

Cultivation of *Arthrospira platensis* in heterotrophic and mixotrophic conditions with different concentrations of whey

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ABSTRACT

Wastes left over from human food production is commonly used to produce feed for animals, which is an important issue for a rational utilization of food sources globally, and a topic that attracts researcher for the establishment of best food production management. Whey as a side product from cheese production has great potentials in terms of nutritional value for both human food and animal feed production. This study aimed to investigate the possible use of whey (1, 10 and 30%, v/v) as an external carbon source for mixotrophic and heterotrophic cultivation of the cyanobacterium *Arthrospira platensis*. The highest specific growth rate ($\mu = 0.2 \text{ day}^{-1}$), protein ($3.76 \pm 0.14 \text{ mg/g cell}$) and lipid ($4.67 \pm 0.18 \text{ mg/g cell}$) contents were detected in heterotrophic culture while the highest chlorophyll-a ($292.39 \pm 1.31 \text{ mg/g cell}$) and total carbohydrate ($1.42 \pm 0.07 \text{ mg/g cell}$) contents were found in mixotrophic culture. In heterotrophic cultivation, it can be noted that the absorbed organic carbon source increased cell counts and triggered especially lipid production. In the mixotrophic cultivation, carbon absorbed from the culture medium or CO_2 captured with chlorophyll was utilized in the production of total carbohydrate. This study provides evidence that a cyanobacterium can adapt to heterotrophic conditions without light, creating an example for an economic and ecological production model for biochemical components.

Keywords: *Arthrospira platensis*, Biochemical composition, Heterotrophic cultivation, Mixotrophic cultivation, Whey

Introduction

Agricultural activities are remarkably influenced by the increasing environmental problems. The demand for high utility food for the increasing world population is a challenging issue for the food production industry. Whey is a side product of cheese production. Approximately 80-90 L of whey is formed from cheese produced from 100 L of milk (Božanić, Barukčić, and Lisak, 2014; Ghobrini et al., 2020). Nutritional composition of whey is dependent on cheese type. In average, it may contain lactose (46-52 %), protein (6-10 %), calcium (0.4-0.6 %), and phosphate (1-3 %). About 70 % of whey is used as raw material in different industries, while the remaining part is generally considered as waste (Božanić, Barukčić, and Lisak, 2014). This proportion of waste may have negative influences on the environment, due to its remarkably high biological oxygen demand (>35,000 ppm) and chemical oxygen demand (>60,000 ppm), which in fact can be further converted into a value product through microbial growth process (Bentahar et al., 2019; Smithers, 2008).

The cyanobacteria *Arthrospira* sp. has important nutritional properties with high protein, essential amino acid and vitamin contents (Rosas et al., 2018; Sivakumar et al., 2018). Production type for cyanobacteria is usually called phototrophic culture (Ozturk Urek & Kerimoglu, 2019). Heterotrophic cultivation is an alternative culture type with an organic carbon source but without light (Meireles et al., 2017). Another option for cyanobacteria production is mixotrophic cultivation that contains organic and inorganic carbon sources and also light (Joannesa et al., 2016; Velioglu Tosuner & Ozturk Urek, 2021). In our previous study the biomass, chlorophyll, and total lipid production of *A. platensis* was investigated with mixotrophic production in presence of sucrose (Velioglu Tosuner & Öztürk Ürek, 2020).

Heterotrophic and mixotrophic cultures have some advantages over phototrophic cultivation in terms of better growth rate, higher biomass, protein, lipid production etc. Despite of many advantages of these culture types, there are some problems such as higher cost due to organic carbon source and contamination risk (Wang et al., 2017; Zhan et al., 2017). The cost for carbon source is approximately 50% of total microalgae cultivation medium (Chandra et al., 2014), hence it also affects the choice of carbon source type (Lutzu et al., 2016). Whey is seen as an important carbon source candidate due to its low cost, high amount and rich content.

In this study, *A. platensis* was grown in heterotrophic and mixotrophic cultivation conditions with different concentrations of whey. Effects of whey concentrations and trophic culture types on biomass increase chlorophyll, protein, total carbohydrate and total lipid were investigated. This study

provides comparative beneficials from biotechnological application of mixotrophic and heterotrophic cultivations.

Material and Methods

Microalgae and Growth Media

The microalgae *Arthrospira platensis* (Gamont) Geitler 1952 was provided by Çukurova University, Faculty of Aquaculture, Türkiye. For the sustenance of cyanobacteria under photoautotrophic culture, it was grown in Zarrouk's Medium (pH 9.0) (Zarrouk, 1966). Batch cultivation was implemented in 750 mL working volume/1 L serum bottle with continuous illumination (2500 lux (33.75 $\mu\text{mol photon m}^{-2} \text{s}^{-2}$) by white fluorescent lamps), at 30°C and the cultures were mixed and aerated using filtered air continuously.

Mixotrophic and Heterotrophic Cultivation

Mixotrophic and heterotrophic cultures were applied in Zarrouk's Medium (pH 9.0) which contained different concentration of whey (1, 10 and 30%, v/v) as organic carbon source. Whey was provided by Balkan Süt Ürünleri, Izmir, Türkiye. Culture was inoculated to an initial optical density (OD= 600 nm) of 0.2. Since *A. platensis* is a filamentous microorganism, before reading, the OD the culture was transferred to spectrophotometer cuvette and the cuvette was turned upside down for three times (Velioglu Tosuner & Öztürk Ürek, 2020).

Batch cultivation was operated in 100 mL working volume/250 mL Erlenmeyer at 100 rpm, 30°C for both cultivations. For mixotrophic culture, continuous illumination (1500 lux or 20.25 $\mu\text{mol photon m}^{-2} \text{s}^{-2}$) was provided by white fluorescent lamps.

Cyanobacteria was incubated in dark environment for heterotrophic culture. Specific growth rate (μ) was calculated according to the equation below.

$$\mu = \ln \frac{X_1 - X_0}{t_1 - t_0} \quad (X: \text{ amount of microorganism, } t: \text{ time as day}).$$

Determination of Total Lipid Content

Total lipid content of cyanobacteria was determined by using freshly prepared phospho-vanillin reagent and the absorbance was measured at 530 nm against a reference sample (Mishra et al., 2014).

Determination of Chlorophyll a and b Content

Chlorophyll a and b contents were measured as described by Lichtenthaler and Wellburn (1983). The algal suspension was collected by centrifuged (5000 rpm, 15 min, 4°C) and then

homogenized in absolute ethanol by 8000 rpm for 1 min and 9500 rpm for 1 min with 30 seconds intervals (Esen and Ozturk Urek, 2015). The obtained supernatant (12000 rpm, 10 min, 4°C) was measured at 664.2 and 648.6 nm. Chlorophyll contents were calculated according to the equations below.

$$\text{Chl a} = 13.36 \times \text{Abs}_{664.2} - 5.19 \times \text{Abs}_{648.6}$$

$$\text{Chl b} = 27.43 \times \text{Abs}_{648.6} - 8.12 \times \text{Abs}_{664.2}$$

Determination of Total Protein Content

Cells collected by centrifugation were homogenized with 50 mM, pH 7.0 phosphate buffer, followed by centrifugation (12000 rpm, 10 min, 4°C), and the supernatant was used for the analysis of protein content (Esen and Ozturk Urek 2015). Protein quantification was carried out by the Bradford method at 595 nm. Bovine serum albumin in concentrations ranging from 0-250 ppm is used as standard (Bradford, 1976). To prepare Bradford reagent, 100 mg of Coomassie Brilliant Blue G-250 is dissolved in 50 mL of 95% ethanol. To the solution is added 100 mL of 85% phosphoric acid and complete with water to a total volume of 1000 mL. 100 µL of sample (100 µL of pure water as a reference) is mixed with 900 µL of reagent and allowed to stand at room temperature for 2 minutes and the absorbance is measured at 595 nm against the blank.

Determination of Total Carbohydrate Content

The supernatant of homogenized cell was used to determine total carbohydrate content by phenol-sulphuric acid method (Dubois et al., 1956). Homogenization procedure was applied as explained in the previous section. The absorbance was measured at 470 nm against a reference sample.

FTIR Analysis

The FTIR (Perkin Elmer Spectrum BX) spectra were recorded in the 4000- 400 cm^{-1} spectral region. Cells separated from growth medium were dried at 70°C overnight before analysis. Approximately 1 mg of dried cell sample was milled with approximately 100 mg of dried KBr and then pressed to form a pellet for measurement.

Statistical Analysis

All experiments were carried out in triplicates (n=3) and repeated 3 times. Each value is an average of 3 parallel replicates. Data were presented as mean \pm standard deviation. The data were analyzed by analysis of variance (TUKEY) to identify the significantly different groups at (p<0.05) by one-way TUKEY test using SPSS software statistical program (SPSS for windows ver. 21.00, USA).

Results and Discussion

The cyanobacteria *A. platensis* was incubated under mixotrophic and heterotrophic cultivation conditions, in the presence of different concentrations of whey. The highest optical density value (2.737) was detected in mixotrophic medium containing 1% (v/v) whey and the highest specific growth rate ($\mu = 0.2 \text{ day}^{-1}$) was found in heterotrophic medium containing 30% (v/v) whey (p<0.05). The high organic carbon source concentration provides carbon skeleton and continuous energy supply for the maintenance of cyanobacteria (Chandra et al., 2014). Several earlier investigations reported that mixotrophic culture supports growth more than heterotrophic culture (Wang et al., 2017; Zhan et al., 2017). The heterotrophic medium with higher concentration of whey may have created the favorable condition for the growth of the cyanobacteria, resulting in higher specific growth rate. In the mixotrophic medium containing high whey concentration, high OD and specific growth rates have not been determined. In this medium, the required conditions for the simultaneous work of two metabolisms may not have been met. In mixotrophic cultivation, cells require a lower organic carbon source than heterotrophic cultivation because higher carbon source concentration can have an inhibition effect (Joanessa et al., 2016).

When chlorophyll change was examined during the incubation period, chlorophyll-a values increased in the last days of incubation in mixotrophic cultures containing 1% (v/v) and 10% (v/v) whey, but did not show a significant change in the medium containing 30% (v/v) whey (Figure 1). The highest chlorophyll-a ($292.39 \pm 1.31 \text{ mg/g cell}$) was determined on the 28th day in the medium containing 10% (v/v) whey, and the chlorophyll-b ($67.585 \pm 0.31 \text{ mg/g cell}$) value was determined on the 21st day of the incubation in the heterotrophic medium containing 1% (v/v) whey (p<0.05). The reason for the increase in the amount of chlorophyll in the mixotrophic medium containing 10% (v/v) whey in the last days of incubation could be attributed to its use of organic carbon source in the medium in the first days of incubation and then activated its phototrophic metabolism. This is also supported by the total carbohydrate content data (Figure 3). Chlorophyll content was determined at higher values in mixotrophic culture as expected. In mixotrophic cultures, CO₂, fixed by chlorophyll, in addition to the external carbon source, provides a carbon source that can be used in biochemical components production (Zhu et al., 2016). The low amount of chlorophyll-a in heterotrophic cultivation indicates that the cell is adapted to this type of cultivation and that only the heterotrophic metabolism is active. In the dark environment chlorophyll molecules oxidized and degradation occurs (Maroneze et al., 2019). The cell uses energy to biomass growth instead of

chlorophyll production. Different studies show that more chlorophyll-a degradation occurs while chlorophyll-b oxidation and degradation less happen (Maroneze et al., 2019).

The amount of protein did not change significantly during the incubation in mixotrophic media containing 10% and 30% whey whereas an increase was observed in the medium containing 1% (v/v) whey (Figure 2). This result is also supported by the OD data. In the mixotrophic cultures, the protein content has been detected to be very low. The highest protein value (1.51 ± 0.68 mg/ g cell) was determined on the 21st day of incubation in a mixotrophic medium containing 1% (v/v) whey ($p < 0.05$). The highest protein content was detected as 3.76 ± 0.14 mg/ g cell in heterotrophic culture with 1% (v/v) whey (Figure 2) ($p < 0.05$). The high protein content of whey might have triggered this result. The protein content in the heterotrophic cultivation (1% (v/v) whey) is 2.49 fold higher than the protein content in the mixotrophic cultivation (1% (v/v) whey) ($p < 0.05$). Furthermore, it can be stated that the cells in this medium use the carbon they take from the growth medium in the production of protein causing the lipid level remain low.

When the total carbohydrate change in the growth medium was examined, an increasing trend was observed during the incubation period (Fig 3). The highest value (1.42 ± 0.07 mg/ g cell) was detected on 21st day of incubation in a mixotrophic medium containing 10% (v/v) whey ($p < 0.05$). In the heterotrophic cultures, the maximum total carbohydrate content was detected as 0.72 ± 0.08 mg/ g cell on the 14th day in the presence of 1% (v/v) whey. The total carbohydrate content in the mixotrophic cultivation (10% (v/v) whey) is 1.92 fold higher than the total carbohydrate content in the heterotrophic cultivation (1% (v/v) whey) ($p < 0.05$). While the carbohydrate contents remained low values, the protein level reached higher values in the heterotrophic medium containing whey. The presence of both organic and inorganic carbon sources in the mixotrophic culture caused both metabolisms to work. For this reason, the total carbohydrate amount was determined at higher levels than in the heterotrophic medium. However, 30% (v/v) whey creates a high carbon concentration for the mixotrophic medium. Based on the chlorophyll, protein and total carbohydrate values that the cells could not adapt to this medium (Figure 1, 2 and 3).

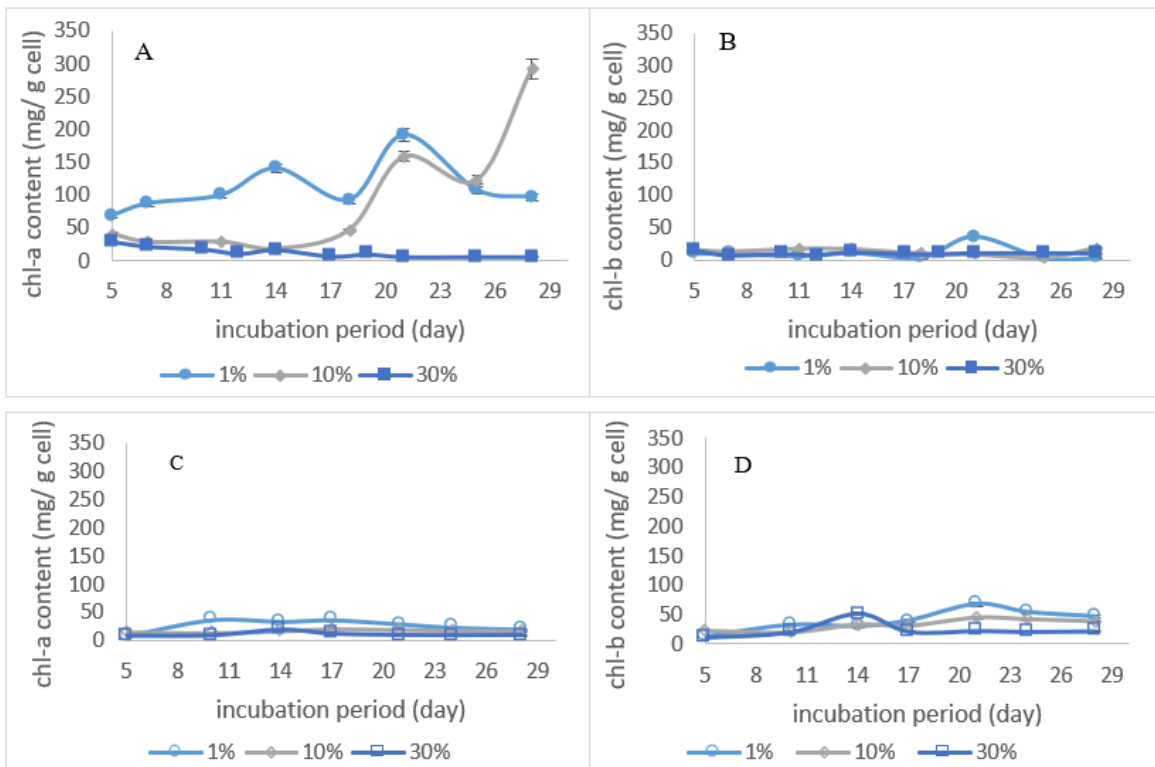


Figure 1. Chlorophyll-a and chlorophyll-b content changes depending on the incubation period of *A. platensis* grown in mixotrophic and heterotrophic cultures containing whey at varying concentrations (1, 10 and 30%, v/v) (A: Chlorophyll-a in mixotrophic culture, B: Chlorophyll-b in mixotrophic culture, C: Chlorophyll-a in heterotrophic culture, D: Chlorophyll-b in heterotrophic culture). The values are the mean \pm SD for experiments of three separate experiments

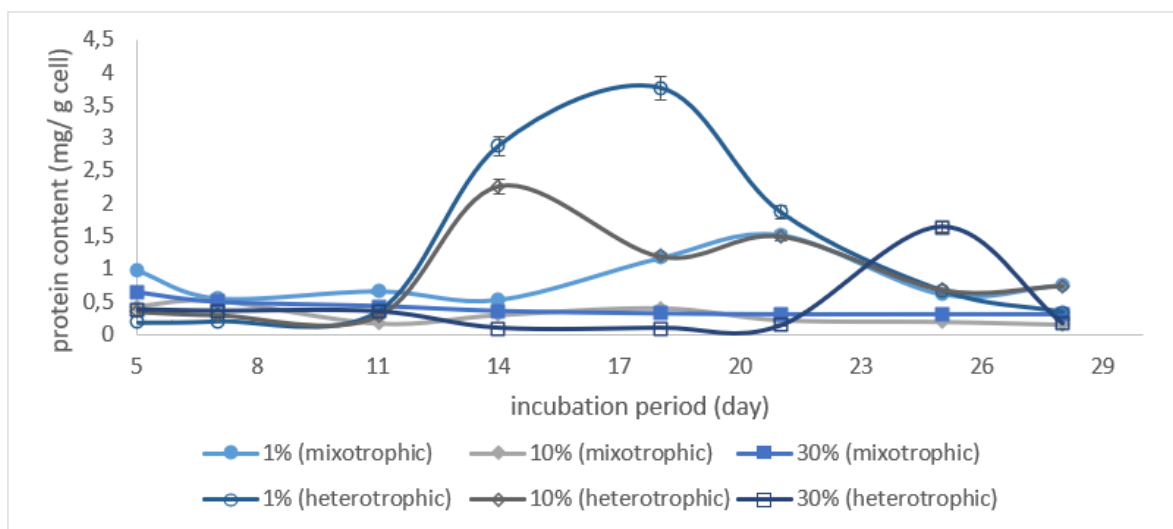


Figure 2. Protein content changes according to the incubation period of *A. platensis* grown in mixotrophic and heterotrophic cultures containing whey at varying concentrations (1, 10 and 30%, v/v). The values are the mean \pm SD for experiments of three separate experiments

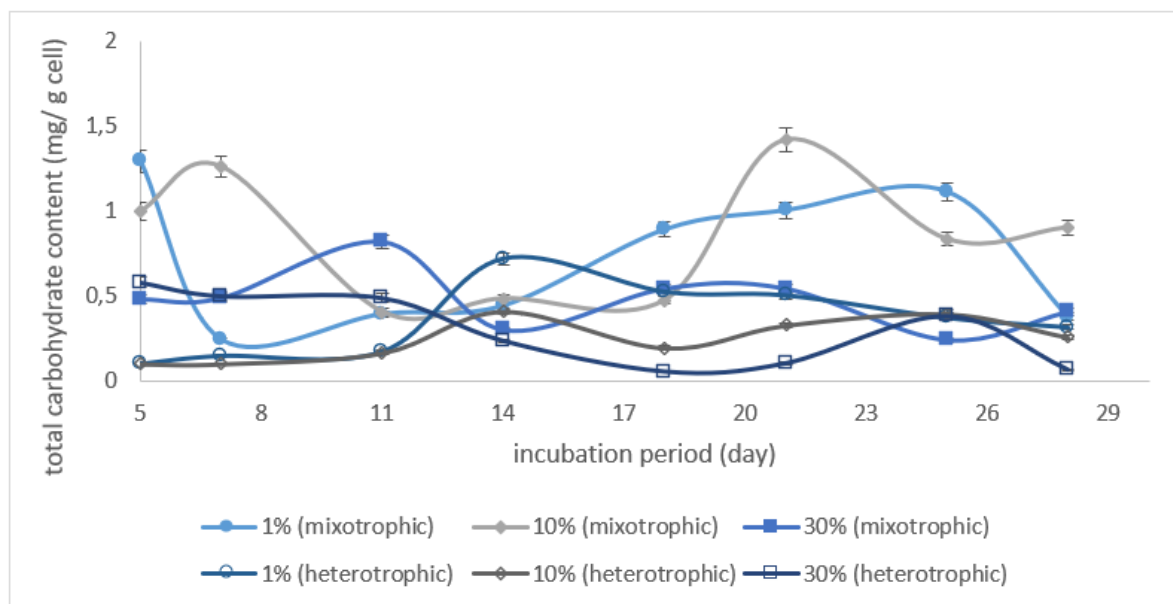


Figure 3. Total carbohydrate content changes depending on the incubation period of *A. platensis* grown in mixotrophic and heterotrophic cultures containing whey at varying concentrations (1, 10 and 30%, v/v). The values are the mean \pm SD for experiments of three separate experiments

In terms of total lipid content, the highest values were detected in heterotrophic cultures (Figure 4). The highest lipid content was detected as 4.67 ± 0.18 mg/g cell with 1% (v/v) whey in heterotrophic cultivation on 18th day (Figure 4) ($p < 0.05$). In the higher whey concentrations, the maximum lipid production was observed in the later days of incubation. In mixotrophic cultures, the maximum lipid production (3.76 ± 0.16 mg/g cell) was detected with 10% (v/v) whey on the 14th day of incubation. The highest lipid values were obtained at the beginning of the stationary phase (Figure 4). The total lipid content in the heterotrophic cultivation (1% (v/v) whey) is 1.24 fold higher than the total lipid content in the mixotrophic cultivation (10% (v/v) whey) ($p < 0.05$). The low chlorophyll production in the heterotrophic culture provides more acetyl CoA which are used in lipid synthesis pathway. Additionally, the amount of produced lipid is 1.27 fold higher than our previous study in which sucrose was used as organic carbon source (Velioglu Tosuner & Öztürk Ürek, 2020).

In a study where *A. platensis* was grown mixotrophically in the presence of whey, increased protein contents and decrease carbohydrates were recorded with the increase of whey concentrations, however no significant changes were found for the lipid levels (Pereira et al., 2019). Although these results are similar to our results, higher whey concentration was tested in our study and substrate inhibition effect was observed (Figure 3). In addition, in our study it was shown that *A. platensis* can adapt to heterotrophic conditions and moreover, it can synthesize lipid and protein at a higher rate.

According to the FTIR data, $-\text{CH}_2\text{OH}$, $-\text{CH}_3$ peaks of carbohydrate structure were determined in cells grown in mixotrophic medium. The cells grown in heterotrophic cultures shows N-H and C-N stretching on protein structure and C=O and CH_2 peaks on lipid structure. These results are supported by the spectroscopic analysis results of total carbohydrate, lipid and protein.

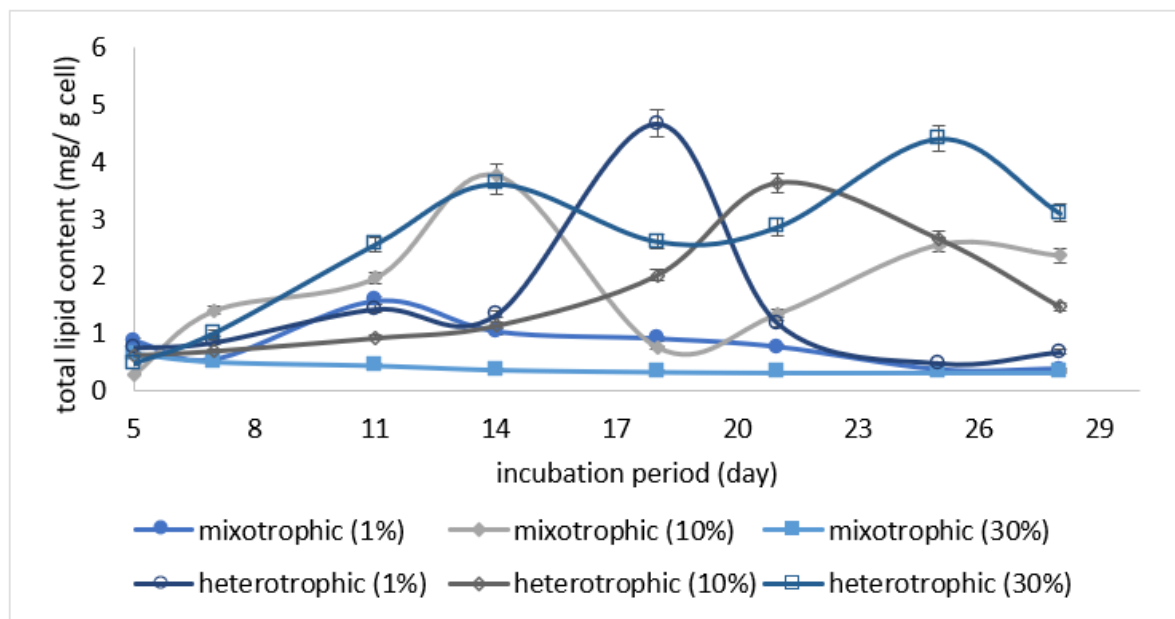


Figure 4. Total lipid content of *A. platensis* cell with different concentration of whey (1, 10 and 30 %, v/v) in mixotrophic or heterotrophic cultures. The values are the mean \pm SD for experiments of three separate experiments

Conclusion

This study provided an alternative way for the disposal of a waste material by turning it into a value-added product. Not all microalgae cells could adapt in mixotrophic and especially heterotrophic cultures. This study shows that *A. platensis* is adapted to mixotrophic and heterotrophic conditions with different whey concentrations. Valuable materials such as protein, lipid and carbohydrate have been produced by using whey in the microbial growth medium as a carbon source. Whey is mentioned as a waste which is difficult to treat and comes out in high amounts.

It is very difficult for cyanobacteria to survive in the absence of light. However, heterotrophic cultivation type, which does not need light, is more economical and easier to implement for large-scale productions. This study has shown that, *A. platensis* adapted to the heterotrophic medium in the presence of whey and produced protein and lipid. It can be concluded that assimilation of organic carbon source by *A. platensis* in mixotrophic and heterotrophic cultures cell growth and biochemical content is not rigidly reliant on photosynthetic pathway. The organic carbon was transferred into cell and redirected towards carbohydrate synthesis in mixotrophic cultivation while it was used in protein and lipid synthesis pathway in heterotrophic cultivation. In the heterotrophic conditions the produced lipid and protein levels were higher than mixotrophic culture 1.24 and 2.49 fold, respectively. Produced lipid and protein are value-added products that can be evaluated in different fields, including human and animal healthy nutrition. Thus, the potential of their both economic and an ecological production system were revealed.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Ethics committee approval is not required for this study.

Funding disclosure: -

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Disclosure: -

References

- Bentahar, J., Doyen, A., Beaulieu, L., Deschênes, J.S. (2019).** Investigation of β -galactosidase production by microalga *Tetrademus obliquus* in determined growth conditions. *Journal of Applied Phycology*, 31(1), 301-308. <https://doi.org/10.1007/s10811-018-1550-y>
- Božanić, R., Barukčić, I., Lisak, K. (2014).** Possibilities of whey utilization. *Austin Journal of Nutrition and Food Sciences*, 2(7), 7.
- Bradford, M.M. (1976).** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chandra, R., Rohit, M. V., Swamy, Y. V., Mohan, S. V. (2014).** Regulatory function of organic carbon supplementation on biodiesel production during growth and nutrient stress phases of mixotrophic microalgae cultivation. *Bioresource Technology*, 165, 279-287. <https://doi.org/10.1016/j.biortech.2014.02.102>
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956).** Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356. <https://doi.org/10.1021/ac60111a017>
- Esen, M., Ozturk Urek, R. (2015).** Ammonium nitrate and iron nutrition effects on some nitrogen assimilation enzymes and metabolites in *Spirulina platensis*. *Biotechnology and Applied Biochemistry*, 62(2), 275-286. <https://doi.org/10.1002/bab.1268>
- Ghobrini, D., Potocar, T., Smolova, J., Krausova, G., Yakoub-Bougdal, S. et al. (2020).** Heterotrophic cultivation of *Chlorella vulgaris* using saline waste water from the demineralization of cheese whey. *Biotechnology Letters*, 42(2), 209-217. <https://doi.org/10.1007/s10529-019-02770-7>
- Joannesa, C., Mansaa, R.F., Yasirb, S.M., Dayouc, J. (2016).** Comparative studies of cell growth of freshwater microalga *Chlorella* sp. in photoautotrophic, heterotrophic and mixotrophic cultures. *Jurnal Teknologi*, 78(7), 83-89. <https://doi.org/10.11113/jt.v78.4349>

- Lichtenthaler, H.K., Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11, 591-592.
<https://doi.org/10.1042/bst0110591>
- Lutzu, G.A., Zhang, W., Liu, T. (2016). Feasibility of using brewery wastewater for biodiesel production and nutrient removal by *Scenedesmus dimorphus*. *Environmental Technology*, 37(12), 1568-1581.
<https://doi.org/10.1080/09593330.2015.1121292>
- Maroneze, M.M., Zepka, L.Q., Lopes, E.J., Pérez-Gálvez, A., Roca, M. (2019). Chlorophyll oxidative metabolism during the phototrophic and heterotrophic growth of *Scenedesmus obliquus*. *Antioxidants*, 8(12), 600.
<https://doi.org/10.3390/antiox8120600>
- Meireles dos Santos, A., Vieira, K.R., Basso Sartori, R., Meireles dos Santos, A., Queiroz, M.I. et al. (2017). Heterotrophic cultivation of cyanobacteria: study of effect of exogenous sources of organic carbon, absolute amount of nutrients, and stirring speed on biomass and lipid productivity. *Frontiers in Bioengineering and Biotechnology*, 5(12), 1-7.
<https://doi.org/10.3389/fbioe.2017.00012>
- Mishra, S.K., Suh, W.I., Farooq, W., Moon, M., Shrivastav, A. et al. (2014). Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. *Bioresource Technology*, 155, 330-333.
<https://doi.org/10.1016/j.biortech.2013.12.077>
- Ozturk Urek, R., Kerimoglu, Y. (2019). Evaluation of effects of Mg^{2+} and Cu^{2+} on pigment-metabolite production and photosystem II activity of *Arthrospira platensis* Gomont 1892. *Turkish Journal of Fisheries and Aquatic Sciences*, 19(10), 873-883.
http://doi.org/10.4194/1303-2712-v19_10_07
- Pereira, M.I., Chagas, B.M., Sassi, R., Medeiros, G.F., Aguiar, E.M., Borba, L.H., Rangel, A.H. (2019). Mixotrophic cultivation of *Spirulina platensis* in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity. *PloS One*, 14(10), e0224294.
<https://doi.org/10.1371/journal.pone.0224294>
- Rosas, V.T., Poersch, L.H., Romano, L.A., Tesser, M.B. (2018). Feasibility of the use of *Spirulina* in aquaculture diets. *Reviews Aquaculture*, 1-12.
<https://doi.org/10.1111/raq.12297>
- Sivakumar, N., Sundararaman, M., Selvakumar, G. (2018). Evaluation of growth performance of *Penaeus monodon* (Fabricius) fed diet with partial replacement of fishmeal by *Spirulina platensis* (Sp) meal. *Indian Journal of Animal Research*, 52(12), 1721-1726.
<https://doi.org/10.18805/ijar.B-3438>
- Smithers, G.W. (2008). Whey and whey proteins—from ‘gutter-to-gold’. *International Dairy Journal*, 18(7), 695-704.
<https://doi.org/10.1016/j.idairyj.2008.03.008>
- Velioglu Tosuner, Z., Öztürk Ürek, R. (2020). Evaluation of sucrose as carbon source in mixotrophic culture of *Arthrospira platensis* Gomont 1892. *Aquatic Research*, 3(1), 1-12.
<https://doi.org/10.3153/AR20001>
- Velioglu Tosuner, Z., Ozturk Urek, R. (2021). The effects of nutrition on lipid production of *Haematococcus pluvialis* and biodiesel potential. *Environmental Engineering and Management Journal*, 20 (8), 1289-1299.
<https://doi.org/10.30638/eemj.2021.119>
- Wang, H., Zhou, W., Shao, H., Liu, T. (2017). A comparative analysis of biomass and lipid content in five *Tribonema* sp. strains at autotrophic, heterotrophic and mixotrophic cultivation. *Algal Research*, 24, 284-289.
<https://doi.org/10.1016/j.algal.2017.04.020>
- Zarrouk, C. (1966). Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. PhD, Université de Paris, Paris, France.
- Zhan, J., Rong, J., Wang, Q. (2017). Mixotrophic cultivation, a preferable cyanobacterium cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect. *International Journal of Hydrogen Energy*, 42(12), 8505-8517.
<https://doi.org/10.1016/j.ijhydene.2016.12.021>
- Zhu, L.D., Li, Z.H., Hiltunen, E. (2016). Strategies for lipid production improvement in microalgae as a biodiesel feedstock. *BioMed Research International*, 2016, 8792548.
<https://doi.org/10.1155/2016/8792548>