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EFFECT OF VARIOUS CARBON SOURCES ON THE GROWTH PROPERTIES AND MORPHOLOGY OF *SPIRULINA PLATENSIS*

A. A. HARUTYUNYAN [1,3*], J. G. MANOYAN [1**], L. R. HAMBARYAN [2***], L. S. GABRIELYAN [1****]

[1] *Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia*
[2] *Chair of Ecology and Nature Protection, YSU, Armenia*
[3] *Research Institute of Biology, Laboratory of Microbiology, Bioenergetics and Biotechnology, YSU, Armenia*

Cyanobacteria generate biomass under photoautotrophic conditions during photosynthesis. Cultivation of cyanobacteria under photoheterotrophic conditions using various organic carbon sources can increase yield of biomass. In the current study, the effect of organic carbon sources on the growth properties and morphology of cyanobacteria *Spirulina platensis*, as well as the production of the photosynthetic pigments: chlorophyll *a*, carotenoids, and phycocyanin were investigated. Carbon sources, such as glucose, fructose and succinate, caused not only an increase in the cyanobacterial growth rate, but also morphological changes in *S. platensis* trichomes. An increase in the amounts of photosynthetic pigments was also observed. Thus, glucose, fructose, and succinate are more efficient substrates for photoheterotrophic cultivation of these cyanobacteria.

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***Keywords*:** *Spirulina platensis*, carbon sources, photoheterotrophic growth, morphology, pigments content.

Introduction. *Spirulina platensis* is filamentous cyanobacteria, which belongs to Phylum Cyanobacteria, class Cyanophyceae, order Oscillatoriales, family Microcoleaceae, and genus *Spirulina (Artrospira)*. *Spirulina* is widely used in biotechnology as a most nutritious and healthy food, which contains a high content of proteins, fatty acids, carbohydrates, vitamins, macro- and microelements [1–3].

S. platensis is composed of individual cells organized into trichomes and characterized by regularly coiled filaments. Under certain conditions, its helical filaments can transform into abnormal morphologies, such as irregularly curved and even linear forms, which are already considered irreversible [4]. Microscopy of samples of *S. platensis* from different places reveals features in the development of length of filaments, their color, shape, saturation of filaments, and viability of developing filaments [2].

It is well known that many parameters affect the growth of microorganisms: cultivation conditions, temperature, pH, light intensity, carbon, and nitrogen sources,

[*] E-mail: aniharutyunyan@ysu.am
[**] E-mail: jmanoyan@ysu.am
[***] E-mail: lusine.hambaryan@ysu.am
[****] E-mail: lgabrielyan@ysu.am

the content of microelements in the medium, etc. [2, 5–7]. At the same time, the nature of the carbon source in the culture medium is one of the main factors determining the productivity of microalgae and their accumulation of the main groups of biologically active substances [7, 8]. Microalgae are capable of both photoautotrophic and photoheterotrophic growth [5, 7, 8]. The choice of the carbon source for microalgae cultivation is very important, because it can be easily utilized by cyanobacteria, affect the cyanobacteria growth properties and enhance biomass yield. Under photoheterotrophic conditions the growth rate and biomass yield of green algae are higher than under photoautotrophic conditions [5, 8]. In some green microalgae carbon sources such as acetate play an important role in algae metabolism and increase yield of biomass and respiration rates [5, 7, 8].

The present study aims to study the effects of organic carbon sources (glucose, fructose, lactose, succinate, acetate, lactate, and malate) on growth properties, content of photosynthetic pigments (chlorophyll *a*, carotenoids, and phycocyanin), and morphology of *S. platensis* IBCE S-2 was investigated.

Materials and Methods. *S. platensis* IBCE S-2 (Algae Collection, Institute of Biophysics and Cell Engineering, NAS, Minsk, Belarus) was cultivated in the presence of organic carbon sources (acetate, succinate, malate, lactate, glucose, fructose, lactose) under aerobic conditions and continuous illumination ~50 $W \cdot m^{-2}$ in standard Zarrouk medium at 25 ± 2℃, pH 9.0 ± 0.2 [5]. Carbon sources at a concentration of 1 g/L were added to the cultivation medium (Fig. 1). Cyanobacteria cultivated under photoautotrophic condition were used as a control. The pH of the growth medium was measured by a standard pH meter (“HANNA Instruments”, Portugal) [5, 9].

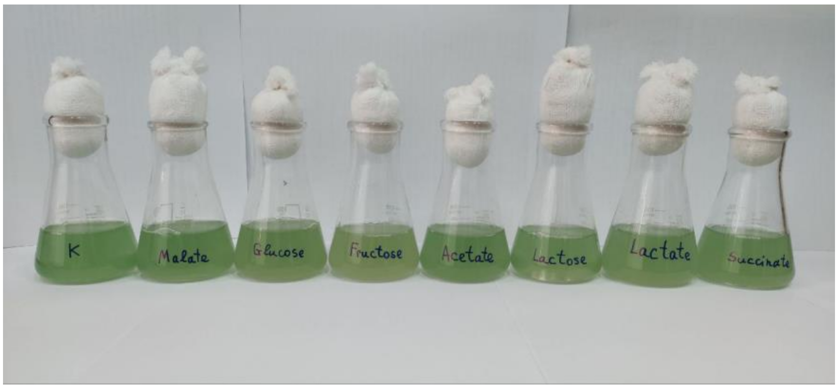

Fig. 1. Cultivation of *S. platensis* in photoautotrophic (control) and photoheterotrophic (in the presence of various organic carbon sources) conditions.

The growth of *S. platensis* was monitored by optical density (OD_{680}) changes on an SP-2000 UV-Vis spectrophotometer (“Ningbo Hinotek Instrument”, China). The specific growth rate was calculated as previously described: growth rate = $(\ln OD_t - \ln OD_0)/t$, where OD_0 is the initial value of OD; OD_t is the value of OD after *t* days and expressed in d^{-1} [5, 9].

The absorption spectra of *S. platensis* whole cells were recorded in the range 400–800 *nm* wavelength regions by UV-Vis spectrophotometer (Genesys 10S

UV-VIS-Thermo Fisher Scientific and UV 2700, USA). Concentrations of chlorophyll *a*, phycocyanin, and total carotenoids were calculated as described [10, 11] and expressed in *μg*/*mL*.

The morphology and number of cells of *S. platensis* were determined using a Motic Binocular M10LB-S light microscope with digital camera in a Nageotte chamber (0.01 *mL*). The appearance of the cells, the presence of pigments, the structure and length of the filaments were evaluated, which were conditionally divided into: short – up to 20 cells, medium – up to 60 cells, and long – more than 60 cells.

Results and Discussion. In this work, *S. platensis* IBCE S-2 was cultivated under photoautotrophic and photoheterotrophic conditions. Photoheterotrophic conditions were created by addition organic carbon source (acetate, succinate, malate, lactate, glucose, fructose, lactose) in Zarrouk medium. *S. platensis* grown under photoautotrophic condition was used as a control.

All carbon sources, except lactose, stimulated cyanobacterial growth rate (Fig. 2). The highest growth rate of *S. platensis* was observed after 4 day cultivation in the presence of glucose, fructose, and succinate. The growth rate of *S. platensis* grown under photoautotrophic condition was ~0.46 d^{-1}, whereas cultivation of cyanobacteria in glucose, fructose and succinate containing media led to an increase in the specific growth rate by ~2 fold (Fig. 2).

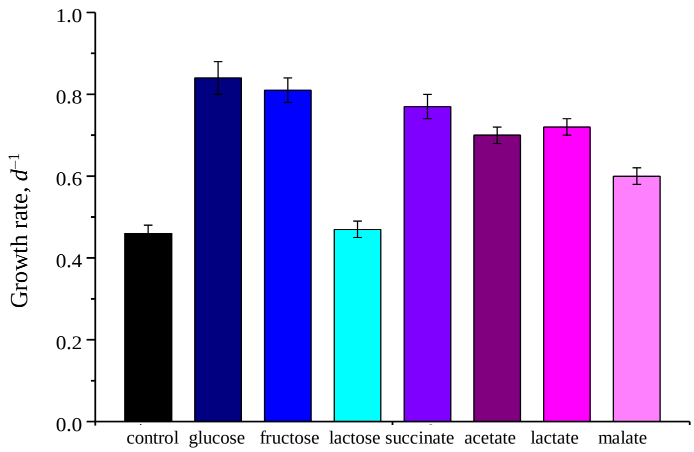

Fig. 2. Growth rates of *S. platensis* grown in the presence of organic carbon sources. The control was *S. platensis* cultivated under photoautotrophic condition.

Fig. 3 represents absorption spectra of *S. platensis* cultures cultivated under photoautotrophic and photoheterotrophic conditions. The absorption peaks in the wavelength range from 400 *nm* to 800 *nm* correspond to carotenoids with absorbance at ~400–500 *nm*, chlorophyll *a* with absorbance at ~440 *nm* and ~680 *nm*, and phycocyanin with absorbance at ~620 *nm* [12]. These spectra indicate that the photoheterotrophic cultivation with glucose, fructose and succinate as a carbon source leads to increase the content of photosynthetic pigments in *S. platensis*.

Carbon sources used stimulated the synthesis of photosynthetic pigments in *S. platensis*. The photosynthetic pigments of cyanobacteria include only one type of chlorophyll – chlorophyll *a*, as well as carotenoids and main phycobiliprotein – phycocyanin [12].

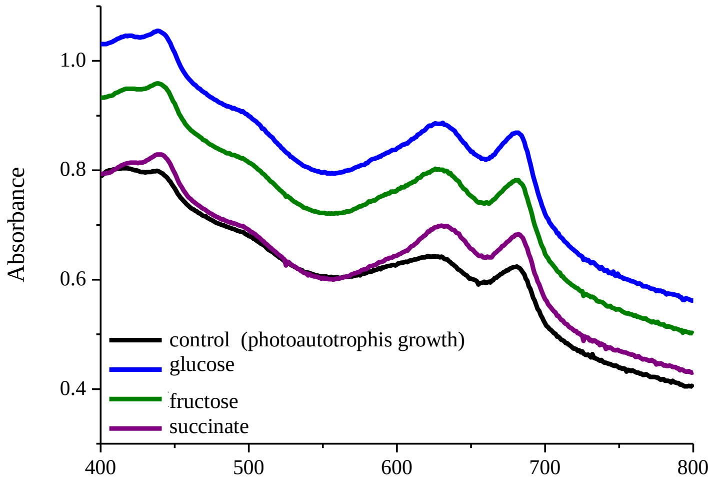

Fig. 3. The absorption spectra of *S. platensis* cultures cultivated under photoautotrophic (control) and photoheterotrophic (with organic carbon sources) conditions.

The changes in the photosynthetic pigments content in cyanobacteria are shown in Fig. 4. The highest content of chlorophyll *a* (Fig. 4, a) and phycocyanin (Fig. 4, b) were determined in *S. platensis* cultivated in glucose and fructose containing media. In the presence of glucose and fructose the content of chlorophyll *a* and phycocyanin increased by ~1.4 and ~1.2 fold, respectively, compared to the control. The carotenoids contents were ~1.3 and ~1.2 fold more in *S. platensis* cultivated in glucose and fructose containing media, respectively, in comparison with control (Fig. 4, c). Other carbon sources did not show any significant effect on the photosynthetic pigments content. In our previous study have been found stimulatory effect of carbon sources on growth rate and photosynthetic pigments amount in green alga *Parachlorella kessleri* PA-002 isolated in Armenia [5]. The highest amounts of chlorophyll *a* and total carotenoids were observed during cultivation of *P. kessleri* in the presence of acetate and fructose, respectively; whereas chlorophyll *b* content was not changed significantly [5].

Carbon sources used also caused morphological changes in *S. platensis* trichomes (Fig. 5, a). The presence of long filaments (more than 60 cells) and intense pigmentation, as well as the visual density of cyanobacteria is considered favorable for the development of the species. In the presence of malate and lactose, the filaments of *S. platensis* were short with rounded small cells at the ends; 20% of cells in trichomes were colorless and don’t contain pigments (Fig. 5, b). On the contrary, in glucose and fructose containing media, many long filaments with full, large and pigmented cells were observed (Fig. 5 c, d). In the presence of acetate and lactate, filaments of medium size (10–15 cells) with expanded cells prevailed (not shown). In the presence of succinate, many long developed trichomes with whole cells having a dark green color were observed (Fig. 5, e). Changes in *S. platensis* trichomes can be caused by various environmental factors, such as the amount of oxygen, nutrient availability, ultraviolet irradiation, and light conditions [2, 6].

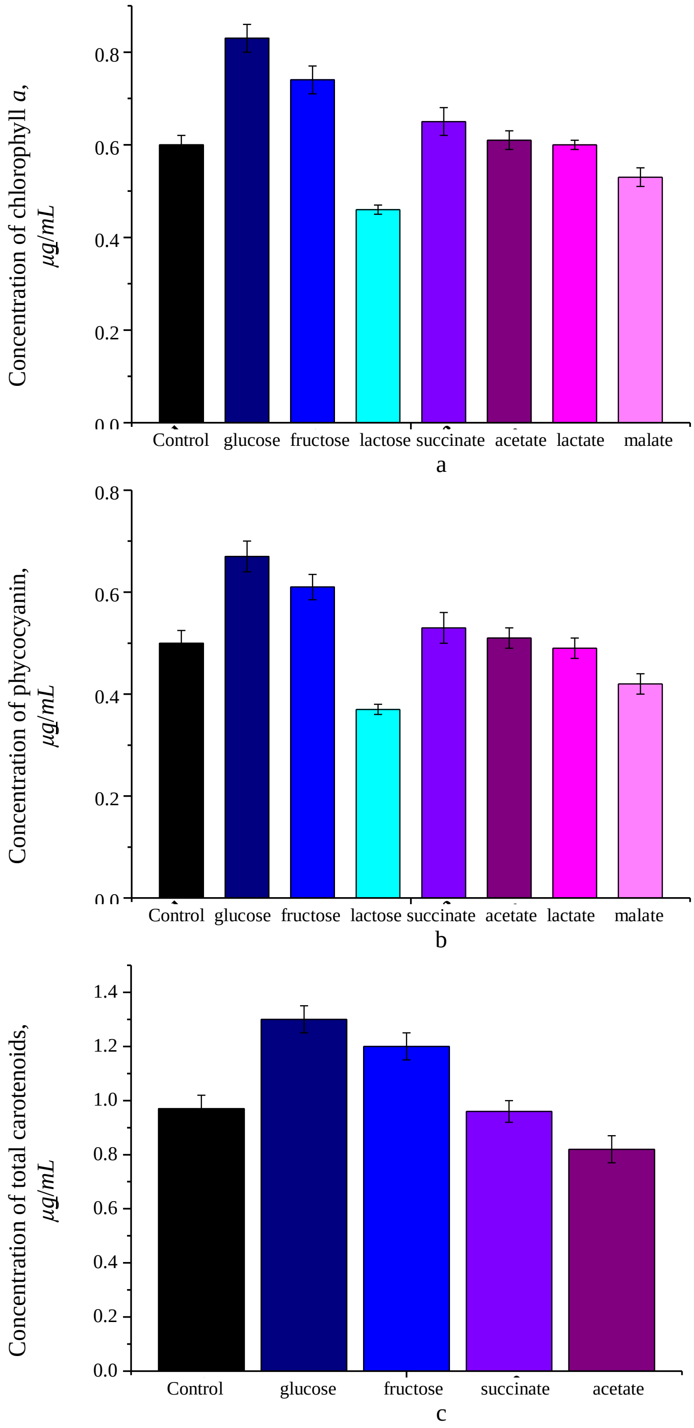

Fig. 4. Effects of organic carbon sources on chlorophyll *a* (a), phycocyanin (b), and total carotenoids (c) content. The control is *S. platensis* cultivated under photoautotrophic condition.

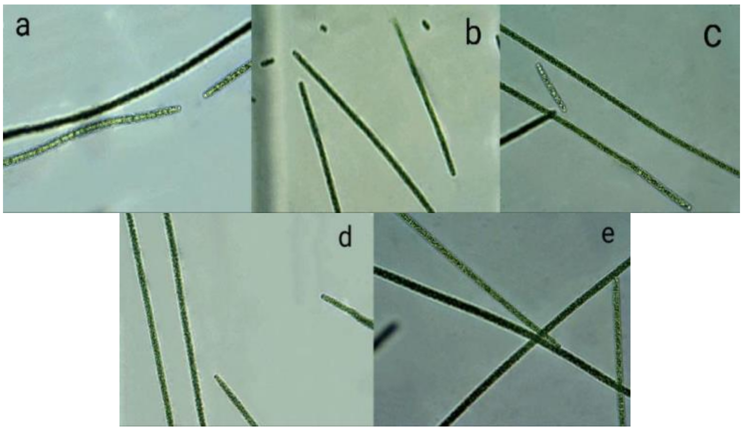

Fig. 5. Morphology of *S. platensis* cultivated (a) under photoautotrophic conditions, and in (b) malate, (c) glucose, (d) fructose, and (e) succinate containing media.

Conclusion. The presented data demonstrate that the various ogranic carbon sources have different effects on growth properties and morphology of *S. platensis*. During cultivation in glucose, fructose and succinate containing media, the long trichomes of *S. platensis* with large and well pigmented cells were detected. The results obtained indicate that glucose, fructose, and succinate are the most preferable carbon sources for the photoheterotrophic cultivation of *S. platensis*, and can be used to increase the growth rate and yield of biomass of cyanobacteria, as well as the production of photosynthetic pigments.

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Ա. Ա. ՀԱՐՈՒԹՅՈՒՆՅԱՆ, Զ. Գ. ՄԱՆՈՅԱՆ, Լ. Ռ. ՀԱՄԲԱՐՅԱՆ, Լ. Ս. ԳԱԲՐԻԵԼՅԱՆ

ԱԾԽԱԾՆԻ ՏԱՐԲԵՐ ԱՂԲՅՈՒՐՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ *SPIRULINA PLATENSIS*-Ի ԱՃՄԱՆ ՉԱՓԱՆԻՇՆԵՐԻ ԵՎ ՁԵՎԱԲԱՆՈՒԹՅԱՆ ՎՐԱ

Ցիանոբակտերիաները կենսազանգված են առաջացնում ֆոտոավտոտրոֆ պայմաններում ֆոտոսինթեզի ընթացքում: Ֆոտոհետերոտրոֆ պայմաններում ցիանոբակտերիաների աճեցումը ածխածնի օրգանական տարբեր աղբյուրների կիրառմամբ կարող է խթանել կենսազանգվածի ելքը: Տվյալ աշխատանքում ուսումնասիրվել է ածխածնի օրգանական աղբյուրների ազդեցությունը ցիանոբակտերիա *Spirulina platensis*-ի աճման չափանիշների և ձևաբանության, ինչպես նաև ֆոտոսինթեզային գունակների՝ քլորոֆիլի *a*-ի, կարոտինոիդների և ֆիկոցիանինի, արտադրության վրա: Ածխածնի աղբյուրները, մասնավորապես գլյուկոզը, ֆրուկտոզը և սուկցինատը, ազդել են ոչ միայն ցիանոբակտերիայի աճման արագության վրա՝ խթանելով այն, այլ նաև առաջացրել են *S. platensis*-ի ձևաբանական փոփոխություններ: Գրանցվել է

նաև ֆոտոսինթեզային գունակների քանակի ավելացում: Այդպիսով, գլյուկոզը, ֆրուկտոզը և սուկցինատը արդյունավետ սուբստրատներ են տվյալ ցիանոբակտերիաների ֆոտոհետերոտրոֆ աճեցման համար:

А. А. АРУТЮНЯН, Дж. Г. МАНОЯН, Л. Р. ГАМБАРЯН, Л. С. ГАБРИЕЛЯН

ВЛИЯНИЕ РАЗЛИЧНЫХ ИСТОЧНИКОВ УГЛЕРОДА НА ПАРАМЕТРЫ РОСТА И МОРФОЛОГИЮ *SPIRULINA PLATENSIS*

Цианобактерии генерируют биомассу в процессе фотосинтеза в фотоавтотрофных условиях. Культивирование цианобактерий в фотогетеротрофных условиях с использованием различных органических источников углерода может увеличить выход биомассы. В данной работе исследовано влияние органических источников углерода на параметры роста и морфологию цианобактерий *Spirulina platensis*, а также на содержание фотосинтетических пигментов: хлорофилла *a*, каротиноидов и фикоцианина. Источники углерода, в частности глюкоза, фруктоза и сукцинат, вызывали не только увеличение скорости роста цианобактерий, но и морфологические изменения трихом *S. platensis*. Также наблюдалось увеличение количества фотосинтетических пигментов. Таким образом, глюкоза, фруктоза и сукцинат являются наиболее эффективными субстратами для фотогетеротрофного культивирования данных цианобактерий.