugation at 4000 × g for 10 min. The recovered pellet were then washed with 50 mL of sterile distilled water and the suspension was then recentrifuged. The pellet were then oven-dried at 70-90 °C for 24 h and weighed. Biomass obtained was expressed as gram dried cell per liter of growth medium. As the microorganism accumulate substantial amounts of lipid, lipidless biomass concentration was used as a more accurate representation of growth by subtracting the value of lipid concentration (g/L) from the biomass concentration obtained.

Lipid extraction and fatty acid analysis

Lipid extraction was done using chloroform-methanol (2:1, v/v), as described by Folch et al. (1957). The extract was vaporized at room temperature and dried in a vacum desiccator for constant weight. Fatty acid compositions of the samples were determined as fatty acid methyl esters (FAMEs) by gas chromatography (HP 5890, USA) equipped with a capillary column (BPX 70, 30 m, 0.32 μm) at 200 °C as detection temperature using helium as a carrier gas with flow rate of 40 cm^3min^{-1}. FAMEs were prepared by dissolving 0.05 g of the sample in 0.95 mL hexane and the mixture was added to 0.05 mL of 1M sodium methoxide.

Residual fructose determination

The measurement of the residual fructose concentration was determined by using Dinitrosalicyclic Colorimetric Method (DNS) (Garriga et al. 2017). The centrifugated supernatant of each samples were appropriately diluted in Falcoon tubes with DI H_2O to an expected concentration of 1 g/L sugar. A 3 mL of diluted samples obtained was added with 3 mL DNS reagent and heated at 90 ºC for 5-15 min. The solution was added with 1 mL of sodium potassium tartrate and the OD at 575 nm was determined.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (one-way ANOVA) using SigmaStat 2.03 (SPSS, Chicago, IL, USA). Where significant difference were identified ($P<0.05$), all pairwise multiple comparisons between each treatment were carried out using a Tukey test.

Results and Discussion

Growth, Lipid and DHA Production of *Aurantiochytrium* sp. SW1 by Batch Cultivation

Batch fermentation proved to be an affective cultivation mode for the cultivation of *Aurantiochytrium* sp. SW1 as biomass concentration, lipid content, DHA content and DHA productivity achieved were shown to be similar with previously reported work using *Schizochytrium* and other *Aurantiochytrium* species (Manikan et al. 2014, 2015; Nazir et al. 2018; Qu et al. 2013). As illustrated in Figure 1, growth occurred until 48 h where the residual fructose concentration significantly decreased as li pidless biomass concentration increased until 48 h of cultivation, achieving 11.1 g/L. The lipidless biomass concentration obtained was similar to what was achived by Valcenir et al. (2016) where up to 12.3 g/L lipidless biomass was observed when *Aurantiochytrium* sp. ATCC PRA-276 was grown in batch cultivation. Similar profile of growth was also reported by Gao et al. (2013) where active growth of *Aurantiochytrium* sp. SD116 stopped at 48 h of cultivation and followed by stationary phase until 96 h of cultivation.

Throughout the 120 h cultivation period, SW1 was found to accumulate lipid at the early stage of log phase until the late stationary phase. Lipid concentration and content increased from 1.12 g lipid/L and 21.37% (g/g biomass) at 24 h cultivation to 11.22 g lipid/L and 48.91% at 96 h, respectively. This shows that, *Aurantiochytrium* sp. SW1 had similar lipid accumulation profile as *Schizochytrium* sp. HX-308 that started at the early stage of growth but with a lower lipid content of 10.7 g/L (Ren et al. 2009). This observation was different to what has been established in oleaginous fungi where substantial lipid accumulation was only initiated during stationary phase (Wynn et. al 1999). These may probably be caused by the involvement of two lipid biosynthesis pathway in oleaginous microalgae, polyketide synthase (PKS), and fatty acid synthase (FAS), whereas only the latter exists in oleaginous fungi (Shuib et al. 2018). Lipid biosynthesis through the FAS pathway is known to only be initiated after depletion of nitrogen, thereby, increasing lipid content in oleaginous fungi is only evident concurrent with stationary phase (Ratledge 2005).

The concentration of DHA increased at the early phase of cultivation, reaching its maximum by 96 h, achieving 6.0 g/L DHA (53.44% of total fatty acids) with the highest DHA productivity of 0.063 g/L/h. *Aurantiochytrium* sp. SW1 shows similar DHA accumulation patern to those reported by Chang et al. (2013) and Yu et al. (2015) of *Schizochytrium* sp. S31 and *Aurantiochytrium* sp. YLH70 grown on glycerol and high-fructose corn syrup, respectively. This findings also showed that, fructose was efficiently utilized by SW1 where up to 83.0% of fructose was used through out the growth and lipid accumulation phase.

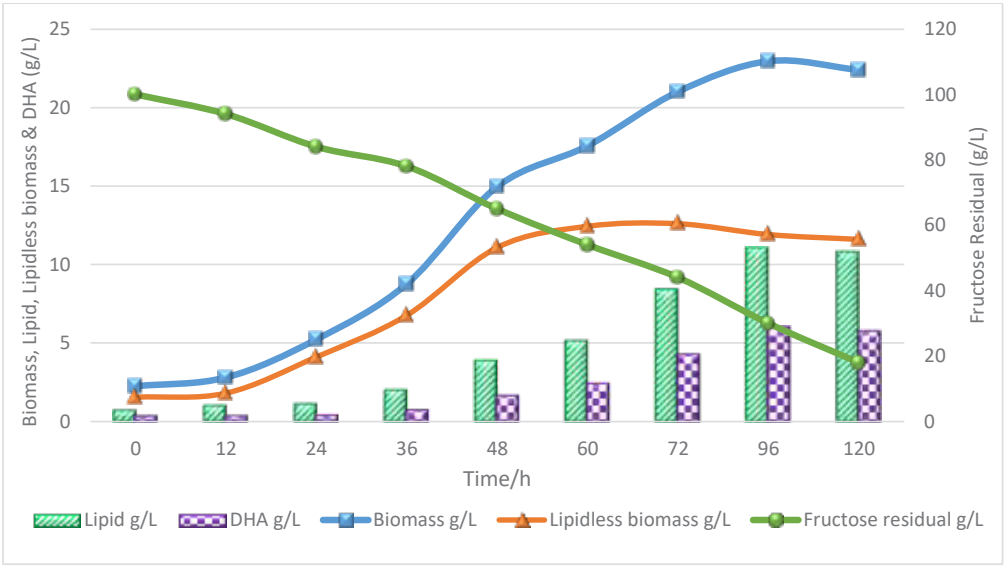

FIGURE 1. Time profile for growth, lipid and DHA production by *Aurantiochytrium* sp. SW1 within 120 h batch cultivation

FED-BATCH CULTIVATION PROCESS

Fed-batch cultivation was carried out in 1 L Erlenmeyer flask to evaluate the effects of different feeding strategies of fructose on the lipid and DHA production by *Aurantiochytrium* sp. SW1. Concentration of cell dry weight, lipidless biomass, residual fructose, DHA and total lipid content within 120 h fermentation were analysed at intervals. Figure 2 shows the growth, lipid and DHA production profiles for feeding strategy 1 and 2. When feeding strategy 1 (cultivation carried out with an initial concentration of fructose of 35 g/L and fed with fructose at 24 h intervals) was employed, slower rate of growth compared to batch cultures were observed. Lipidless biomass concentration achieved at 24 and 48 h were 2.8 and 7.0 g/L, which were 32.0% and 36.9% lower than in batch cultivation, respectively. The maximum lipid, DHA and lipidless biomass (weight of biomass without lipid content) concentration was also 29.3%, 19.5%, and 21.4% lower compared to what was achived in batch cultivation. This could be due to the lower initial and residual glucose concentration during the fermentation process which results in limited supply of carbon source for cell growth, accumulation of lipids and DHA (Qu et al.2013).

Different response were observed when feeding strategy 2 was carried out, where improved growth was evident as the maximum lipidless biomass achieved was 44.16% and 37.24% higher compared to those observed in fed-batch feeding strategy 1 and batch cultivation, respectively. The final cell dry weight, total lipid content and DHA content of feeding strategy 2 were 30.56 g/L, 39.46% of the DCW (12.06 g/L lipid) and 50.61% of the TFA (6.1 g/L DHA), approximately 60.3%, 38.1%, and 22% improvement compared to feeding strategy 1. The differences in biomass, lipid and DHA concentrations achieved in fed-batch feeding strategy 1 and 2 were significant ($P<0.05$) with p values of 0.031, 0.015 and 0.017, respectively. Table 1 summarises the yield and productivity of dry cell weight, lipid and DHA concentration for batch cultivation and fed-batch strategy 1 and 2 of *Aurantiochytrium* sp. SW1.

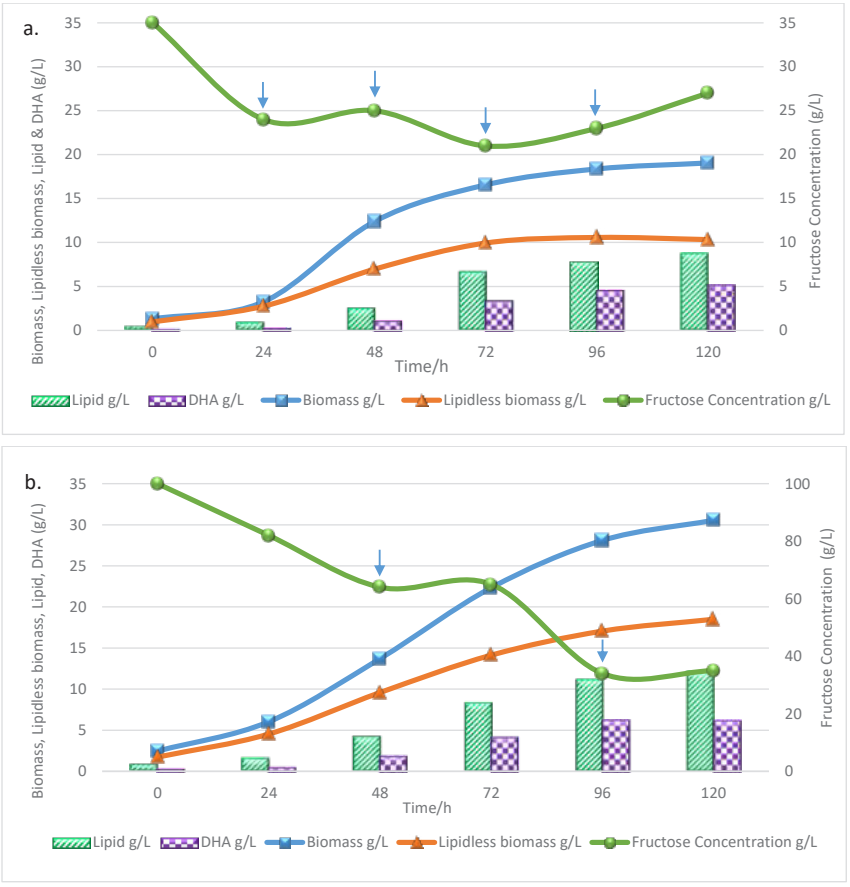

FIGURE 2. Time course of biomass, lipid, DHA and lipidless biomass production in fed-batch cultivations by *Aurantiochytrium* sp. SW1 using different time feeding mode. a) feeding strategy 1, b) feeding strategy 2. The arrows were used: fructose time feeding

TABLE 1. Yield and productivity of biomass, lipid and DHA

Response parameters	Batch	Fed-batch (Feeding strategy 1)	Fed-batch (Feeding strategy 2)
DCW (g/L/h)	0.19	0.16	**0.25**
Lipid (g/L/h)	0.09	0.07	**0.10**
DHA (g/L/h)	**0.05**	0.04	**0.05**
Yield $_{x/s}$	**0.33**	0.29	0.28
Yield $_{L/s}$	**0.16**	0.12	0.11
Yield $_{DHA/s}$	**0.09**	0.07	0.06

Therefore, although higher biomass concentration and productivity was achieved in the cultures supplemented with higher fructose content of fed-batch feeding strategy 2, it did not result in any increase of the lipid and DHA yield and productivity. No improvement in terms of lipid and DHA production compared to batch cultivation were observed as indicated by the similar level of lipid and DHA productivity and lower yield for cell mass and product formation (Table 1). This indicates that higher content of fructose was being used for cell growth rather than contributing to lipid accumulation. It was known that excessive supply of carbons cource and nitrogen starvation was essential for lipid accumulation in oleaginous microorganism (Wong et al. 2008). However, increasing the fructose concentration up to a certain high concentration was not effective for the lipid and DHA production. This was shown to be caused by inhibitory effects of high fructose concentration through catabolite repression. Fructose is a rapidly metabolizable carbon-energy that will increase in the intracellular ATP concentration, causing the repression of enzyme biosynthesis and results in slower metabolization of energy source (Yamane & Shimizu 1984). This results is parallel to what was reported by Wong et al. (2008) where the DHA yield by *Aurantiochytrium mangrovei* MP2 was not linearly improved under high carbon source concentration. Another report by Ganuza et al. (2008) showed that with an increase in the initial glucose concentration from 2 to 6 g/L, the dry cell weight of *Schizochytrium* sp. dramatically increased in the first 3 days of cultivation but with no effects on the lipid and DHA yield.

The fed-batch with feeding strategy 1 resulted in the lowest yield and productivity of cell dry weight, lipid, and DHA which might be due to the fructose concentration kept low during the fermentation. However, this is different with the experiment of batch, fed-batch and repeated fed-batch by *Schizochytrium* sp. (Qu et al. 2013). The final results showed that the fed-batch fermentation process in feed-back feeding strategy had the highest biomass, lipid, DHA production, and DHA productivity among the different mode of cultivation. In term of statistical analysis using Tukey test (one-way ANOVA), no significance difference ($P>0.05$) was founded between batch and two feeding strategies of fed-batch cultivation, where the average of p value for biomass, lipid, and DHA concentration were 0.076, 0.104, and 0.110, respectively. As the data indicates no improvement was achieved in the fed-batch strategies, further experiments were carried out to evaluate the impact of repeated-batch cultivation to lipid and DHA production by *Aurantiochytrium* sp. SW1.

REPEATED-BATCH CULTIVATION PROCESS AND THE DIFFERENCES BETWEEN MOD OF FERMENTATION

Repeated-batch fermentation is an efficient strategy accomplished by substituting certain portion of the mature culture with fresh medium and this mode is an efficient strategy to improve the productivity as it saves the time

for seed culture, inoculation, cleaning and sterilization of the fermentor between each fermentation cycle (Zhao et al. 2011). Repeated-batch cultivation of different total harvesting volume (95%, 90%, and 80% v/v) was carried out by harvesting at 96 h intervals (two cycles), each followed by subsequent replacement with fresh medium. The replacement time of 96 h was employed as the highest lipid and DHA production was achieved at 96 h in batch fermentation mode.

Table 2 shows the effects of different harvesting volume on growth, lipid, and DHA production at the end of the second cycle. Results indicate that as the harvesting volume were decreased, higher final cell dry weight, and total lipid content were achieved. The final DHA production and productivity were also higher when harvesting volume percentage of 90% and 80% were employed compared to that of 95%. Based on these results, the replacement ratio of 90% was the best for the repeated-batch process due to the highest DHA concentration and DHA productivity of 15.04 g/L and 0.054 g/L/h achieved, respectively (Table 2). These results agrees with those reported by Qu et al. (2013), where the final DHA content and DHA productivity of *Schizochytrium* sp. were the highest at the replacement ratio of 90% and 80% compared to at 95%. Barajas-Solano et al. (2016) also reported that in order to maintain a stable culture production of *Aurantiochytrium* sp. using repeated fed-batch cultivation, a culture volume between 75% and 90% must be removed every 2-3 days over a total time of 12 days.

TABLE 2. Effects of different harvesting volume on growth, lipid, and DHA production by *Aurantiochytrium* sp. SW1 in the repeated-batch fermentation

Harvesting volume (%)	Cell dry weight (g/L)	Total lipids (g/L)	DHA (g/L)	DHA productivity (g/L/h)
95	62.80 ± 1.83	26.06 ± 1.15	11.95 ± 2.51	0.041 ± 0.18
90	67.62 ± 1.35	29.87 ± 2.13	**15.04 ± 1.76**	**0.052 ± 0.20**
80	67.93 ± 0.52	30.28 ± 1.27	14.78 ± 1.97	0.051 ± 0.14

Growth and fermentation characteristics of the first and second fermentation cycles of the repeated-batch process of SW1 with 90% harvesting volume are shown in Figure 3. As shown in Figure 3, all the parameters including the dry cell weight, lipid concentration, DHA concentration, lipid percentage and DHA percentage decreased through the cycles. Compared to the initial culture (batch), a 15.32% decrease in DHA concentration was observed in the first cycle and a further 17.58% decrease occurred in the second cycle. This may have been caused by the effects of the age of the cultures that were retained to initiate the subsequent cycle which have reached 96 h and were already in the early stationary phase when the first and second cycle were initiated.

As shown in Table 3, the fatty acids composition of lipids produced from the two cycles of this fermentation process was shown to be consistent, with 16:0 and DHA being the most abundant. Qu et al. (2013) also reported similar observations where both fatty acids being dominantly present in the lipids produced by *Schizochytrium* sp. in cycles of repeated fed-batch process. The consistency of the lipid composition in each cycle indicates the potential of the repeated-batch process over the fed-batch process which would allow immediate subsequent production run to be performed.

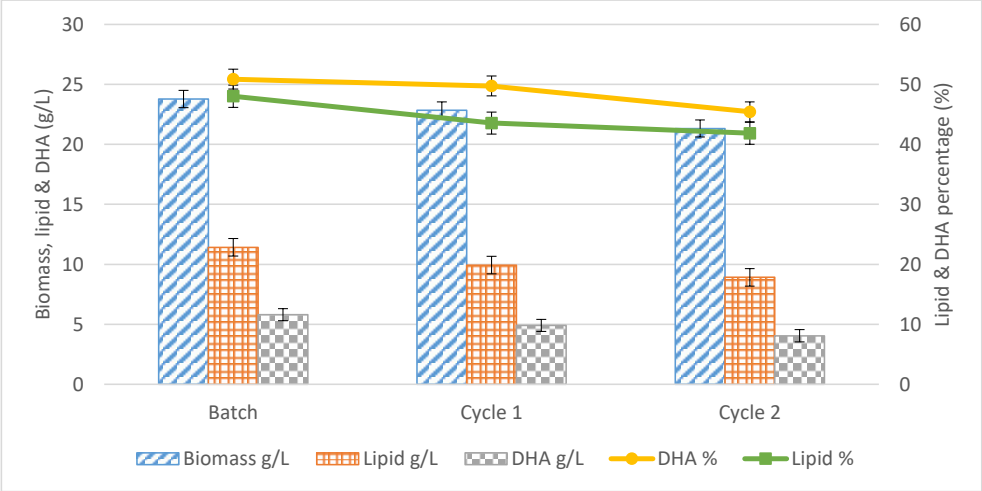

FIGURE 3. Biomass, lipid and DHA concentration of a repeated-batch culture of *Aurantiochytrium* SW1 with 90% harvesting volume

TABLE 3. Fatty acids compositions of lipids of *Aurantiochytrium* sp. SW1 produced in cycle 1 and 2 of a repeated-batch fermentation

Lipids composition	Percent of fatty acids (%)		
	Cycle 0	Cycle 1	Cycle 2
C 14	3.515	4.117	4.505
C 14:1	0.246	0.291	0.073
C 16	40.504	42.482	45.012
C 17:1	0.131	0.166	0.200
C 18	1.198	1.175	2.145
C 20:4n6 (AA)	0.620	0.629	0.561
C 20:5n3 (EPA)	0.409	0.507	0.477
C 22:2	0.978	0.504	0.330
C 22:6n3 (DHA)	47.819	44.751	39.957

The overall comparisons of the efficiency of the three different modes of fermentation process for DHA-rich lipids production of *Aurantiochytrium* sp. SW1 are shown in Table 4. Results showed that the batch process is the most preferable cultivation strategy compared to the other two modes carried out as outlined in this work. This is due to two reasons: first, there was no significance difference ($P<0.05$) founded between batch and fed-batch

cultivation and second, the value of DHA yield over the total fructose consumed (Y)/(g/L fructose used) showed that batch cultivation (0.06) was higher that fed-batch cultivation strategy 2 (0.04).

TABLE 4. Respond parameters for different modes of cultivation for DHA production by *Aurantiochytrium* sp. SW1

Cultivation mode	Cultivation time (h)	Cell dry weight (g/L)	Total lipid (g/L)	DHA (g/L)	DHA productivity (g/L/h)
Batch	96	22.94 ± 0.96	11.22 ± 1.45	6.0 ± 0.75	0.063 ± 0.05
Feed-batch (strategy 1)	96	28.11 ± 1.13	11.04 ± 2.14	6.17 ± 0.82	0.064 ± 0.07
*Repeated-batch (90%)	288	67.62 ± 1.35	29.87 ± 2.13	15.04 ± 1.76	0.052 ± 0.20

*Data represents the total sum of each of the parameters harvested at the end of initial culture (batch), cycle 1 and 2

CONCLUSION

Comparative experimental results of lipid and DHA production by *Aurantiochytrium* sp. SW1 under different modes of cultivation strategies are presented in this paper. It demonstrated that batch cultivation is the most efficient and suitable for lipid and DHA production. There were no significant differences in lipid concentration, DHA concentration, and DHA productivity were achieved when fed-batch process with two different feeding stretegies were conducted. Repeated-batch cultivation was unsuitable as it shows the lowest DHA productivity despite the highest DHA concentration achieved. However, the consistent of DHA yield and fatty acids composition lipids produced in each of the fermentation cycles indicate the potential for its application in large-scale production of DHA-rich lipid.

ACKNOWLEDGEMENTS

We thank Universiti Kebangsaan Malaysia for funding this research under the grant GUP-2016-054.

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Received: 13 February 2020
Accepted: 16 November 2020