Iranian Journal of Fisheries Sciences 19(6) 3112-3126 2020
DOI: 10.22092/ijfs.2020.122942

Research Article

The effect of temperature and different carbon and nitrogen sources on the growth and fatty acid profile of a newly isolated microorganism *Aurantiochytrium* sp. strain SHY

Pahlavanyali M.[1]; Jalili H.[2*]; Noroozi M.[3]; Moradi Y.[1]; Hallajisani A.[4]

Received: September 2019 Accepted: November 2019

Abstract

Thraustochytrids have received a great deal of attention in recent years for producing a significant amount of omega-3 fatty acids. However, their commercial and sustainable production from microalgae sources faces technical and economic limitations. In this study, the effect of temperature and different carbon and nitrogen sources in YEP calture on the growth and fatty acids profile in a newly isolated strain of *Aurantiochytrium* sp. SHY has been conducted. Glucose produced more biomass, but galactose was more suitable for lipid formation. Galactose promoted the highest production of fatty acids (36.4%), but the ratio of docosahexaenoic acid to fatty acids was 19.05% which was less than glycerol. With an increased glucose concentration, EPA is considerably higher and DHA is only slightly increased. This is the first study that has been carried out on this new isolate wherein a mixed carbon source was used in the culture media and the results showed the microorganism's preferences for carbohydrate consumption is in the following pattern: glucose > galactose > fructose> sucrose. The meat extract was the most suitable nitrogen source for biomass development (7 g L^{-1}), but the maximum DHA to fatty acids ratio (25%) was achieved when the microorganism consumed monosodium glutamate as a nitrogen source. The strain produced more biomass at 25°C, and lowering the temperature achieved a higher DHA formation. The results of this study show that if this strain is going to introduce as a good potential candidate for food industries and aquaculture, it needs more study in bioreactor that's going on.

Keywords: Fatty acids, Mangrove forest, Nitrogen sources, Persian Gulf, Temperature

1-Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
2-Department of Biological Engineering, Faculty of New Sciences and Technologies,University of Tehran, Iran.
3- Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.
4-Caspian Faculty of Engineering, College of Engineering, University of Tehran, Tehran, Iran.
*Corresponding author's Email: hjalili@ut.ac.ir

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

Introduction

The docosahexaenoic acid (DHA) is an essential fatty acid that plays a significant role in human health, mainly in performance efficiency of the brain and retina, reduction of risk in cardiovascular disorders as well as a recommended dietary intake in infancy (Devarshi *et al.*, 2019). Fish oil is the main source of DHA. However, its commercial use is restricted because of the odor and flavor which is unappealing to consumers. Fish oil requires a multi-step purification process for production of DHA that consequently enhances the product's cost (Kim *et al.*, 2013). For these reasons, the DHA production from marine microorganisms has increasingly received more attention (Chang *et al.*, 2014; Manikan *et al.*, 2015b). Although oil from the microorganism is free of contaminations, its commercial production is restricted because of the high cost process. Much efforts have been made by scientists to facilitate the process and increase the yield (Hong *et al.*, 2011).

Marine microorganisms such as *Aurantiochytrium* and the microalgae *Crypthecodinium cohnii* are among the best sources that produce DHA (Rumiani *et al.*, 2018). Thraustochytrides are a group of non-photosynthetic unicellular protist that are heterotrophic and produce zoospores. Their isolation environments from algae and plant matter are decomposing and sediments in coastal areas. They are widely distributed and their aggregation is mostly found in the coastal areas of the seas, oceans, bays and estuaries, mangrove forests, algal beaches along the seas and also marine sediments. *Aurantiochytrium* is a sister genus of *schizochytrium* and all were originally considered to form one genus (Yokoyama and Honda, 2007). The zoospores released are similar in shape and vegetative cells are generally dispersed as single cells (Marchan *et al.*, 2017). In Iran, these strains exist on the shores of the Persian Gulf.

The culture conditions, including temperature, salinity, pH, dissolved oxygen and nutrients such as type and concentration of carbon and nitrogen sources have a considerable impact on both the quality and quantity of the produced fatty acids by the microalgae strains (Pahlavan Yali *et al.*, 2017). It was found that glucose at a concentration of 20 g L^{-1} and temperature of 28°C are the most optimized conditions for biomass, lipid, and DHA production by *Aurantiochytrium* sp. SD116 (Gao *et al.*, 2013). Monosodium glutamate and peptone were shown to be the best nitrogen sources for biomass and squalene production by *Aurantiochytrium* sp. (Chen *et al.*, 2010). The maximum biomass and lipid production of the microorganism strain of *Aurantiochytrium* sp. strain 4W-1b were found at temperatures of 15–25°C and a glucose concentration of 45 g L^{-1} (Nakazawa *et al.*, 2012). The effects of temperature on the biomass, lipid, and

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

DHA production by *Aurantiochytrium mangrovei* Sk-02 were investigated and 30°C was reported as the best temperature for cell growth, while the highest production of PUFAs occurred at 12°C (Chodchoey and Verduyn, 2012).

The aim of this study was to investigate the preferences of carbohydrate consumption by a newly isolated strain of *Aurantiochytrium* sp. SHY from the Persian Gulf and the impact of various culture parameters on the growth and fatty acid profile of this microorganism.

Materials and methods

Microorganism isolation and cultivation

The leaves and sediment of the mangrove plant (*Avicennia marina* (Forsk) Vierh) were collected from the shore of the Persian Gulf in Assaluyeh City of Bushehr province in summer and winter and transferred in sterile plastic containers to the Microalgae Laboratory at the Iranian Biological Resource Center. The leaves were washed 4–5 times in sterile water with penicillin G and streptomycin sulfate in a concentration of 0.25–0.5 mg L^{-1} to suppress bacterial growth (Kamlangdee and Fan, 2003). The washed leaves were cut into 0.5-1 cm pieces and placed in a YEP medium**.** Immediately after incubation the zoospores were formed on the plates. The formed zoospores were removed and transferred to GYP culture medium. A complete description of the isolation and identification of *Aurantiochytrium* microalgae is given in the previous article (Pahlavan Yali *et al.*, 2017).

Experimental design

In order to examine the impact of various culture media on the production of biomass, FA, and DHA by *Aurantiochytrium* sp. strain SHY, seven carbon sources (glucose, fructose, lactose, galactose, sucrose, ethanol, and glycerol) each at a concentration of 20 g L^{-1} were added to the media. In a second experiment, four sources of nitrogen comprising yeast extract, meat extracts, peptone and MSG, each at a concentration of 10 g L^{-1} were added to each culture medium which also included glucose at a concentration of 60 g L^{-1} as a carbon source. In a third experiment, the mixed culture media containing four carbon sources including glucose 12.5 g L^{-1}, fructose 12.5 g L^{-1}, galactose 12.5 g L^{-1}, and sucrose 12.5 g L^{-1} as well as meat extract 8 g L^{-1} as a nitrogen source was evaluated. To study the effect of temperature on the microorganism's growth and DHA's production, the microorganism was cultured in a medium containing glucose and meat extract at concentrations of 60 and 10 g L^{-1}, respectively, and at the temperatures of 15, 20, 25, 30, and 35°C for a period of eight days.

A fourth experiment was conducted in order to investigate the effects of four glucose concentration levels (40, 60, 80, and 100 g L^{-1}) as the carbon source on the biomass and DHA production of

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

the microorganism. The meat extract at a ratio of 6:1 (carbon: nitrogen) was added to each medium as the nitrogen source (Pahlavan Yali *et al.*, 2017). All of the experiments were repeated in triplicate. The statistical analyses of the biomass results were performed using statistical analysis system version 9.1 (SAS).

Biomass determination

The samples of the microorganism culture were taken daily from all the containers. A volume of 10 mL of each sample was centrifuged in 50 ml Falcon tubes for 5 min at 7000 g. The obtained algal biomass was dried for 12 hr at 60°C in an oven. The dried biomass is reported as DCW. The maximum specific growth rate was calculated. The biomass was then lyophilized at -55°C using an OPERON lyophilizer (South Korea).

Determination of the fatty acid profile of lipids

The lipid extraction was performed according to the Bligh and Dyer method. For this purpose, 1 g dry sample was mixed with 4 ml chloroform–methanol 2:1 (v:v) as a solvent. After centrifugation and evaporation of the solvent, the remaining organic phase was stored in an oven until the sample reached a constant weight. 0.1g of extracted lipid was injected into the gas chromatograph (Agilent 6890, USA) to analyze the content of fatty acids. The GC was equipped with a capillary column (100m×0.25µm×0.2µm). The oven temperature was increased from 110 to 230 °C at a rate of 4°C min^{-1} and was maintained constant at 230°C for 10 min. Hydrogen was used as a carrier gas (Bligh and Dyer, 1959).

Carbohydrates measurement

The carbohydrate measurement was performed according to derivatization method using a GC (Varian CP-3800, USA). The aliquots were evaporated completely and then added to pyridine, hexamethyldisilazane, and trichloromethylsilane (1:3:9, v:v:v) for 3 hr at 70°C. The oven temperature was increased from 150 to 210°C at a rate of 5°C min^{-1}, then increased to 300°C at a rate of 10°C min^{-1} and was maintained constant at this temperature for 3 min (Medeiros and Simoneit, 2007).

Results

Morphological description

The spherical vegetative cells of *Aurantiochytrium* sp. strain SHY with two flagellates and a diameter of 3 to 7 µm were observed under the microscope. The triad and tetrad forms of the cells were seen due to binary division. The globose or ovoid zoospores lose their flagellum and developed into the vegetative cells. The vegetative cells shifted to cell cluster or zoosporangium. The cell cluster changed into the amoeboid cells and the zoosporangium released zoospore thus repeating the cycle (Fig. 1).

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

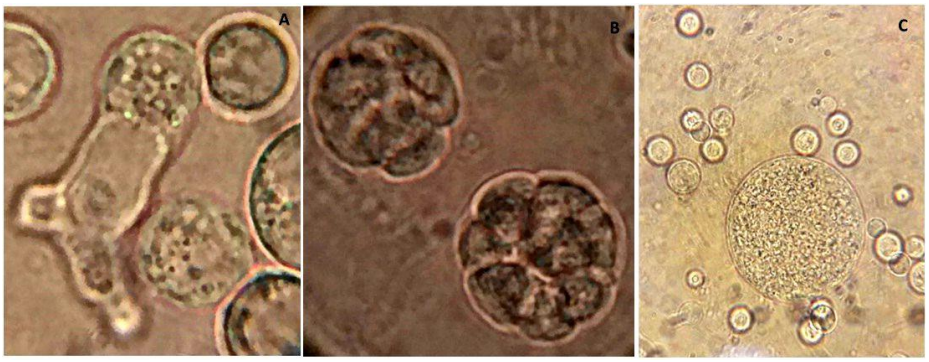

Figure 1: The morphological image of *Aurantiochytrium* strain SHY: A) amoeboid cells; B) cell cluster; C) zoospores.

Molecular identification

The BLAST analysis showed that this strain had high similarity (97 bootstrap) to *Aurantiochytrium* sp. strain TA4. The *Aurantiochytrium* separated and differentiated from the other genera in the cladogram. Besides the *Aurantiochytrium* sp. TA4, the isolated *Aurantiochytrium* belonged to this big clad as depicted in the phylogenetic tree (Fig. 2). Following its purification and morphological and molecular (using the 18SrRNA gene region) examinations, the microorganism was identified as *Aurantiochytrium* sp. SHY strain (Accession number-ky677759).

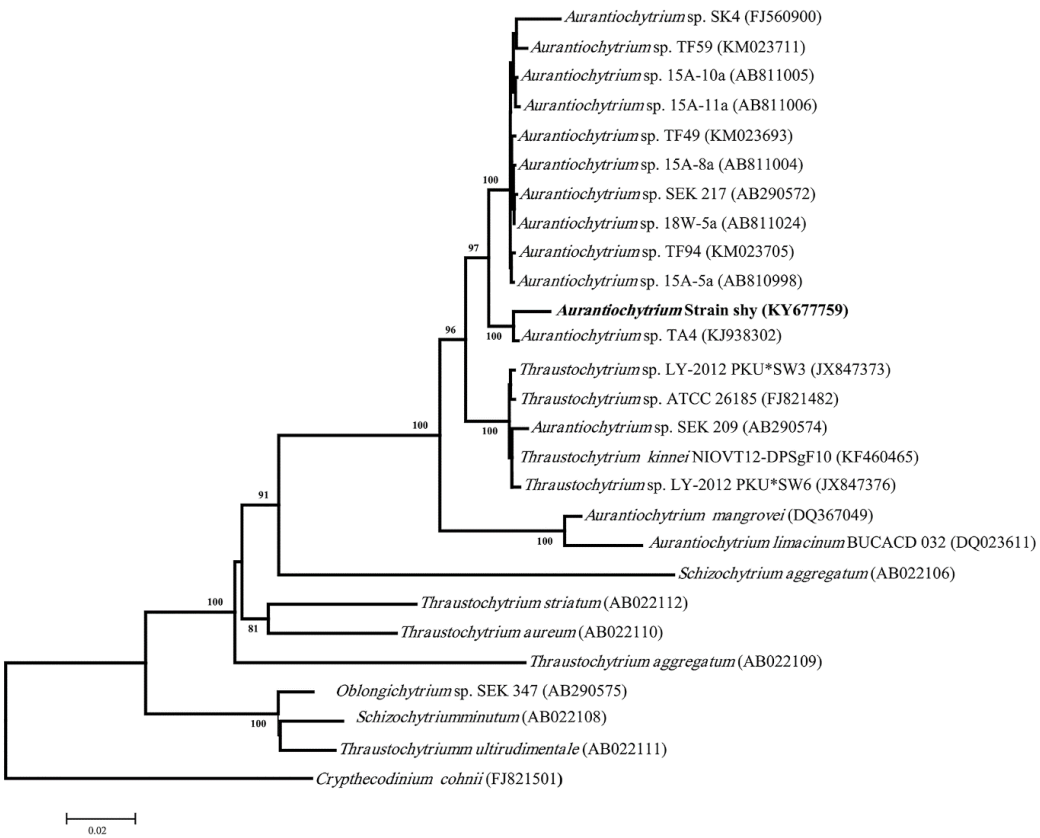

Figure 2: Molecular identification and phylogenetic tree.

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

Effect of carbon sources on growth and fatty acids profile

The effect of various carbon sources on the growth rate, biomass production, and fatty acids composition of *Aurantiochytrium* sp. SHY has been studied. The fatty acids composition and DHA levels in the fatty acids are shown in Tables 1 and 2. The maximum biomass production using glucose was 6.95 g L^{-1}. The highest biomass concentration following the glucose was observed during fructose consumption. The lag phase times for all carbon sources are similar during *Aurantiochytrium* sp. SHY growth. The maximum specific growth rate belonged to glucose and fructose. Regarding biomass production, the arrangement for carbon sources can be described as: glucose, fructose, glycerol, galactose, andsucrose. Statistical analysis shows that there is a significant difference between ethanol and sucrose with other carbon sources (p>0.01).

Saturated fatty acids to the FA produced by all the carbon sources was 60–75%. As results show, the microorganism produced much lower DHA and FA from glucose and fructose respectively. The maximum FA and DHA/FA were 36.44% and 21.2%, respectively, obtained by using galactose and glycerol as carbon sources, while the highest DHA production was achieved using glucose at 371 mg L^{-1} (Table 1).

Table 1: Maximum specific growth rate, fatty acid, and DHA production by *Aurantiochytrium* sp. SHY using different carbon sources.

Carbon Sources	FA%	DHA/FA (%)	DHA/DCW mg/g	DHA (mg/L)	μ_{max} h^{-1}	C16:0/FA %	DPA/FA %	SFA/FA %	EPA/FA %	DHA/DPA
Glucose	31.24	17.1	53.42	371	0.024	63.24	7.21	71.82	0.4	2.37
Fructose	22.02	18.16	39.98	251.1	0.023	54.28	9.34	66.91	1.0	1.94
Galactose	36.44	19.05	69.4	324.2	0.018	56.46	8.21	68.3	0.9	2.32
Glycerol	31.32	21.2	66.4	338	0.022	53.67	6.87	63.25	0.48	3.08

Effect of glucose concentrations

The maximum biomass production (6.87 g L^{-1}) was observed at a glucose concentration of 40 g L^{-1}. There was a significant difference between the mean of biomass of 40 and 100 g L^{-1} (p<0.01) and 40 and 80 g L^{-1} glucose (p<0.05) during the eight days. With an increase in glucose concentration from 40 to 100 g L^{-1}, the biomass production reduced from 6.87 to 5.5 g L^{-1}. The fatty acids production was almost constant (34%) at glucose concentrations of 40–100 g L^{-1} (Table 2).

Effect of mixed culture

To find the preference of carbon source consumption in *Aurantiochytrium* sp. SHY strain, a culture mix of four carbon source, including fructose, glucose, galactose, and sucrose was prepared and used as the substrate for

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

the microorganism's growth. The results of the biomass formation and carbon source consumption are shown in figure 3. The maximum biomass (8.5 g L^{-1}) was obtained on day 9 of cultivation. Compared to the growth on glucose as a single carbon source, the biomass formation in the mixed culture was greater. The microorganism simultaneously assimilated glucose and galactose faster than the other carbon sources during the first two days of cultivation. After that time, the glucose diminished quicker than the galactose. Therefore, the microorganism's preferences for carbohydrate consumption follow the pattern: glucose > galactose > fructose > sucrose.

Table 2: Lipid and DHA production by *Aurantiochytrium* sp. SHY in different glucose concentrations.

Glucose Concentration (g/L)	FA %	DHA/FA (%)	DHA/DCW mg/g	DHA (mg/L)	μ_{max} h^{-1}	C16:0/FA %	EPA/FA %	DPA/FA %	DHA/DPA	SFA/FA %
40	34.01	16.6	56.45	359.6	0.0116	54.57	0.9	12.69	1.31	65.62
60	34.11	14.51	49.5	295	0.0111	52.5	2.55	16.92	0.86	60.99
80	33.92	17.41	59.05	324.2	0.01058	43.01	5.28	15.53	1.12	57.8
100	34.79	17.09	59.45	327	0.01057	40.15	10.58	13.88	1.23	54.06

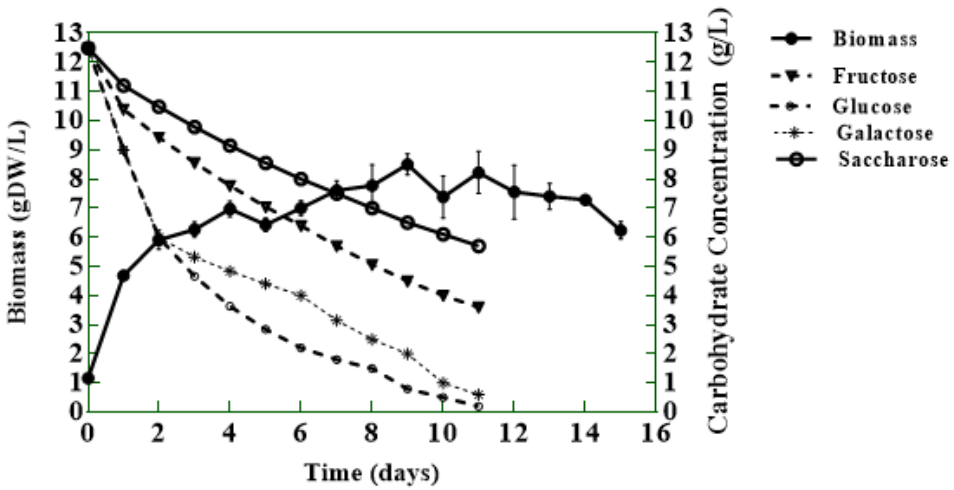

Figure 3: Biomass formation and carbohydrates assimilation with *Aurantiochytrium* sp. SHY during growth on mixed carbon sources.

Effect of nitrogen sources

The results of producing biomass from *Aurantiochytrium* sp. SHY by using various nitrogen sources is shown in Table 3. The maximum biomass production (7 g L^{-1}) was observed on

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

meat extract, while MSG medium had a minimum biomass (2.36 g L^{-1}). There was a significant difference between the mean of biomass of meat extract and MSG (p<0.05) during the eight days. The highest FA content (34.91%), DHA/FA (25%), and DHA (370.3 mg L^{-1}) were obtained when peptone, MSG, and meat extract were used as nitrogen sources, respectively (Table 3). The highest μ_{max} was 0.024 hr^{-1} that was observed in the meat extract.

Table 3: Lipid and DHA production by *Aurantiochytrium* sp. SHY on different nitrogen sources

Nitrogen Source	FA (%)	DHA/FA %	DHA/DCW (mg/g)	DHA (mg/L)	μ_{max} h^{-1}	C16:0/FA %	EPA/FA %	DPA/FA %	DHA/DPA	SFA/FA %
Meat Extract	31.08	17.02	52.9	370.3	0.024	58.4	0.5	9.88	1.72	65.31
Yeast Extract	32.42	16.36	53	287.9	0.022	47.74	2.98	9.64	1.7	60.32
Peptone	34.91	15.26	53.2	283.02	0.024	60.47	1.71	9.10	1.67	66.95
MSG	32.73	25	81.8	193.05	0.011	31.24	3.0	6.9	3.62	47.21

Effect of different temperatures

The biomass production, composition of the produced fatty acids, and DHA levels at different temperatures is exhibited in Fig. 4 and Table 4.

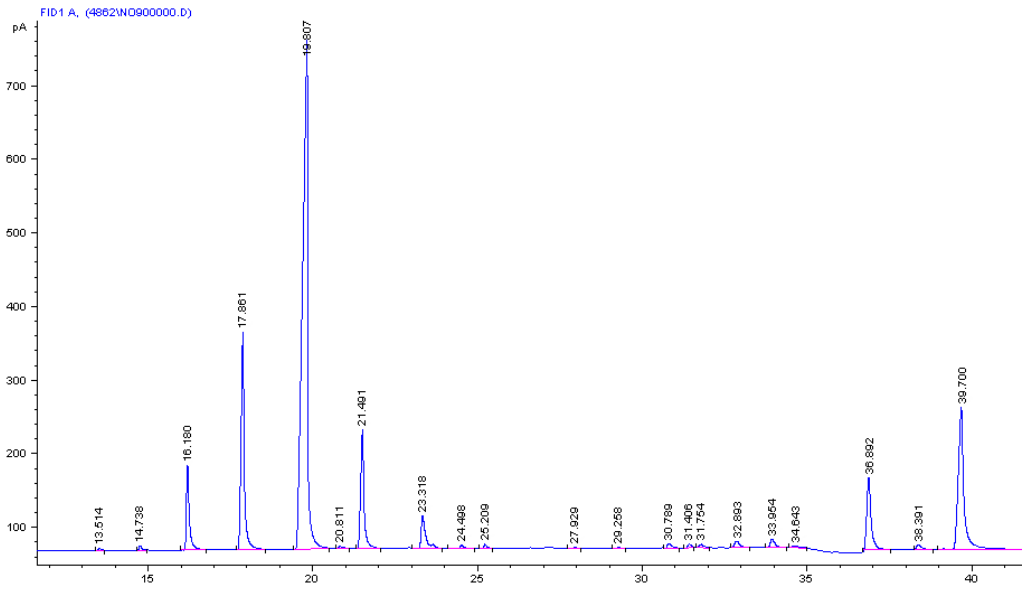

Figure 4: GC analyses of oil at 25°C.

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

Table 4: Fatty acid and DHA production rate at various temperatures by *Aurantiochytrium* sp. SHY.

T (°C)	FA (%)	DHA/DCW mg /g	DHA/FA (%f)	DHA (mg/L)	μ_{max} h^{-1}	C16:0/FA %	EPA/FA %	DPA/FA %	DHA/DPA	SFA/FA %
15	28.1	63.81	22.71	357.9	0.02	39.4	3.4	16.76	1.36	52.36
20	25.3	55.71	22.02	406.12	0.018	38.8	1.67	12.07	1.8	54.38
25	29.28	41	14	328.41	0.026	47.26	0.75	5.67	2.45	73.17
30	29.98	23.91	12.63	208.23	0.021	32.64	0.15	1.57	8.04	78.35
35	24.4	37.86	9.8	139.15	0.017	44.19	0.49	4.5	2.17	81.69

In this study, within 20–25°C temperature range, the growth rate picked up and the highest biomass production (8.28 g L^{-1}) was observed at 25°C after seven days. There was a significant difference between the biomass mean at 15°C and other temperatures (p<0.05). The produced DCW at 15 and 35°C was approximately 30% lower than its maximum. The maximum specific growth rate was observed at 25°C.

The highest FA content (29.98%) was observed at 30°C. DHA/DPA ratio was also increased with increase in the temperature. The highest DHA/FA rate (22.71) was observed at 15°C, however, the highest DHA production (406 mg L^{-1}) was seen at 20°C. As expected, with increase in temperature, the DHA decreased from 22.7 to 9.8%.

Discussion

According to the results, the monosaccharide carbon sources (glucose, galactose, and fructose) produced more biomass, fatty acids, and DHA, while biomass production was low when disaccharide and ethanol were used. By using glycerol, the production of biomass was better than the disaccharide but less than the other monosaccharides. These results are in compliance with those obtained from the microorganism strains of the same genus, *Aurantiochytrium* sp. strain SD116 (Gao *et al.*, 2013), *Aurantiochytrium limacinum* SR21, and *Aurantiochytrium limacinum* SR31 (Wu *et al.*, 2005; Cho *et al.*, 2018). Glucose as a carbon source showed the highest production both for the biomass and DHA in comparison to the other carbon sources, meanwhile the DHA and FA contents were maximum in glycerol and galactose, respectively. The effect of different carbon sources on the cell growth of *Aurantiochytrium limacinum* mh0186 was studied and it was reported that mannose and glucose have the most cell growth, while galactose has 70% biomass of glucose (Nagano *et al.*, 2009; Park *et al.*, 2017). Therefore, a combination of glucose and glycerol can be a good feed strategy for maximizing omega-3 production.

Yokochi found that DCW in *Aurantiochytrium limacinum* SR21 increased to the maximum level when glucose concentration increased to 90 g

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

L^{-1}, but it was followed by a decrease in DCW level (Cho *et al.*, 2018). In the study performed on *Aurantiochytrium* sp. strain KRS101 by Hong, when the glucose concentration increased to 60 g L^{-1}, the biomass increased once and then reduced slightly. Consequently, it seems that increasing glucose concentration has a limiting role in the growth of microalgae and probably a reason related to environmental high osmotic pressure. In this study, with increasing the glucose concentration, the EPA is considerable and DHA is slightly increased (Menogol *et al.*, 2019). Glucose is found to be suitable for EPA production. According to the authors' literature, there is no report about the effect of glucose concentration on the shift of DHA to EPA. Therefore, this interesting result needs a more biochemical-based experiment.

In mixed carbon sources, glucose and galactose consumption rates were nearly similar during the first two days, but later glucose consumption increased and it was depleted quicker from the medium. Glucose has more energy content (2.8 kj mol^{-1}) than the other substrates (Boyle and Morgan, 2009; Heo *et al.*, 2020). These trends for *Aurantiochytrium* sp. SHY showed that the tendency for monosaccharide consumption is higher than disaccharides that in compliance with the other previous studies (Yokochi *et al.*, 1998, Ju *et al.*, 2018). Based on the published data, glucose might be a preferred substrate for heterotrophic microorganism cultivation, because it appears that the microorganism's growth on other substrates requires a lag period to develop the specific transport system that is necessary for up-taking of the other substrates (Perez-Garcia *et al.*, 2011).

The nitrogen in culture medium is essential for the rapid cell growth and biomass production during the primary stages of the growth phase. Due to the nitrogen deficiency, the cells are not able to proliferate (Bellou *et al.*, 2016; Kaya *et al.*, 2020). Nitrogen sources limitation and continued consumption of carbon sources causes the cell proliferation and increment lipid accumulation. In the complex nitrogen culture media, in addition to nitrogen, carbon, enzymes and cofactors are essential for cell growth. As a result, the biomass production and growth rate are high during their utilization as a nitrogen source. The maximum DHA/FA and yield of DHA were observed during the utilization of MSG. These results are in compliance with the previous studies (Unagul *et al.*, 2006; Jakobsen *et al.*, 2008; Patel *et al.*, 2019). Chen *et al.* (2012) reported that a combination of MSG with other nitrogen sources may provide suitable conditions for microalgal cell growth and DHA production.

The increased temperature led to the increase in saturated fatty acid content from 52.4% at 15°C to 81.7% at 35°C. These results revealed that the saturation rate of fatty acids is directly correlated with temperature, so that at

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

lower temperatures, unsaturated fatty acids have a higher contribution which is in compliance with the previous findings (Taoka *et al.*, 2011; Bellou *et al.*, 2012). The solubility of oxygen in the culture medium decreases with temperature boosting, on the other hand the rate of oxygen delivery or mass transfer coefficient magnified. Therefore, for maximal biomass production an optimal environmental temperature will be expected. The maximum percentage of total fatty acids (including EPA and DHA) were observed at 15°C (26.1%) and decreased (10.25%) with increasing temperature to 35°C, as expected. This result is also in compliance with most of researches (Metz *et al.*, 2001; Zhu *et al.*, 2007; Chodchoey and Verduyn, 2012). Although the highest biomass production was found at the 25-30^{o}C temperature range, and also the highest percentage of DHA content is occurred at lower temperatures, therefore the temperature shift during the cultivation period is a good strategy for maximize DHA production.

A major fraction of the saturated fatty acids consisted of palmitic and stearic acids, while DHA formed the major part of the unsaturated fatty acids. Yielding this fatty acid composition is one of the prominent attributes of the microorganism *Aurantiochytrium* sp. (Manikan *et al.*, 2015a). The increase in temperature also elevated the DHA/DPA ratio. These results show that DPA is not a precursor of DHA, because with the rising temperature and the reduction in production and amount of DHA, rationally, the DHA/DPA ratio must decrease but it didn't (Hong *et al.*, 2011). The EPA/FA ratio in this strain is in the range of 0.45–4.22%. This ratio reduces with the rising temperature. However, the EPA in this strain is significantly higher than in *Aurantiochytrium* sp. (Manikan *et al.*, 2015b).

Acknowledgments

This study has been conducted at the Iranian National Biological Research Center. The authors would like to thank the center's authorities and personnel for their support and assistance.

References

Bellou, S., Moustogianni, A., Makri, A. and Aggelis, G., 2012. Lipids containing polyunsaturated fatty acids synthesized by Zygomycetes grown on glycerol. *Applied Biochemistry and Biotechnology*, 166, 146-158. https://doi.org/10.1007/s12010-011-9411-z

Bellou, S., Triantaphyllidou, I.E., Aggeli, D., Elazzazy, A. M., Baeshen, M.N. and Aggelis, G., 2016. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. *Current Opinion in Biotechnology*, 37, 24-35. https://doi.org/10.1016/j.copbio.2015.09.005

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian journal of*

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

biochemistry and physiology, 37, 911-917. https://doi.org/10.1139/o59-099

Boyle, N.R. and Morgan, J.A., 2009. Flux balance analysis of primary metabolism in Chlamydomonas reinhardtii. *BMC systems biology*, 3, 4. https://doi.org/10.1186/1752-0509-3-4

Chang, K.J.L., Nichols, C.M., Blackburn, S.I., Dunstan, G.A., Koutoulis, A. and Nichols, P.D., 2014. Comparison of thraustochytrids Aurantiochytrium sp., Schizochytrium sp., Thraustochytrium sp., and Ulkenia sp. for production of biodiesel, long-chain omega-3 oils, and exopolysaccharide. *Marine Biotechnology*, 16, 396-411. https://doi.org/10.1007/s10126-014-9560-5

Chen, G., Fan, K.W., Lu, F.P., Li, Q., Aki, T., Chen, F. and Jiang, Y., 2010. Optimization of nitrogen source for enhanced production of squalene from thraustochytrid *Aurantiochytrium* sp. *New Biotechnology*, 27, 382-389. https://doi.org/10.1016/j.nbt.2010.04.005

Chen, X.S., Ren, X.D., Dong, N., Li, S., Li, F., Zhao, F.L., Tang, L., Zhang, J.H. and Mao, Z.G., 2012. Culture medium containing glucose and glycerol as a mixed carbon source improves ε-poly-l-lysine production by *Streptomyces* sp. M-Z18. Bioprocess and biosystems *engineering*, 35, 469-475. https://doi.org/10.1007/s00449-011-0586-z

Cho, C., Shin, W., Woo, D. *et al.*, 2018. Growth medium sterilization using decomposition of peracetic acid for more cost-efficient production of omega-3 fatty acids by Aurantiochytrium. Bioprocess Biosyst Eng 41, 803–809. https://doi.org/10.1007/s00449-018-1914-3

Chodchoey, K. and Verduyn, C., 2012. Growth, fatty acid profile in major lipid classes and lipid fluidity of Aurantiochytrium mangrovei Sk-02 as a function of growth temperature. *Brazilian Journal of Microbiology*, 43, 187-200. http://dx.doi.org/10.1590/S1517-83822012000100020

Devarshi, P.P., Grant, R.W., Ikonte, C.J., Hazels Mitmesser, S., 2019. Maternal Omega-3 Nutrition, Placental Transfer and Fetal Brain Development in Gestational Diabetes and Preeclampsia. *Nutrients*, 11, 1107P. https://doi.org/10.3390/nu11051107

Gao, M., Song, X., Feng, Y., Li, W. and Cui, Q., 2013. Isolation and characterization of Aurantiochytrium species: high docosahexaenoic acid (DHA) production by the newly isolated microalga, Aurantiochytrium sp. SD116. *Journal of Oleo Science*, 62, 143-151. https://doi.org/10.5650/jos.62.143

Heo, S., Oh, Y.T., Kim, Z. *et al.*,2020. Application of Jerusalem artichoke and lipid-extracted algae hydrolysate for docosahexaenoic acid production by *Aurantiochytrium* sp. KRS101. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-020-02207-z.

Hong, W.K., Rairakhwada, D., Seo, P.S., Park, S.Y., Hur, B.K., Kim, C.H. and Seo, J.W., 2011. Production of Lipids Containing High Levels of Docosahexaenoic Acid by a Newly Isolated Microalga, Aurantiochytrium sp. KRS101. *Applied Biochemistry and Biotechnology*, 164, 1468-1480.

http://dx.doi.org/10.1007/s12010-011-9227-x

Jakobsen, A.N., Aasen, I.M., Josefsen, K.D. and Strøm, A.R., 2008. Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid *Aurantiochytrium* sp. strain T66: effects of N and P starvation and O2 limitation. *Applied Microbiology and Biotechnology*, 80, 297. http://dx.doi.org/10.1007/s00253-008-1537-8

Ju, J., Oh, B., Ryu, S. *et al.*, 2018. Production of Lipid Containing High Levels of Docosahexaenoic Acid by Cultivation of Aurantiochytrium sp. KRS101 Using Jerusalem Artichoke Extract. *Biotechnol Bioproc E*, 23, 726–732. https://doi.org/10.1007/s12257-018-0419-x

Kamlangdee, N. and Fan, K., 2003. Polyunsaturated fatty acids production by Schizochytrium sp. isolated from mangrove. *Songklanakarin Journal of Science and Technology*, 25, 643-650.

Kaya, K., Kazama, Y., Abe, T. *et al.*, 2020. Influence of medium components and pH on the production of odd-carbon fatty acids by Aurantiochytrium sp. SA-96. *Journal of Applied Phycology*, 32, 1597–1606. https://doi.org/10.1007/s10811-020-02111-6

Kim, K., Jung Kim, E., Ryu, B.G., Park, S., Choi, Y.E. and Yang, J.W., 2013. A novel fed-batch process based on the biology of Aurantiochytrium sp. KRS101 for the production of biodiesel and docosahexaenoic acid. *Bioresource technology*, 135, 269-274. https://doi.org/10.1016/j.biortech.2012.10.139

Manikan, V., Kailil, M.S. and Hamid, A.A., 2015a. Response surface optimization of culture medium for enhanced docosahexaenoic acid production by a Malaysian thraustochytrid. *Scientific Reports*, 5. https://doi.org/10.1038/srep08611

Manikan, V., Nazir, M.Y.M., Kalil, M.S., Isa, M.H.M., Kader, A.J.A., Yusoff, W.M.W. and Hamid, A.A., 2015b. A new strain of docosahexaenoic acid producing microalga from Malaysian coastal waters. *Algal Research*, 9, 40-47. https://doi.org/10.1016/j.algal.2015.02.023

Marchan, L.F., Chang, K.J.L., Nichols, P.D., Mitchell, W.J., Polglase, J.L. and Gutierrez, T., 2017. Taxonomy, ecology and biotechnological applications of thraustochytrids: a review. *Biotechnology Advances*, 36, 26-46. https://doi.org/10.1016/j.biotechadv.2017.09.003

Medeiros, P.M. and Simoneit, B.R.T., 2007. Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1141, 271-278. https://doi.org/10.1016/j.chroma.2006.12.017

Menegol, T., Romero-Villegas, G.I., López-Rodríguez, M. *et al.*, 2019. Mixotrophic production of polyunsaturated fatty acids and carotenoids by the microalga *Nannochloropsis gaditana*. *Journal of Applied Phycology*, 31, 2823–2832. https://doi.org/10.1007/s10811-019-01828-3

Metz, J.G., Roessler, P., Facciotti, D., Levering, C., Dttrich, F., Lassner, M., Valentine, R., Lardizabal, K., Domergue, F. and Yamada, A. 2001.

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science*, 293, 290-293. 10.1126/science.1059593

Nagano, N., Taoka, Y., Honda, D. and Hayashi, M., 2009. Optimization of Culture Conditions for Growth and Docosahexaenoic Acid Production by a Marine Thraustochytrid, <I>Aurantiochytrium limacinum</I> mh0186. *Journal of Oleo Science*, 58, 623-628. 10.5650/jos.58.623

Nakazawa, A., Matsuura, H., Kose, R., Ito, K., Ueda, M., Honda, D., Inouye, I., Kaya, K. and Watanabe, M.M., 2012. Optimization of Biomass and Fatty Acid Production by Aurantiochytrium sp. Strain 4W-1b. *Procedia Environmental Sciences*, 15, 27-33. https://doi.org/10.1016/j.proenv.2012.05.006

Pahlavan Yali, M., Jalili, H., Noroozi, M., Moradi, Y. and Saba, F., 2017. optimization of culture condition for Growth of the *Aurantiochytrium* sp.shy Isolated from persian Gulf. *Proceeding of 2th International and 10th national Biotechnology congress of Islamic Repulic of Iran* August 29-31 2017 Karaj-Iran, 78. 10.22092/IJFS.2018.117491

Park, S., Kim, K., Han, S. *et al.*, 2017. Organic solvent-free lipid extraction from wet Aurantiochytrium sp. biomass for co-production of biodiesel and value-added products. *Applied Biological Chemistry*, 60, 101–108. https://doi.org/10.1007/s13765-017-0258-z

Patel, A., Rova, U., Christakopoulos, P. *et al.*, 2019. Simultaneous production of DHA and squalene from Aurantiochytrium sp. grown on forest biomass hydrolysates. *Biotechnol Biofuels*, 12, 255. https://doi.org/10.1186/s13068-019-1593-6.

Perez-Garcia, O., Escalante, F. M., De-Bashan, L.E. and Bashan, Y., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. *Water Research*, 45, 11-36. https://doi.org/10.1016/j.watres.2010.08.037

Raghukumar, S., 2002. Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). *European Journal of Protistology*, 38, 127-145. https://doi.org/10.1078/0932-4739-00832

Rumiani, L.A., Jalili, H. and Amrane, A., 2018. Enhanced docosahexaenoic acid production by Crypthecodinium cohnii under combined stress in two-stage cultivation with date syrup based medium. *Algal Research*, 34, 75-81. https://doi.org/10.1016/j.algal.2018.07.010

Taoka, Y., Nagano, N., Oita, Y., Izumida, H., Sugimoto, S. and Hayashi, M., 2011. Effects of Cold Shock Treatment on Total Lipid Content and Fatty Acid Composition of Aurantiochytrium limacinum strain mh0186. *Journal of Oleo Science*, 60, 217-220. 10.5650/jos.60.217

Unagul, P., Assantachai, C., Phadungruengluij, S., Pongsuteeragul, T., Suphantharika, M. and Verduyn, C., 2006. Biomass and docosahexaenoic acid formation by Schizochytrium mangrovei Sk-02 at low salt concentrations. *Botanica marina*, 49, 182-190. https://doi.org/10.1515/BOT.2006.023

[DOR: 20.1001.1.15622916.2020.19.6.28.0]

Wu, S.T., Yu, S.T. and Lin, L.P., 2005. Effect of culture conditions on docosahexaenoic acid production by *Schizochytrium* sp. S31. *Process Biochemistry*, 40, 3103-3108. https://doi.org/10.1016/j.procbio.2005.03.007

Yokochi, T., Honda, D., Highshihara, T. and Nakahara, T., 1998. Optimization of docosahexaenoic acid production by Schizochytrium limacinum SR21. *Applied Microbiology and Biotechnology*, 49, 72-76. https://doi.org/10.1007/s002530051139

Yokoyama, R. and Honda, D., 2007. Taxonomic rearrangement of the genus Schizochytrium sensu lato based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for Schizochytrium and erection of Aurantiochytrium and Oblonnov. *Mycoscience*, 48, 199. http://dx.doi.org/10.1007/S10267-006-0362-0

Zhu, L., Zhang, X., Ji, L., Song, X. and Kuang, C., 2007. Changes of lipid content and fatty acid composition of *Schizochytrium limacinum* in response to different temperatures and salinities. *Process Biochemistry*, 42, 210-214. https://doi.org/10.1016/j.procbio.2006.08.002

[DOR: 20.1001.1.15622916.2020.19.6.28.0]