Cumhuriyet Science Journal
e-ISSN: 2587-246X
ISSN: 2587-2680
Cumhuriyet Sci. J., 42(3) (2021) 545-552
http://dx.doi.org/10.17776/csj.931137

Effects of co-culturing *Schizochytrium* sp. and *Escherichia coli* cells on biomass and Docosahexaenoic acid (DHA) production

Deniz ŞAHİN [1,*]

[1]*İstanbul Technical University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, İstanbul/TURKEY*

Abstract

Heterotrophic marine microalga *Schizochytrium* sp. is one of the most studied microorganisms for docosahexaenoic acid (DHA) production. Severeal strategies were reported to enhance DHA production, including co-culturing algal cells with different microorganisms. In this study, *Schizochytrium* sp. and *Escherichia coli* were co-cultured to examine the effect of bacterial cells on the algal growth and DHA production. The cells were incubated for 168 h and recovered to analyze biomass production, lipid content and DHA yield in the mixed culture medium. Cultivation of algal and bacterial species together decreased the biomass production (g/L), total lipid concentration (ml/L), DHA yield (g/L) and DHA percentage in lipid content about 4.1, 1.7, 3.8 and 2.2 folds, respectively, compared to algal monoculture. The only increasing amount was obtained with DHA yield per biomass (mg/gCDW) which was about 1.1 fold higher in the mixed culture. The results showed that presence of *Escherichia coli* cells in the medium affected the growth of *Schizochytrium* sp. cells and DHA production negatively. It was estimated that the interaction between algal and bacterial cells were competition instead of mutualistic interaction in which bacterial cells outcompeted the algal cells and limited the cell density increase of algal cells in the mixed culture.

Article info

History:
Received: 01.05.2021
Accepted: 15.09.2021

Keywords:
Docosahexaenoic acid,
Omega-3,
Schizochytrium sp.,
Escherichia coli,
Cocultivation.

1. Introduction

Docosahexaenoic acid (DHA) is one of the most important omega-3 fatty acids with their beneficial effects on human development and health [1, 2]. Although the most widely used source for DHA is cold water fish, increasing human population and consumption and decreasing fish stocks make development of alternative sources inevitable [3, 4].

Schizochytrium is a genus of heterotrophic marine microalgae and one of the most studied microorganism for DHA production. Obtained strains of *Schizochytrium* genus may be used to produce high amount of DHA (~100 g/L biomass production, 50-70% of cell dry weight as fatty acid and 30-70 % of lipids as DHA) [5]. Several studies were reported to enhance DHA production in *Schizochytrium* sp. including optimization of growth medium [6-8] using different carbon and nitrogen sources and co-culturing algal cells with different organisms [9-11].

Co-culturing microalgal cells with different organisms including other algal cells, yeast cells and bacterial cells is a promising strategy to increase biomass production and DHA yield. The interaction between each pair of organisms needs to be studied carefully to analyze the outcome of co-culturing [10, 11]. Moreover, bacterial contamination is also an unintentionally formed co-cultured environment which needs to be analyzed in terms of effect on the DHA production. Although using organic-rich media may increase algal growth rate and lipid content, heterotrophic bacteria may proliferate quickly which may affect the growth of algae and production of lipid and DHA [12, 13].

In this study, *Schizochytrium sp.* and *Escherichia coli* cells were co-cultured to investigate the effect of microalgae-bacteria co-cultivation on algal growth and production yield of omega-3 fatty acids. Microalgal and bacterial cells were cultured together for 168 h of incubation. pH change of the growth medium, cell densities and biomass production for each cell type were recorded. Lipid production, fatty acid composition and DHA yield were calculated at the end of incubation period. The results indicated that co-cultivation of algal and bacterial species decreased the biomass production (g/L), total lipid concentration (ml/L), DHA yield (g/L) and DHA percentage in lipid content about 4.1, 1.7, 3.8 and 2.2 folds, respectively.

*Corresponding author. *e-mail address: sahinden@itu.edu.tr*

DHA yield (mg/gCDW) increased about 1.1 fold in the mixed culture compared to algal monoculture. The interaction between algal and bacterial cells were estimated as competition instead of mutualistic interaction in which bacterial cells outcompeted the algal cells and limited the cell density increase of algal cells in the mixed culture.

2. Materials and Methods

2.1. Algae and bacteria cells and growth conditions

Schizochytrium sp. S31 and *Escherichia coli* K12 strains were obtained from American Type Culture Collection (ATCC® 20888™ and ATCC® 10798™, respectively). The growth medium (Complex medium-CM) including glucose (40 g/L), yeast extract (5 g/L), peptone (8 g/L), NaCl (25 g/L) and MOPS (21 g/L) was used for the cultivation of the cells at 28ºC on a shaker (200 rpm) [6]. pH of the starting medium was adjusted to 6.0. The samples were scaled up to 500 ml flasks containing 100 ml of CM (pH:6.0) with initial OD_{600} and OD_{660} for bacterial and algal cells, respectively, at 0.01 as starting cell concentration for monoculture and co-culture samples.

2.2. Measuring biomass production and growth rates

Microalgal and bacterial monocultures and the co-culture were incubated for 168 h at 28ºC on a shaker (200 rpm). Samples were taken every 24 h to measure cell dry weight (CDW) and cell densities at OD_{600} and OD_{660} for *Escherichia coli* and *Schizochytrium sp.*, respectively. Growth curves were formed for algal and bacteral monocultures and co-culture sample. Cell densities and daily biomass production were given in Figure 1. The change in pH values for algal and bacterial monocultures and the co-culture sample during incubation period were recorded and given in Figure 2.

2.3. Fatty acid composition analysis by Gas Chromatography

After 168 h of incubation, the cultures were centrifuged for 15 minutes at 3000xg (Optima MAX Ultracentrifuge) to precipitate the cells. Freeze-drying was applied on the precipitated cells (Christ-Alpha 1-2 LDplus) for 48 h to measure CDW. Following protocol was used for the lipid extraction process [6]. Briefly, n-hexane (FisherScientific) was mixed with culture samples in 6:1 ratio (v/CDW) and sonicated for disruption of the cells (three bursts of 20 s). Disrupted cells were put on an orbital shaker (150 rpm) and incubated for 6 h at 27°C. Then, the cells were centrifuged at 3000g for 10 minutes to obtain supernatants. The supernatant samples were kept under fume hood until a viscous liquid was left the bottom of the tubes.

For the fatty acid composition determination, the extracted lipids from each sample were analyzed using GC-FID (Agilent Technologies 6890N), gas chromatography with flame ionization detector. The protocol which was used in our previous study was followed to prepare fatty acid methyl esters, cold esterification method and the conditions for the GC analysis [6].

3. Results and Discussion

3.1. Growth curves for *Schizochytrium* sp. and *Esherichia coli* monocultures and microalgae-bacteria co-culture

OD_{660} and OD_{600} absorbance values for *Schizochytrium* sp. and *Escherichia coli*, respectively, were measured every 24 h to plot growth curves for monoculture and co-culture samples. Figure 1 indicates both cell densities and cell dry weight measurements during the incubation period. Initial cell densities for each monoculture were arranged to OD value of 0.01. The co-culture sample was initiated with the same cell densities from both microalgae and bacteria cells, each having final OD value of 0.01.

At 24 h, algal monoculture had the lowest OD_{660} value, around 0.13. On the other hand, cell densities in bacterial monoculture and co-culture increased quickly to OD 2.24 and 1.89, respectively. The growth in the co-culture was slower than the growth in the bacterial monoculture. The cell density increase in the co-culture sample was mainly due to bacterial cells which outcompeted the microalgal cells. After 24 h, cell density for algal monoculture started to increase and reached OD_{660} of 2.03 at 48 h and kept increasing steadily till the end of the 168 h to OD_{660} of 4.41. For the bacterial monoculture, cell density entered relatively a stationary phase after the 24 h and increased slowly to OD_{600} value of 2.9 at the 168 h. Similar cell density trend was observed for the co-culture sample after the 24 h which increased slowly from 2.09 to 2.69 (OD_{600} values) and 1.89 to 2.4 (OD_{660} values).

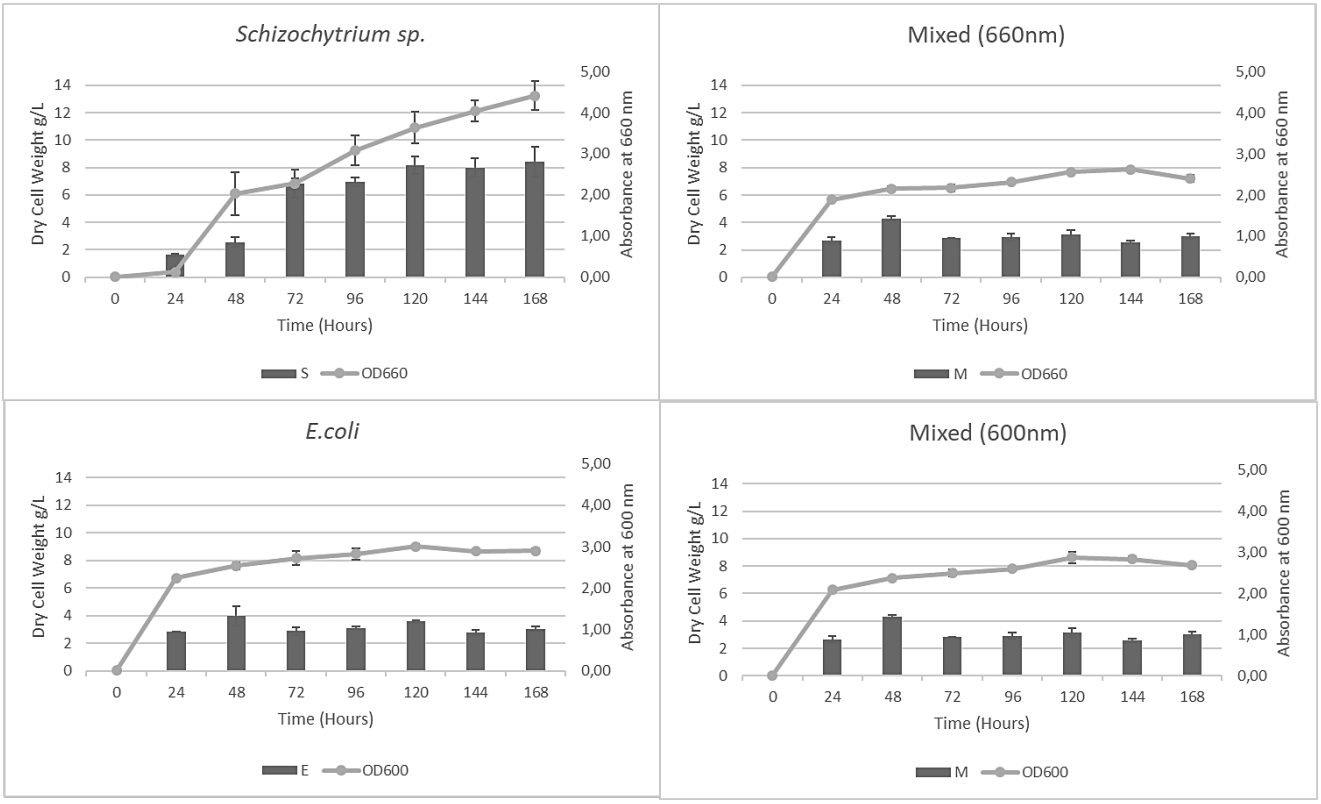

Figure 1. Cell densities (OD) and cell dry weights (CDW) for algal and bacterial monocultures and mixed co-culture sample. *Schizochytrium* sp. and *Escherichia coli* monocultures and co-culture sample were grown for 168 h at 28ºC on a shaker (200 rpm). To measure cell density and cell dry weight for each culture, samples were collected from each flask every 24 h. OD_{660} and OD_{600} values were recorded for algal and bacterial cells, respectively. For the mixed culture, OD values (600-660 nm) were recorded.

For the algal monoculture, cell dry weight was measured as 1.62 g/L and 2.55 g/L at 24 and 48 h, respectively. After 48 h, CDW increased quickly to 6.82 g/L and entered relatively a stationary phase reaching 8.42 at the end of the 168 h. For the bacterial monoculture, CDW increased from 2.83 g/L at 24 h to 4 g/L at 48 h and then fluctuated between 2 and 4 g/L and ending with 3.05 g/L at 168 h. The maximum CDW was obtained at 48 h. Similar CDW pattern was observed for the co-culture sample, in which CDW increased from 2.68 g/L at 24 h to 4.30 g/L at 48 h which was the maximum for the co-culture sample. CDW for the co-culture was almost same with CDW of bacterial monoculture but less than CDW of algal monoculture.

Several studies in literature reports the effect of co-culturing the microalgae cells with different cell types on DHA production. The nature of the interaction between the cells in the mixed culture will determine the outcome of the growing environment. In our previous study [9], we co-cultured *Schizochytrium sp.* cells with *Rhodotorula glutinis* yeast cells and enhanced biomass, DHA and β-carotene production by ~2.6, ~1.18 and 1.76 fold, respectively. In that study, unlike the current study, the increase in biomass was high for both cell types, which was confirmed by microscope examination. After all, we suggested that the interaction between these two heterotrophic species could be mainly competition between cells instead of the mutualistic interaction and the stress conditions due to quick increase in bacterial cell density caused an increase in DHA production by algal cells. Chierslip *et al.* (2011) and Dong and Zhao (2004) suggested that when photosynthetic algae and heterotrophic yeast were mixed, the algae could take a role of an oxygen generator while the yeast provides CO_2 to the algae. The increase in the growth of both cells may be due to the exchange of oxygen and carbon dioxide. We do not expect such an exchange-based cooperation here and in our previous work.

Microalgae and bacteria share same environments in nature and play crucial roles in ecosystem. Bacteria may affect the algal growth under autotrophic conditions either positively or negatively [15-19]. Bacterial cells promote growth of algae cells by reducing dissolved oxygen concentration and consuming the organic materials excreted by algae [20] and secreting biotin, cobalt amine and thiamine [21]. In return, algal cells provide oxygen and extracellular compounds which promotes the bacterial cells [15, 16]. This can be considered as a mutualistic relationship

between algal and bacterial cells based on mutual exchange of materials.

Higgins et al. (2014) co-cultured photosynthetic microalgae *Chlorella minutissima* with *Escherichia coli* under mixotrophic conditions to assess the effects of bacterial contamination on algal biofuel production. It was estimated that *E. coli* would dominate the microalgae cells for the nutrient sources. However, microalgal cells grew more rapidly than bacterial cells in co-cultured environment which was explained by symbiotic relationship between organisms. In a review by Subashchandrabose *et al.* (2013), mixotrophic cyanobacteria and microalgae co-culture was presented as bioremediation agents. The cells form a symbiotic interaction and cell densities of the both cell types increase. In our current study, the opposite was observed, with bacterial cells dominating, restricting the growth of algal cells.

In heterotrophic cultures, the species may primarily compete for the resources instead of forming a mutualistic interaction which may increase CDW and lipid production [23]. Cheirslip *et al*. (2011) suggests that increase in lipid production comes with increase in biomass. In the co-culture, first the cells increase in number in log phase benefiting from the rich medium [12, 24]. When one of the nutrients, particularly nitrogen, is limited, lipid production will initiate indicating that the cells are in stress. The change in pH due to growth and depletion of medium, will cause a decrease in O_2 levels, increase in CO_2 levels and decrease in cell growth in algae causing for lipid production.

Here, the aim of the study is to investigate the effects of co-culturing *Schizochytrium sp.* and *Escherichia coli* cells on the biomass and DHA production. Growth curves and CDW values indicate that bacterial cells outcompete the microalgal cells in the mixed culture. Indeed, observation of the mixed culture under microscopy verified that about 10-fold more surface coverage of bacterial cells than microalgal cells was observed. Although most of the biomass production in the mixed culture was due to the bacterial cell density, there was still a slow increase in cell density of microalgae cells. Bacterial cells were also affected negatively from the microalgal cells in the mixed environment which was observed from relatively lower OD_{600} values. Although both cells were affected by the co-culture medium, algal cells were more affected. The result of the co-culture environment can be directly observed in the slowdown in the growth of algae cells.

3.2. Analysis of total biomass and total fatty acid production

After 168 h of incubation, the cells were collected by centrifugation, disrupted by sonication and finally freeze dried to obtain the cell dry weight. The samples were subjected to hexane extraction protocol to extract the fatty acid content of the cells for the gas chromatography analysis. Total biomass, total lipid concentration, and DHA yield after 168 h of incubation are listed in Table 1.

Table 1: Biomass production, lipid concentration and DHA yields for *Schizochytrium sp.* and *Escherichia coli* monocultures and the mixed culture. Co-culturing algal and bacterial species together decreased the biomass production (g/L), total lipid concentration (ml/L) and DHA yield (g/L) about 4.1, 1.7 and 3.8 folds, respectively, compared to algal monoculture. The only increasing amount was obtained with DHA yield per biomass (mg/gCDW) which was about 1.1 fold higher in the mixed culture.

	Total Biomass (g / L)	Total Lipid concentration (ml / L)	DHA Yield (g / L)	DHA Yield (mg / gCDW)
Schizochytrium sp. (S)	7.74 ± 1.207	0.92	0.249	32.27
E.coli (E)	2.74 ± 1.61	0.51	0.055	20.19
Co-cultivation (S+E)	1.89 ± 0.26	0.53	0.066	35.05

The highest biomass production was observed in algal monoculture at 7.74 g/L CDW which was about three times more than that of bacterial monoculture at 2.74 g/L CDW. Mixed co-culture sample had the lowest biomass production at 1.89 g/L. Both species in the mixed culture sample were affected negatively by the presence of the other species.

Total lipid production for algal monoculture was 0.92 ml/L which was higher than bacterial and mixed cultures at 0.51 ml/L and 0.53 ml/L, respectively. On the other hand, lipid production per CDW for the

mixed culture was more than algal and bacterial monocultures. CDW values indicate that co-culturing *Schizochytrium sp.* and *E. coli* cells did not enhance the total biomass production. The bacterial density increased rapidly and dominated the algal cells. Therefore, the biomass contribution of algal cells was constrained. In our previous study [9], we achieved the maximum biomass production in *Schizochytrium sp.* and *Rhodotorula glutinis* co-cultured medium, although total lipid production was lower in co-cultured medium than that of algal monoculture.

In mixed culture, each cell type will contribute to total lipid production at different rates. Densities of algal and bacterial cells and the lipid production from each cell type will determine the contribution of algal and bacterial cells in the final lipid production. Here, we had about 4.1-fold more biomass production in algal monoculture then the mixed culture, yet the lipid production in algal monoculture was just about 1.8-fold more than both bacterial monoculture and the mixed culture. Even though 1.45 fold less biomass production was observed in the mixed culture than bacterial monoculture, lipid productions were almost same which shows the contribution of algal cells on the lipid content in the mixed sample. Several parameters such as culture medium, pH and temperature of the medium and the nature of each cell type will be effective on the lipid accumulation. A balanced environment is needed for the maximum biomass production and final lipid content otherwise one of the species in the co-cultured medium could predominate the system and affect the growth of others negatively [9].

3.3. Fatty acid composition analysis and DHA yield determination

Fatty acid composition analysis for algal and bacterial monocultures and the mixed culture at the end of 168 h of incubation was shown in Table 2. The table includes omega-3, omega-6 and other fatty acids. Among the omega-3 fatty acids, DHA amounts (% w/v) were 27.15%, 10.85% and 12.50% for algal monoculture, bacterial monoculture and the co-culture samples, respectively. The highest DHA content was achieved with algal monoculture. On the other hand, there was about 2.17-fold decrease in DHA content in the mixed culture than algal monoculture.

Other omega-3 fatty acids, Eicosapentaenoic acid, α-Linolenic acid and Eicosatrienoic acid, were also produced in smaller amounts compared to DHA production. In algal monoculture, EPA production was 1.92% which decreased about 2.5 fold in the mixed culture. α-Linolenic acid production increased in mixed culture which was not detected in algal monoculture.

Schizochytrium sp. is known as a high DHA producer species [6]. Here, DHA content in total extracted fatty acid was 27.15% (%w/v) in the algal monoculture medium which can be enhanced by different medium conditions [6]. Although total lipid production in bacterial monoculture and mixed co-culture samples are almost same, the percentage of DHA in the total fatty acid content is higher in co-culture sample possibly due to contribution of slowly growing algal cells in the medium.

In our previous study [9], DHA production was enhanced in *Schizochytrium* sp. and yeast *Rhodotorula glutinis* mixture. The interaction was possibly competition instead of mutualism which put the algal cells in stress. On the other hand, algal cells kept increasing their density which was not observed here. The increase in algal density in the mixed co-culture was very slow in the current study. In our previous study, both of the cells increased their numbers and kept producing DHA under stress conditions. On the contrary, the algal cells in the mixed culture here was in stress conditions contributing DHA production but cell density increase was limited.

DHA yield (g/L) decreased about 4 fold in the mixed culture which was about 1.25 fold more than bacterial culture (Table 1). On the other hand, DHA yield (mg/gCDW) in the mixed culture was about 1.75 fold higher than that of bacterial monoculture indicating that *E. coli* cells in the mixed culture contribute to the fatty acid production but DHA production is due to algal contribution.

Altogether, growing algal cells with bacterial cells caused a decrease in cell density, biomass production and DHA percentage in lipid content. On the other hand, DHA yield was enhanced per CDW indicating higher fatty acid production in a decreasing biomass.

Table 2: Fatty acid composition (%: w/v) of oil extracts from *Schizochytrium sp*. (S), *Escherichia coli* (E) and the co-culture (S+E) samples according to Gas chromatography-FID analysis. DHA content for S+E sample decreased ~2.2 fold compared to S sample. (ND: Not detected)

	S	E	S+E
Omega-3			
Docosahexaenoic acid *(C22:6 n-3)*	27.15	10.85	12.5
Eicosapentaenoic acid *(C20:5 n-3)*	1.92	ND	0.77
α-Linolenic acid *(C18:3 n-3)*	0.05	0.41	0.69
Eicosatrienoic acid *(C20:3n-3)*	0.47	ND	ND
Omega-6			
γ-linolenic acid *(C18:3 n-6)*	0.21	ND	0.11
Others			
Palmitic acid *(C16:0)*	39.05	25	26.55
Myristic acid *(C14:0)*	15.35	6.8	8.42
Palmitoloic acid *(C16:1)*	3.68	1.59	1.98
Oleic acid *(C18:1n+9c)*	3.59	2.42	3.08
Stearic acid *(C18:0)*	2.82	27.16	14.58
Pentadecanoic acid *(C15:0)*	2.62	0.96	1.33
Lauric acid *(C12:0)*	0.75	1.27	1.42
Margaric acid *(C17:0)*	0.57	18.46	24.58
Erucic acid C22 1n-9	0.56	0.3	0.27
Tridecanoic acid *(C13:0)*	0.24	0.16	0.99
Behenic acid *(C22:0)*	0.22	0.11	0.16
Lignoceric acid *(C24:0)*	0.2	0.22	0.35
Nervonic acid *(C24:1)*	0.19	0.59	ND
Arachidic acid *(C20:0)*	0.09	0.71	0.32
Myristoleic acid *(C14:1)*	0.08	0.15	0.19
Heptadecenoic acid *(C17:1)*	ND	2.48	ND
γ-linoleic acid (*C18:2n+6c)*	ND	0.23	1.14
Heleicosanoic acid *(C21:0)*	ND	0.11	0.17
Eicosenoic acid (*C20:1)*	ND	ND	0.25
Eicosadienoic *(C20:2)*	ND	ND	0.17

3.4 pH variations

pH values for algal and bacterial monocultures and the mixed culture during 168 h of incubation period were given in Figure 2. pH value for the algal monoculture remained between 5.4 and 6.0 which was higher than pH values of bacterial monoculture and the mixed culture samples. For both of the bacterial monoculture and the mixed culture, pH of the media decreased quickly below 5.0 after 24 h of incubation and then fluctuated between pH 4.2 and 5.0, following a similar pattern during incubation period.

Initial decrease in pH in all of the samples can be explained with the initial increase in cell density in all monoculture and mixed culture species. Wu et al. (2005) suggested that reduction in pH was due to the secretion of organic acids such as succinic acid, pyruvic acid, and malic acid. Here, we observed a quick increase in cell densities of bacterial cells in monoculture and mixed co-culture which may cause an increase in CO_2 concentration decreasing pH of the medium. Increase in pH between 24 and 72 h in all mediums, specifically algal medium, indicates that CO_2 was used for lipid production metabolism [26]. Addition of bacterial cells to the growth medium after 24 h of algal incubation may change the nature of the interaction between the cells and in this way different pH and lipid production can be observed.

Here, the aim of the study was to investigate the effects of co-culturing *Schizochytrium sp.* and *Escherichia coli* cells on the growth and biomass production of algal cells and DHA yield. The results indicated that algal and bacterial cells did not form a mutualistic interaction and the presence of bacterial cells affected the algal cells negatively. The density of the bacterial cells increased quickly which caused a decrease in the pH of the medium and growth and biomass production of algal cells. DHA yield per liter of culturing medium also decreased in the mixed culture compared to algal monoculture.

For further studies, bacterial cells can be added to the growth medium after algal cells have passed the lag phase and reached a certain cell density. Similarly, starting cell density of the algal cells can be increased in the co-culture medium. For both conditions, the interaction between bacterial and algal cells may be affected by different cell densities of each cell type, resulting in different amounts of lipid and DHA production. Additionally, using different growth medium conditions as we reported in our previous studies may affect CDW and lipid production and DHA content.

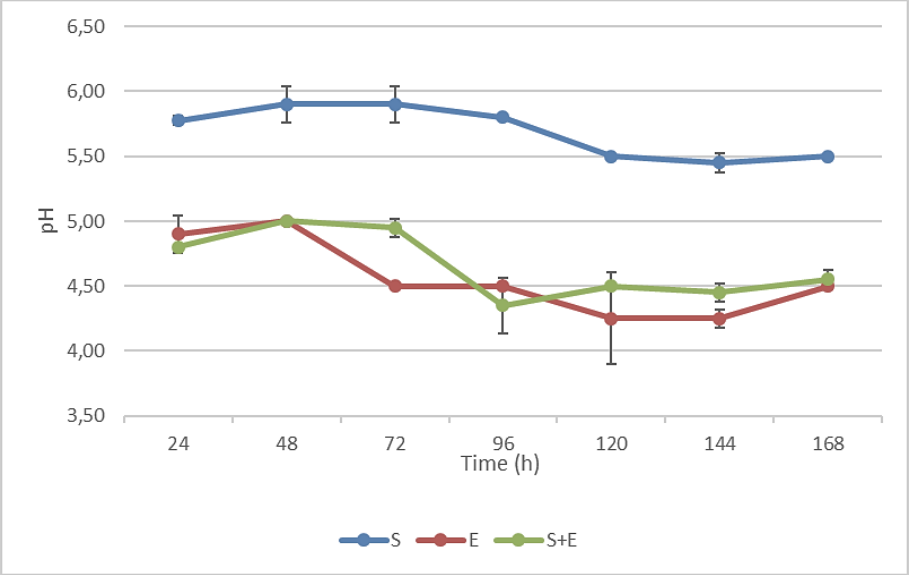

Figure 2.: pH change of the incubation mediums with time. pH values for *Schizochytrium* sp. (S), *Escherichia coli* (E) monocultures and the mixed culture sample (S+E) were measured every 24 h. pH for each medium was arranged to 6.0 at t=0 h. pH of the S medium was between 5.4 and 6, while for E and S+E mediums, pH values decreased quickly in the first 24 h and fluctuated between 4.2 and 5.0 values till the end of 168 h of incubation.

Conflicts of interest

The author declares that there is no conflict of interest.

References

[1] Connor W.E., Importance of n-3 fatty acids in health and disease, *Am. J. Clin. Nutr.*, 71 (2000) 171-175.

[2] Simopoulos A.P., Omega-3 fatty acids in inflammation and autoimmune diseases, *J. Am. Coll. Nutr.,* 21(6) (2002) 495-505.

[3] Leite G.B., Abdelaziz A.E.M., Hallenbeck P.C., Algal biofuels: Challenges and opportunities, *Bioresour. Technol.*, 145 (2013) 134-141.

[4] Ochsenreitheri K., Gluck C., Stressler T., Fischer L., Syldatk C., Production Strategies and Applications of Microbial Single Cell Oils, *Front Microbiol*, 7 (2016) 1539.

[5] Aasen I.M., Ertesvåg H., Heggeset T.M., Liu B., Brautaset T., Vadstein O., Ellingsen T.E., Thraustochytrids as production organisms for docosahexaenoic acid (DHA), squalene, and carotenoids, *Appl. Microbiol. Biotechnol.*, 100(10) (2016) 4309-21.

[6] Sahin D., Tas E., Altindag U.H., Enhancement of docosahexaenoic acid (DHA) production from *Schizochytrium* sp. S31 using different growth medium conditions, *AMB Express*., 8(7) (2018).

[7] Chatdumrong, W., Yongmanitchai W., Limtong S., Worawattanamateekul S., Optimization of docosahexaenoic acid (DHA) production and improvement of astaxanthin content in a mutant *Schizochytrium limacinum* isolated from mangrove forest in Thailand, *Nat. Sci.,* 41 (2007) 324–334.

[8] Patil K.P., Gogate P.R., Improved synthesis of docosahexaenoic acid (DHA) using *Schizochytrium limacinum* SR21 and sustainable media, *J. Chem. Eng.,* 268 (2015) 187–196.

[9] Sahin D., Altindag U.H., Tas E., Enhancement of

docosahexaenoic acid (DHA) and beta-carotene production in *Schizochytrium* sp. using symbiotic relationship with *Rhodotorula glutinis, Process Biochemistry*, 75 (2018) 10-15.

[10] Cheirsilp B, Suwannarat W, Niyomdecha R, Mixed culture of oleginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* for lipis production from industrial wastes and its use as biodiesel feedstock, *N. Biotechnol.*, 28(4) (2011) 362-368.

[11] Santos C.A., Reis A., Microalgal symbiosis in biotechnology, *Appl. Microbiol. Biotechnol.*, 98(13) (2014) 5839-5846.

[12] Zhang Y., Su H., Zhong Y., Zhang C., Shen Z., Sang W., Yan G., Zhou X., The effect of bacterial contamination on the heterotrophic cultivation of *Chlorella pyrenoidosa* in wastewater from the production of soybean products, *Water Research*, 46(17) (2012) 5509-5516.

[13] Han J., Zhang L., Wang S., Yang G., Zhao L., Pan K., Co-culturing bacteria and microalgae in organic carbon containing medium, *J. Biol. Res. (Thessalon)*. 23 (2016) s40709.

[14] Dong Q.L., Zhao X.M., In situ carbon dioxide fixation in the process of natural astaxanthin production by a mixed culture of *Haematococcus pluvialis* and *Phaffa rhodozyma. Catal. Today*, 98(4) (2004) 537-544.

[15] de-Bashan L.E., Bashan Y., Moreno M., Lebsky V.K., Bustillos J.J., Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. When coimmobilized in alginate beads with the microalgae growth promoting bacterium *Azospirillium brasilense*, *Can. J. Microbiol*, 48 (2002) 514-21.

[16] Ethier S., Woisard K., Vaughan D., Wen Z., Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid, *Bioresour Technol.* 102 (2011) 88–93.

[17] Lorincz Z., Preininger E., Kósa A., Pónyi T., Nyitrai T., Sarkadi L., Artificial tripartite symbiosis involving a green alga (Chlamydomonas), a bacterium (Azotobacter) and a fungus (Alternaria): morphological and physiological characterization, *Folia Microbiol. (Praha)*, 55 (2010) 393–400.

[18] Ortiz-Marquez J.C., Do Nascimento M., Dublan Mde L., Curatti L., Association with an ammonium-excreting bacterium allows diazotrophic culture of oil-rich eukaryotic microalgae, *Appl. Environ. Microbiol.*, 78 (2012) 2345–52.

[19] Subashchandrabose S.R., Ramakrishnan B., Megharaj M., Venkateswarlu K., Naidu R., Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation, *Environ. Int.,* 51 (2013) 59-72.

[20] Mouget J.L., Dakhama A., Lavoie M.C., de la Noüe J., Algal growth enhancement by bacteria: is consumption of photosynthetic oxygen involved?, *FEMS Microbiol. Ecol.*, 18 (1995) 35–43.

[21] Croft M.T., Lawrence A.D., Raux-Deery E., Warren M.J., Smith A.G., Algae acquire vitamin B12 through a symbiotic relationship with bacteria, *Nature*, 438 (2005) 90–3.

[22] Higgins B.T., VanderGheynst J.S., Effects of *Escherichia coli* on mixotrophic growth of *Chlorella minutissima* and production of biofuel precursors, *PLoS One,* 9(5) (2014) e96807.

[23] Perez-Garcia O., Escalante F.M.E., de-Bashan L.E., Bashan Y., Heterotrophic cultures of microalgae: Metabolism and potential products, *Water Res.*, 45(1) (2011) 11-36.

[24] Kot A.M., Blazejak S., Kurcz A., Gientka I., Kieliszek M.,*Rhodotorula glutinis*-potential source of lipids, carotenoids, and enzymes for use in industries, *Appl. Microbiol. Biotechnol,* 100(14) (2016) 6103-6117.

[25] Wu S.T., Yu S.T., Lin L.P., Effect of culture conditions on docosahexaenoic acid production by *Schizochytrium* sp S31, *Process Biochem.,* 40(9) (2005) 3103-3108.

[26] Magdouli S., Brar S.K., Blais J.F., Co-culture for lipid production: Advances and challenges, *Biomass Bioenergy,* 92 (2016) 20-30.