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OPTIMIZATION OF CULTIVATION CONDITIONS OF THE HETEROTROPHIC MARINE MICROALGA *Thraustochytrium aureum* BT6 ORIENTED TO EXPLOIT BIOACTIVE COMPOUNDS

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ABSTRACT

Thraustochytrium is a heterotrophic marine microalgae genus belonging to the Labyrinthula class, Thraustochytriaceae (Thraustochytrids) family. Recently, studied results have showed that some species/strains of *Thraustochytrium* genus are potential candidates to produce biomass rich in lipid containing high polyunsaturated fatty acid (PUFAs), especially docosahexaenoic (DHA C22: 6 ω-3), carotenoid, extracellular polysaccharides (EPSs) and enzymes which play a crucial role in human health. Therefore, throughout many decades, numerous studies have been conducted in exploiting bioactive compounds, especially PUFAs. Biomass and PUFAs yield of some strains *Thraustochytrium* spp. depend on environmental conditions. In this paper, the results on the effects of different culture conditions on the growth of *Thraustochytrium aureum* BT6 (isolated from coastal regions of Binh Thuan in 2010) in 500 mL erlenmeyer flask and 30 L fermentors is presented. Suitable conditions for the growth of strain BT6 were: Bajpai medium, 2% glucose, 0.5% yeast extract, temperature of 20−28 ^{o}C, salt concentration 0.5%; and initial algal cell concentration of 2 g/L. The dry biomass, lipid and DHA contents of strain BT6 in 500 mL erlenmeyer flasks and 30 L fermentor reached the highest levels of 8.56 ± 0.12 and 6.92 ± 0.27 g/L, 18.36 ± 1.15 and 5.50 ± 0.133% DCW, 2.00 ± 0.05 and 0.14 ± 0.01% DCW, respectively, after 4 days of cultivation. Carotenoid content in flask and 30L fermentor achieved 44.45 ± 1.4 and 28.84 ± 1.12 mg/kg DCW, respectively after 4 days of cultivation. Obtained results have indicated that the algae biomass met the requirements in orientation to exploit valuable bioactive compounds.

Keywords: *Thraustochytrium aureum*, cultivation conditions, DHA, PUFAs.

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INTRODUCTION

Thraustochytrids are unicellular heterotrophic marine microalgae with the highest abundance in nutrient-rich areas such as mangroves forest. Thraustochytrids accumulate large quantities of docosahexaenoic acid (C22: 6 ω-3, DHA) and eicosapentaenoic acid (C20: 5 ω-3, EPA) (Singh et al., 2014). Besides, some strains also contain high squalene and carotenoid contents including β-carotene, zeaxanthin, echinenone, canthaxanthin and astaxanthin (Aki et al., 2003). After 192 h of the fermentation of *Thraustochytrium* sp. strain ATCC 26185 using 30 g/L glucose and 2.4 g/L nitrogen, the highest carotenoid content reached 2.22 mg/L (Furlan et al., 2019). Moreover, they are also capable of secreting extracellular polysaccharides and enzymes. Therefore, Thrausotochytrids mainly including extensively studied strains as *T. arueum, T. roseum, T. striatum* and *Thraustochytrium* sp. ONC-T18 (Shene et al., 2013) are widely used in food, pharmaceutical and animal feed (Singh et al., 2014). The algal cell dry weight is low and often less than 10 g/L (Jeh et al., 2008) except for *Thraustochytrium* sp. ONC-T18 that showed the best performance with cell mass concentration up to 28 g/L and DHA content of 31.4% of total fatty acid (TFA) (Burja et al., 2007), *Thraustochytrium* sp. ATCC 26185 contained maximum biomass and DHA content up to 30.2 g/L and 1.16 g/L, respectively (Furlan et al., 2017).

In Vietnam, strains *Thraustochytrium* rich in DHA and carotenoid successfully isolated from mangrove forest Thi Nai, Binh Dinh province were published (Hoang Thi Lan Anh et al., 2010). Study on the biological characteristics of some potential species belonging to the *Thraustochytrium* genus isolated from mangrove forests located in northern Vietnam to exploit PUFAs (polyunsaturated fatty acids) was also published by Dang Diem Hong & Hoang Thi Lan Anh (2016). Isolation and phylogenetic identification of carotenoid producing heterotrophic marine microalgae Thraustochytrid were published by Tran Thi Xuan Mai et al. (2015). Due to the classification of genus *Thraustochytrium* based on morphological features is not exactly, designing specific primers for identification of microalgal Thraustochytrid isolated in Ben Tre and Tra Vinh province was conducted by Nguyen Lam Minh et al. (2018).

In addition to PUFAs and squalene exploitation (Dang Diem Hong & Hoang Thi Lan Anh, 2016), the study of Hang et al. (2019) is the first publication on bioactive compounds extracted from *Schizochytrium mangrovei* PQ6 including ten compounds as 4α-methyl-5α-ergosta-7,22-dien-3β-ol (1) and cholesterol (2), 3 compounds containing nitrogen such as uracil (6), uridine (7), guanosine (8) and 5 other compounds. Compounds 1–8 possessed antimicrobial activity against gram-positive bacteria as *Enterococcus faeccalis* and gram-negative bacteria as *Pseudomonas aegusinosa* with minimum inhibitory concentration (MIC) value range of 32–128 μg/mL and 32–256 μg/mL, respectively. Compounds 1 and 9 was found to have cytotoxic activity against KB cell lines with IC_{50} values of 93.65 ± 5.63 μg/mL and 69.72 ± 7.88 μg/mL, respectively.

However, exploiting of bioactive compounds from *Thraustochytrium* has not yet been studied in Viet Nam. Therefore, we continued to study the appropriate culture conditions of *Thraustochytrium aureum* strain BT6 for the harvesting microalgal biomass to meet the requirements in orientation to exploit precious bioactive compounds.

MATERIAL AND METHODS

Material

Thraustochytrium aureum Goldstein 1963 (strain BT6) was isolated in coastal waters of Binh Thuan province in 2010, belongs to the microalgal culture collection of the Algal Technology Department, Institute of Biotechnology, Vietnam Academy of Science and Technology. The strain was deposited on medium GPY with 1.5% agar (Dang Diem Hong & Hoang Thi Lan Anh, 2016).

Method

Determination of algae growth

Determination of algae growth through dry cell weight (DCW)

At certain experimental times, 10 mL of culture broth are centrifuged to obtain biomass. After centrifugation, cell biomass was transferred to a cup of known mass and dried at 105 ^{o}C until constant weight for three consecutive weighing times. The dry cell weight (DCW) of the sample was determined by the following formula:

DCW (g) = Weight (cup + algal biomass) – Weight (cup)

Determine the specific growth rate

The specific growth rate of the algal population was be determined by the following formula: $\mu = (\ln N_1 - \ln N_0)/(t_1 - t_0)$; Where μ is the typical growth rate of algae cells with unit (/day); N_1 is DCW at time t_1, N_o is DCW at time t_0 (Imamoglu et al., 2007; Dang Diem Hong, 2019).

Algal cell morphology was taken using Japanese Canon IXY 7.0 digital camera under Olympus CX21 optical microscope with 400 times magnification and camera with 3 times magnification (Dang Diem Hong, 2019).

Analysis of total lipid content

Total lipid content was analyzed according to the method of Bligh & Dyer (1959) with some modification to suit the conditions of Vietnam as published by Dang et al. (2011). The total lipid content is calculated by dry biomass using the following formula:

Total lipids (%) = (m2/m1) × 100

Where: m1 (g) is DCW used for lipid analysis; m2 (g) is the total lipid obtained from m1 g DCW.

Determination of residual glucose

Residual glucose concentration in the medium during fermentation was determined according to Miller's method (Miller, 1959).

Determination of total carotenoid

The total carotenoid content was determined as described by Furlan et al. (2019)**.** The total carotenoid content (µg/g of biomass) was calculated by the following formula:

$$\text{Total carotenoid content} = \frac{A_{477nm} \cdot V_{extract} \cdot DF}{0.2 \cdot W_{sample}}$$

Where: A_{477nm} is the optical density absorbed at wavelength of 477 nm; $V_{extract}$ is extract volume (mL); DF is the dilution factor (final volume divided by the initial volume); 0.2 is the A_{477nm} value of carotenoid solution of 1 µg/mL; and W_{sample} is sample weight (g).

Analysis of fatty acid composition in algae biomass

10 mg of algal biomass were dissolved with 1 mL n-hexane, shaken thoroughly in a stoppered vial. Then, the mixture was added 25 µL of CH_3ONa solution in methanol (2 mol/L) and shaken thoroughly for 1 minute. The mixture was added 1 mL of distilled water, shaken thoroughly and centrifuged at 3,000 rpm for 7 minutes at 4 ^{o}C to separate the layers. The lower unreacted wax layer was removed. The mixture was added 100 mL of HCl, shaken well and centrifuged at 3,000 rpm for 5 minutes at 4 ^{o}C. After centrifugation, the mixture was layered. The lower layer was removed, upper solvent layer was collected and dried by adding anhydrous sodium sulfate and continue to be layered by centrifugation at 3,000 rpm for 7 minutes at 4 ^{o}C. After methyl esterification, the samples were injected by an autoinjector (injection volume of 0.9 µL) on gas chromatograph: HP-6890 coupled with Mass Selective Detector Agilent 5973; Columns: HP-5MS (0.25 m × 30 m × 0.25 mm); Helium as a carrier gas; Column temperature program: start at 80 ^{o}C for 1 minute, increase to 150 ^{o}C at speed 40 ^{o}C/min; then increase the temperature to 260 ^{o}C and hold for 10 minutes at rate of 10 ^{o}C/min. Mass spectral libraries was WILEY275. L and NIST 98. L according to ISO/FDIS 5590: 1998, Germany as described in Dang et al. (2011). The parameter was analyzed at the Vietnam

Certification Center (Quacert), Technical Center of Standards Metrology and Quality, Ministry of Science and Technology.

Methods for determining suitable culture conditions in flask and 30 L fermentor scales

In order to determine the suitable culture conditions of the BT6 strain in 500 mL flask, the effects of nutritional media such as Bajpai, M1 and GPY were studied. The culture media had the following composition: GPY medium (g/L): 2 - glucose, 1 - polypepton, 0.5 - yeast extract (YE), 15 - agar, 17.5 - artificial sea salt (Dang Diem Hong & Hoang Thi Lan Anh, 2016). Bajpai medium (g/L): 5 - NaCl, 5 - $MgSO_4.7H_2O$, 1 - KCl, 0.1 - KH_2PO_4, 0.2 - $CaCO_3$, 0.2 - $(NH_4)_2SO_4$, 2 - sodium glutamate, 0.1 - $NaHCO_3$, 20 - glucose, 10 - yeast extract (Bajpai et al., 1991). M1 medium (g/L): 30 - glucose, 10 - yeast extract, 17.5 - artificial sea salt (Dang Diem Hong & Hoang Thi Lan Anh, 2016). The culture conditions as: glucose concentration (1, 2, 3, 4 and 5%); yeast extract concentrations (0; 0.25; 0.5; 1 and 1.5%); NaCl concentrations (0; 0.5; 1; 1.5 and 2%), temperatures (15, 20, 25, 28, 32 and 37 ^oC), initial algal cell concentrations (1, 2, 3, 4 and 5 g/L) were studied. Thermostatic shaker VS-8480SFN (Korea) with a speed of 200 rpm for 5 days of culture was used for the above experiments. All the experiments were conducted in triplicated (n=3 for each treatment), the algal growth was determined daily. The experiment was conducted at the Algal Technology Department, Institute of Biotechnology, Vietnam Academy of Science and Technology.

Cultivation of BT6 strain was also conducted in 30-liter fermentor according to the publication of Dang Diem Hong & Hoang Thi Lan Anh (2016) with suitable culture conditions as in flasks. However, the fermentation conditions in 30-liter bioreactor were as follows: aeration of 0.5 L/L/min; the stirring speed of 350−400 rpm and industrial yeast extract; glucose and industrial yeast extract concentrations of 5% and 1%, respectively.

Experimental data are processed by Excel software and statistically analyzed by one-way ANOVA at $P < 0.05$ level.

RESULTS AND DISCUSSION

Effect of nutrient media

The effect of three different nutrient media (Bajpai, M1 and GPY) on the growth of a strain of *T. aureum* BT6 was presented in Figure 1. Obtained results in Figure 1 showed that the strain BT6 was able to grow on 3 types of media after 5 days of culture. However, DCW and lipid content reached maximum on Bajpai medium, following in M1 medium and finally in GPY medium with 5.08 ± 0.6; 3.23 ± 0.03; 1.16 ± 0.04 g/L and 18.05 ± 1.37; 18.29 ± 0.39; 5.22 ± 1.09% DCW, respectively. Cultivation in Bajpai and M1 media resulted in algae morphology with large cell size and abundant lipid bodies. In GPY medium, algal cells showed small size, weak color, faint lipid bodies. Therefore, in further experiments, the Bajpai medium was selected as the appropriate medium for the growth of strain BT6.

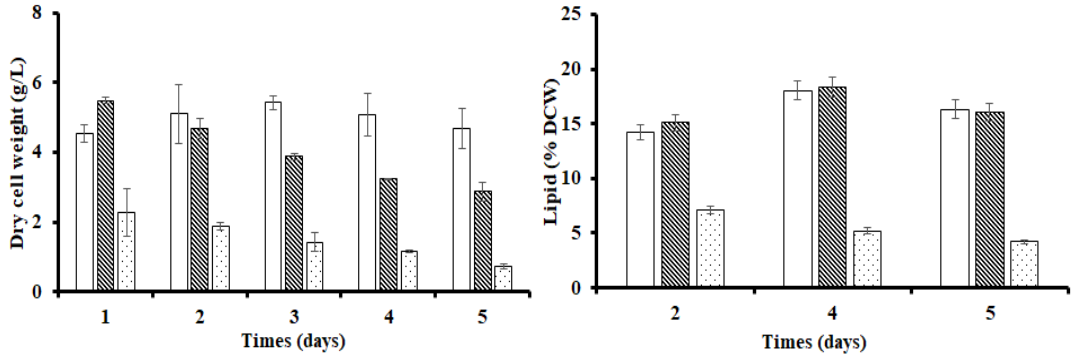

Figure 1. Effect of nutrient media on growth of BT6 strain in 500 mL Erlenmeyer flask

Effect of glucose concentration

According to Xiao et al. (2018), glucose plays an important role in the growth of *Thraustochytrium striatum*. However, this algal cells also only use certain amount of glucose. In the effect of glucose concentration ranging from 1% to 5% on the growth and lipid accumulation of strain BT6 (shown in Fig. 2), DCW and lipid achieved the highest values of 8.42 ± 0.22 g/L and 17.97 ± 0.15% DCW, respectively, at 2% glucose after 4 days of culture. At the same time, the residual glucose content also decreased but not much after the culture days (Fig. 3). At glucose concentration of 2%, residual glucose concetration decreased from 20 g/L to 6 g/L after 5 days of culture. In the increase of glucose up to 4−5%, residual glucose content was consumed at very low levels that only achieved 44−45% of initial glucose concentration after 5 days of culture. Our obtained results also were completely consistent with the report of Xiao et al. (2018), in the cultivation of *T. striatum* at 5% glucose, glucose consumption was only approximately 46% after 6 days of culture. However, at 1% glucose, glucose content level was completely consumed after 5 days of cultivation lead to the decrease in DCW from 6.68 ± 0.20 to 2.68 ± 0.03 g/L. Thus, to obtain high biomass and save chemicals, 2% glucose was used in the next experiments.

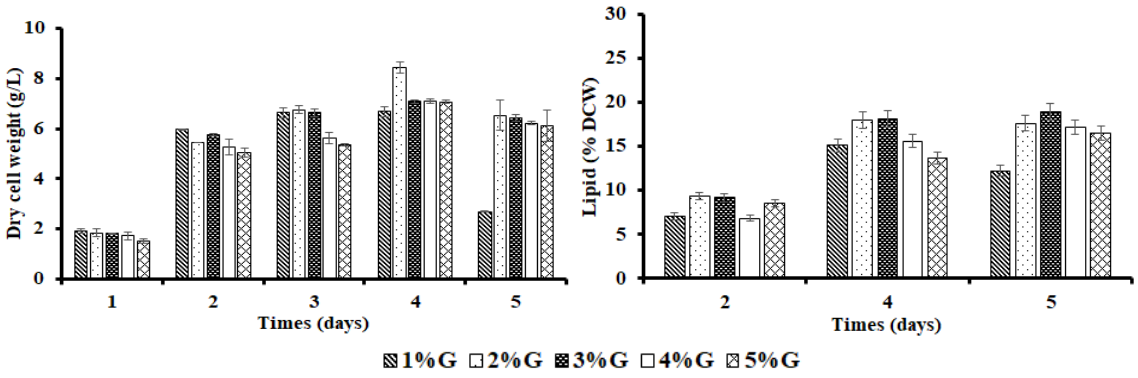

Figure 2. Effect of glucose concentrations on growth of BT6 strain in 500 mL Erlenmeyer flask

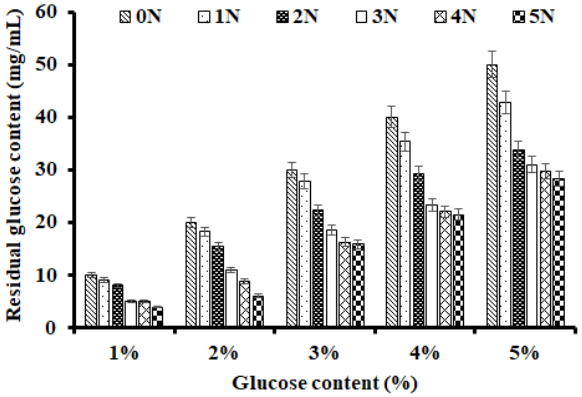

Figure 3. Residual glucose content in culture medium of BT6 strain in 500 mL Erlenmeyer flask

Effect of YE concentration

Effect of YE concentrations of 0; 0.25; 0.5; 1.0 and 1.5% on growth and lipid accumulation of the strain BT6 was shown in Figure. 4. In investigated YE concentrations, the highest DCW and lipid content reached 7.92 ± 0.38 g/L and 18.36 ±

1.15% of DCW at YE concentration of 0.5%, respectively, after 4 days of culture, followed by 1% YE (with 7.24 ± 0.42 g/L). There were no statistically significant differences between the two formulas of 0.5% and 1%. On the other hand, using YE at the high concentration decreased in lipid content in microalgal cells (14.36 ± 1.29 (at 1% YE) and 15.21 ± 0.18 (at 1.5% YE). The obtained results were completely consistent with the report of Anbu et al. (2007). Xiao et al. (2018) showed that high YE level stimulated to cell growth but inhibition to lipid accumulation. Therefore, YE concentration of 0.5% was selected in the next experiments.

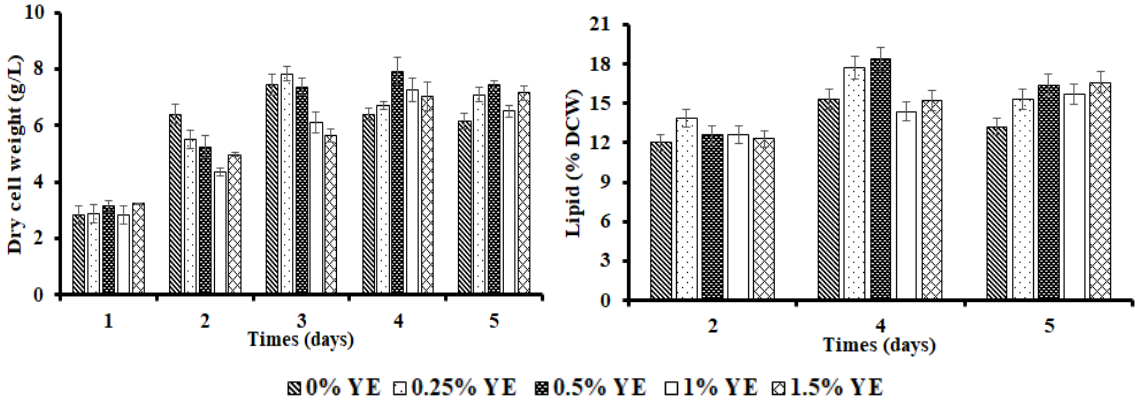

Figure 4. Effect of YE concentrations on growth of BT6 strain in 500 mL Erlenmeyer flask

Effect of NaCl concentration

In Bajpai medium, varying NaCl concentrations ranging from 0% to 2% were investigated. As shown in Figure 5, the growth of BT6 strain did not exhibit significant change with increasing NaCl concentration. The highest DCW of strain BT6 achieved values in the range from 7.99 ± 0.15 to 8.56 ± 0.12 g/L after 4 days of culture. Lipid content tended to increase with increasing NaCl concentration from 0% NaCl (13.56 ± 0.78% of DCW) to 1.5% NaCl (16.46 ± 1.58% of DCW). Thus, strain BT6 tended to increase lipid accumulation for adaptation to increasing concentration of NaCl. However, there were no statistically significant differences. Therefore, to obtain high biomass and save chemicals, NaCL concentration of 0.5% was selected in the next experiments.

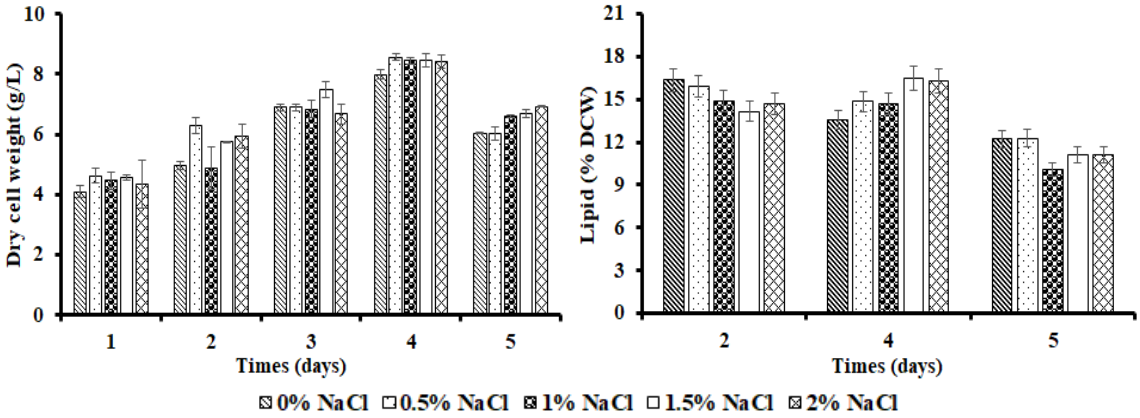

Figure 5. Effect of NaCl concentrations on growth of BT6 strain in 500 mL Erlenmeyer flask

Effect of temperature

The results on the effect of temperature on the growth and lipid content of strain BT6 (Fig. 6) showed that the strain has the best growth at temperatures in the range of 20 to 28 ^{o}C with DCW and lipid content of (8.37 ± 0.07) - (8.69 ± 0.33) g DCW/L and 14−15% DCW, respectively after 4 days of culture. At temperature range of 32−37 ^{o}C, algal cells have thin cell walls, weak color and slow growth after 4 days of culture. At 15 ^{o}C, algal cells have good morphology but slow growth, the highest biomass of 5.46 ± 0.07 g DCW/L and the decrease in lipid content after 4 days of culture. There was no statistically significant difference in growth between temperatures of 20, 25 and 28 ^{o}C ($p > 0.05$). Caamano et al. (2017) also confirmed that the growth of *Thraustochytrium kinnei* strain reached 2.2 g/L at 25 ^{o}C and higher 3.1 times than that at 10 ^{o}C. The result indicated that low temperature inhibited the growth of the strain. Thus, the temperatures in the range of 20 to 28 ^{o}C should be used in the next experiments.

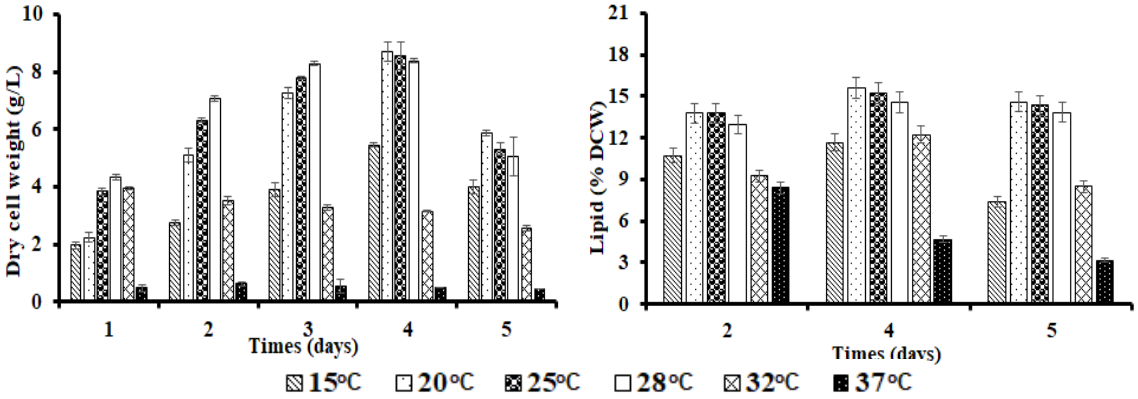

Figure 6. Effect of temperatures on growth of BT6 strain in 500 mL Erlenmeyer flask

Effect of initial algal cell concentration

After 4 days of strain BT6 cultivation on Bajpai medium containing 2% glucose, 0.5% YE, 0.5% NaCl, temperature 28 ^{o}C, the initial algal cell concentrations in the range of 1–5 g/L were investigated. Obtained results showed that the highest DCW reached 8.40 ± 0.05 g/L after 4 days of culture with an initial algal cell concentration of 2 g/L. The algal biomass at initial algal cell concentrations of 3, 4 and 5 g/L was not significantly lower than at 2 g/L with 8.05 ± 0.05, 7.96 ± 0.04 and 8.15 ± 0.15 g/L, respectively. At initial algal cell concentration of 1 g/L, the algal growth (7.19 ± 0.33 g/L) obtained lowest while specific growth rate (μ) reached maximum (0.77/day) and decreased gradually in the following formulas with values ranging from 0.39/day to 0.63/day. There was no significant difference in lipid content between the formulas with values ranging from 14.42% to 16.86% DCW. Thus, an initial algal cell concentration of 2 g/L was selected in the next experiments.

The fatty acid composition in dry biomass of the strain BT6 cultured in Erlenmeyer flask under optimal conditions (Bajpai medium, 2% glucose, 0.5% YE, 0.5% NaCl, temperature 28 ^{o}C, initial algal cell concentration of 2 g/L) was analyzed (Table 1). As shown in Table 1, algal biomass in flask containing unsaturated fatty acids accounted for 6.85 ± 0.03% DCW, in which, C20:5 (ω-3) and C22:6 (ω-3) contents accounted for 4.07 ± 0.11 and 2.00 ± 0.05% of DCW, respectively. Saturated fatty acids accounted for 9.57 ± 0.02% of DCW, in which the C16:0 content accounted for 4.51 ± 0.08% of DCW. Thus, the biomass in the Erlenmeyer flask met the requirements of the orientation to exploit precious bioactive compounds.

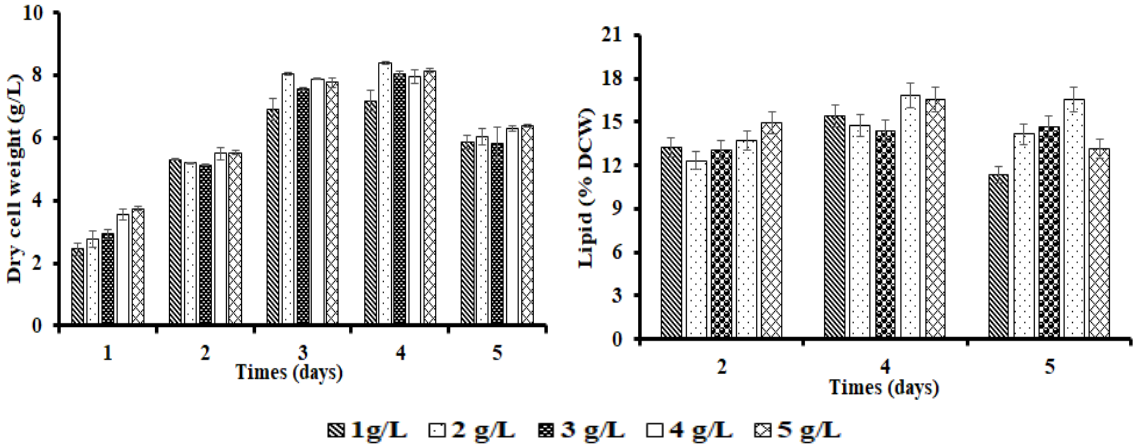

Figure 7. Effect of initial algal cell concentrations growth of BT6 strain in 500 mL Erlenmeyer flask

Table 1. Fatty acid composition in dry biomass of BT6 strain at Erlenmeyer flask and fermentor

Number	Fatty acid	Content (% of DCW)	
1		Erlenmeyer flask	Fermentor
2	C10:0	0.01± 0.01	-
3	C12:0	0.05 ± 0.01	-
4	C13:0	0.12 ± 0.01	0.12 ± 0.01
5	C14:0	0.93 ± 0.02	0.29 ± 0.01
6	C14:2 (ω-3)	-	0.01 ± 0.01
7	**C15:0**	**1.97 ± 0.03**	**0.32 ± 0.01**
8	C16:0	4.51 ± 0.08	1.78 ± 0.02
9	C16:1 (ω-7)	-	0.01 ± 0.01
10	C17:0	1.41 ± 0.02	0.12 ± 0.01
11	**C18:0**	**0.28 ± 0.01**	**0.45 ± 0.01**
12	**C18:1 (ω-9)**	**0.09 ± 0.01**	**2.86 ± 0.10**
13	C18:2 (ω-6)	0.09 ± 0.01	3.94 ± 0.09
14	C18:3 (ω-6)	0.04 ± 0.01	0.03 ± 0.01
15	C20:0	0.02 ± 0.01	0.03 ± 0.01
16	**C20:4 (ω-6)**	**0.56 ± 0.01**	**0.02 ± 0.01**
17	C20:5 (ω-3)	4.07 ± 0.11	0.09 ± 0.01
18	C20:1 (ω-9)	-	0.02 ± 0.01
19	**C22:0**	**0.04 ± 0.01**	**0.03 ± 0.01**
20	C22:6 (n-3)	2.00 ± 0.05	0.14 ± 0.01
21	C23:0	0.07 ± 0.01	0.01 ± 0.01
22	C25:0	0.09 ± 0.01	-
23	C26:0	0.07 ± 0.01	-
	Unsaturated fatty acids	**6.85 ± 0.03**	**7.12 ± 0.03**
	Saturated fatty acids	**9.57 ± 0.02**	**3.03 ± 0.01**

Note: -: No detection.

Cultivation of BT6 strain in 30 L fermentor

Optimum culture conditions for BT6 strain in the Erlenmeyer flask (Bajpai medium, 0.5% NaCl, 20−28 °C temperature, 2 g/L initial cell concentration) were used in 30 L fermentor. The algal medium containing 5% glucose concentration and 1% YE were used in 30 L fermentor at aeration of 0.5 L/L/min and stirring speed of 350−400 rpm. As shown in Figure. 8A, the DCW of BT6 strain increased slowly after 1 day of cultivation, then gradually increased and quickly entered the stationary phase (after 4 days of culture) and maximum reached 6.98 ± 0,04 g/L after 5 days of culture. However, the lipid content achieved low value and only in range of 5.17 ± 0.04 to 5.5 ± 0.13% of DCW after 4-5 days of cultivation. The results of residual glucose content analysis (Fig. 8B) showed that the glucose content decreased with increasing algal biomass. After 5 days of culture, the decrease of the glucose content in the culture medium from 50 mg/mL to 8.97 mg/mL indicated that almost all of the glucose level was used for algal growth in the culture process.

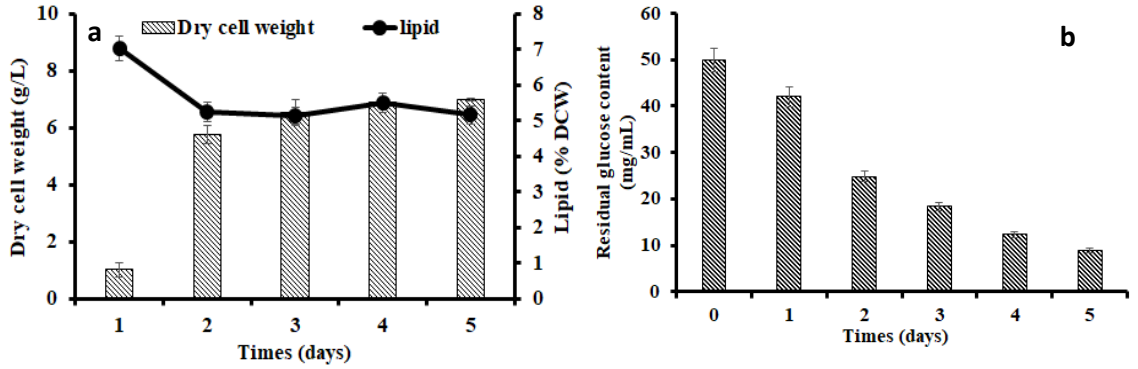

Figure 8. Growth of BT6 strain in 30 L fermentor

Fatty acid composition analysis of BT6 strain biomass in 30 L fermentor after 4 days of culture (Table 1) showed that total fatty acid content reached 10.15 ± 0.02% of DCW, in which saturated and unsaturated fatty acids accounted for 7.12 ± 0.03% of DCW, 3.03 ± 0.01% of DCW, respectively. Among the PUFAs, C18:1 (ω-9) and C18:2 (ω-6) were mainly found, accounted for 2.86 ± 0.10 and 3.94 ± 0.09% of DCW, respectively. The content of C20:5 (ω-3) and C22:6 (ω-3) decreased by only 0.09 ± 0.01 and 0.14 ± 0.01% of DCW, respectively. In saturated fatty acids, C16: 0 mainly accounted for 1.78 ± 0.02% DCW. Thus, the algal biomass cultured in the 30 L fermentation system met the requirements of the orientation to exploit precious bioactive compounds.

Table 2. Dry cell weight, lipid, DHA and EPA contents of BT6 strain in Erlenmeyer flask and 30 L fermentor

Culture condition	Erlenmeyer flask	30 L fermentor
	Bajpai medium, 2% glucose, 0.5% yeast extract, 28 °C, 0.5% NaCl, 200 rpm, 4 days	Bajpai medium, 5% glucose, 1% industrial yeast extract, 25−28 °C, 0.5% NaCl, 350 rpm, 4 days
Dry cell weight (g/L)	8.56 ± 0.12	6.98 ± 0.27
Lipid (% DCW)	18.36 ± 1.15	5.5 ± 0.13
DHA (% DCW)	2.00 ± 0.05	0.14 ± 0.01
EPA (% DCW)	4.07 ± 0.11	0.09 ± 0.01
Carotenoid (mg/kg DCW)	44.45 ± 1.40	28.84 ± 1.12

The obtained results showed that the growth, lipid, DHA, EPA contents of BT6 have changed and depended on the culture conditions and scales (Table 2). In the flask, the highest DCW, lipid, DHA and EPA contents reached 8.56 ± 0.12 g/L, 18.36 ± 1.15% of DCW, 2.00 ± 0.05% of DCW and 4.07 ± 0.11% of DCW, respectively. In 30 L fermentation, the above parameters tended to decrease by 6.92 ± 0.27 g/L, 5.5 ± 0.13% of DCW, 0.14 ± 0.01% of DCW and 0.09 ± 0.01% of DCW, respectively. Besides, carotenoid content also was determined. As shown in Table 2, carotenoid content in the flask and 30 L fermentor reached 44.45 ± 1.4 and 28.84 ± 1.12 g/kg of DCW, respectively after 4 days of culture. Our obtained results were similar to previously published reports for carotenoids. According to Tran Thi Xuan Mai et al. (2015), the total carotenoid of BCM05 strain was found to be 7.6 mg/kg of DCW. The content of carotenoid of this result was 5.8 and 3.8 times lower than that in BT6 train. According to Furlan et al. (2019), the highest total carotenoid content was obtained 77.3 mg/kg of DCW after 192 h of cultivation of *Thraustochytrium* sp. ATCC26185 using initial concentrations of 30 g/L glucose and 2.4 g/L total nitrogen (batch system). The highest production of astaxanthin was achieved in the fed-batch system with a C/N ratio of 6.2 for *Thraustochytrium* sp. ATCC26185 and 40 for *Aurantiochytrium* sp. ATCCPRA-276. Therefore, it was found that the carotenoid profile can be changed by modifying the growth conditions, especially glucose to nitrogen ratio which can be useful for the food industry and biotechnological applications.

CONCLUSIONS

In this research, we selected culture conditions for the heterotrophic marine microalga *Thraustochytrium aureum* BT6 in orientation to exploit bioactive compounds in the flask include**:** Bajpai medium, 2% glucose, 0.5% yeast extract, temperature 20−28 ^{o}C, 0.5% NaCl; initial algae cell concentration of 2 g/L. The highest dry cell weight, lipid content of BT6 strain in the 500 mL flask reached 8.56 ± 0.12 g/L, 18.36 ± 1.15% of dry cell weight after 4 days of cultivation. DHA, EPA and carotenoid contents reached 2.00 ± 0.05 and 4.07 ± 0.11% of dry cell weight and 44.45 ± 1.4 mg/kg of dry cell weight. In the initial cultivation of strain BT6 in 30 L fermentor, the highest dry cell weight and lipid content reached 6.92 ± 0.27 g/L and 5.50 ± 0.133% of dry cell weight, respectively after 4 days of culture. The carotenoid content reached 28.84 ± 1.12 mg/kg of dry cell weight. Thus, the cultured algal biomass met the requirements in the orientation to exploit valuable bioactive compounds.

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REFERENCES

Aki T., Hachida K., Yoshinaga M., Katai y., Yamasaki T., Kawamoto S., Kakizono T., Maoka T., Shigeta S., Suzuki O., Ono K., 2003. Thraustochytrid as a potential source of carotenoids. *J. Am. Oil. Chem. Soc.,* 80(8): 789−794.

Anbu P., Kim D. U., Jeh E. J., Jeong Y. S., Hur B. K., 2007. Investigation of the physiological properties and synthesis of PUFAs from thraustochytrids and its electrophoretic karyotypes. *Biotechnol. Bioprocess. Eng.*, 12(6): 720−729.

Bajpai P. K., Bajpai P., Ward O. P., 1991. Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304. *J. Am. Oil. Chem. Soc.*, 68: 509−514.

Bligh E. G., Dyer W. J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.*, 37(8): 911−917.

Burja A. M., Armenta R. E., Radianingtyas H., Barrow C. J., 2007. Evaluation of fatty acid extraction methods for *Thraustochytrium* sp. ONC-T18. *J. Agric. Food. Chem.,* 55: 4795−4801.

Caamaño E., Loperena L., Hinzpeter I., Pradel P., Gordillo F., Corsini G., Tello M., Lavín P., González A. R., 2017. Isolation and molecular characterization of *Thraustochytrium strain* isolated from Antarctic Peninsula and its biotechnological potential in the production of fatty acids. *Braz. J. Microbiol.,* 48(4): 671−679.

Dang D.H., Hoang T.L.A., Ngo T.H.T., 2011. Study on biological characteristics of heterotrophic marine microalga - *Schizochytrium mangrovei* PQ6 isolated from Phu Quoc Island, Kien Giang province, Vietnam. *J. Phycol.,* 47(4): 944−954.

Dang Diem Hong, 2019. The cultivation of microalgae rich in nutrition used for functional food and animal feed in Vietnam. Monographs. Natural resources and environment of Vietnam. Publisher of Science and Technology, pp. 750 (in Vietnamese).

Dang Diem Hong, Hoang Thi Lan Anh, 2016. New Heterotrophic marine microalgae of

Labyrinthula, Schizochytrium, Traustochytrium in Vietnam: Potential and Challenges. Monographs. Natural resources and environment of Vietnam. *Publisher of Science and Technology*, pp. 625 (in Vietnamese).

Furlan V. J. M., Batista I., Bandarra N., Mendes R., Cardoso C., 2019. Conditions for the Production of Carotenoids by *Thraustochytrium* sp. ATCC 26185 and *Aurantiochytrium* sp. ATCC PRA-276. *Journal of Aquatic Food Product Technology*., 28(5): 465−477.

Furlan V. J. M., Paulo M. D. C., Maus V. W., Ferreira J., Batista I., Bandarra N. M., 2017. Production of docosahexaenoic acid (DHA) from Thraustochytrium sp. ATCC 26185 using differents nitrogen concentrations. *Bol. Cent. Pesqui. Process. Aliment.*, 34(2):1−11

Hang N. T. M., Giap T. H., Thanh N. L., Oanh N. T. T., Hong D. D., Minh C. V., 2019. Metabolies from microalgae *Schizochytrium mangrovei*. *Joural of Natural Compound.*, 55: 978−981.

Hoang Thi Lan Anh, Dinh Thi Ngoc Mai, Ngo Thi Hoai Thu, Dang Diem Hong, 2010. Isolation of a new heterotrophic marine microalgae strain of the genus Thraustochytrium rich in DHA and carotenoid from the Thi Nai - Binh Dinh mangrove forest. *Journal of Biotechnology* 8 (3A): 459−465 (in Vietnamese with English summary).

Imamoglu E., Sukan F.V., Dalay M.C., 2007. Effect on different culture media and light intensities on growth of *Haematococcus pluvialis*. *Int. J. Nat. Eng. Sci.*, 1: 05−09.

Jeh E. J., Kumaran R. S., Hur. B. K., 2008. Lipid body formation by *Thraustochytrium aureum* (ATCC 34304) in response to cell age. *Korean. J. Chem. Eng.*, 25: 1103–1109.

Miller G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.,* 31 (3): 426−428.

Nguyen Lam Minh, Tran Thi Xuan Mai, Nguyen Thi Lien, 2018. Design of specific primers for the identification of Thraustochytrids. *Huaf Journal of Agricultural Science and Technology*, 2(1): 509−518 (in Vietnamese with English summary).

Shene C., Leyton A., Rubilar M., Pinelo M., Acevedo F., Morales E., 2013. Production of lipids and docosahexasaenoic acid (DHA) by a native *Thraustochytrium strain*. *Eur. J. Lipid. Sci. Technol.*, 115: 890−900.

Singh P., Liu Y., Li L. S., Wang G. Y., 2014. Ecological dynamics and biotechnological implications of *Thraustochytrids* from marine habitats. *Appl. Microbiol. Biotechnol.*, 98: 5789−5805.

Tran Thi Xuan Mai, Nguyen Phi Nga, Nguyen Thi Lien, Nguyen Van Be, 2015. Isolation and identification of thraustochytrid heterotrophic microalga for production of carotenoids. *Science Journal of Can Tho University*, 37(1): 57−64 (in Vietnamese with English summary).

Xiao R., Li X., Zheng Y., 2018. Comprehensive study of cultivation conditions and methods on lipid accumulation of a marine protist *Thraustochytrium striatum*. *Protist.*, 169(4): 451−465. https://doi.org/10.1016/j.protis.2018.05.005