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THE INFLUENCE OF EXOGENOUS GLYCINE ON GROWTH AND CYANOBACTERIA *Spirulina platensis* (Gom.) Geitl PHOTOSYNTHETIC PROCESSES

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The aim of the work was to study the impact of glycine on *Spirulina* growth and photosynthetic processes intensity. For research the culture of *Spirulina platensis* (Gom.) Geitl cyanobacterium strain LGU — 603 from collection of Kholodny Institute of Botany of the National Academy of Sciences of Ukraine was used. The cultivation process was carried out in Zaruk nutrient medium in a vertical tubular apparatus of 8 cm in diameter and of volume 2 dm^3 in a constant mixing of the culture medium with air.

Introduction of glycine in a cultural environment resulted in the increase of the *Spirulina* productivity, maintenance of basic photosynthetic pigments and protein. Glycine introduction allowed to increase the growth rate of spirulina and to content achieve the high density of the culture due to the increased length of log-phase. Intensification of *Spirulina platensis* growth and its productivity increase was due to the fragmentation of cyanobacteria trichomes, so the increase of trichomes number with a small number of cells, which grow quickly. The degree of fragmentation depended on the concentration of introduced glycine and on the culture development stadium and also on introduced glycine concentration.

Key words: glycine, spirulina, growth and intensity, photosynthetic activity.

In view of the deterioration of the ecological situation in Ukraine and the decrease in the quality of diets, the foreground problem is the search for new sources of food of natural origin that could provide the human body with a full set of essential amino acids, polyunsaturated fatty acids, vitamins, trace elements and other biologically active substances. It is possible to improve the value of food by using nutritional supplements based on natural ingredients.

The most promising object in this problem solving is cyanobacteria *Spirulina platensis* (hereinafter spirulina), which has many advantages and is therefore considered as the most promising biotechnological object [1, 2]. A number of special substances synthesized by spirulina — bioprotectors, biocorrectors and biostimulants — are not found in any more product of natural origin. This results in the special properties of spirulina as a food product and a broad-spectrum therapeutic and prophylactic agent [3–6].

One of the properties of cyanobacteria *Spirulina platensis,* when the nutritional and lighting conditions are changing, is the ability to switch to photoheterotrophic mode of nutrition. It is able to grow in the presence of some mono- and disaccharides, organic acids, amino acids and some other organic substances, using them as an additional source of carbon and nitrogen [7–10].

The use of various organic sources of carbon or nitrogen leads to a change in metabolic processes, which in turn leads to a change in both the biochemical composition of cyanobacteria and the intensity of its growth.

Since amino acids can act at the same time as a source of carbon and nitrogen, the study

of amino acids effect and mechanism of their action on productivity and photosynthetic activity of spirulina are relevant, this will allow regulating the biosynthetic processes of spirulina and intensifying its growth rate.

The criteria for determining the photosynthetic activity of spirulina and the intensity of its growth are the biomass productivity, the content of the main photosynthetic pigments, namely phycocyanin, chlorophyll, and the main component of biomass — protein. In addition, the content of protein and pigments can be used to a certain extent as a criteria of the biological value of biomass in general, since the biosynthesis of tetrapyrrole pigments, carotenoids and amino acids bound in the central metabolic pathways of the conversion of mevalonic and δ-aminolevulinic acids to the biosynthesis of tocopherols, cyancobalamin, phylloquinone and some other biologically active substances [11].

Since spirulina is a rich source of biologically active substances, the search for new stimulants to increase its productivity and improve the quality of biomass is relevant.

The purpose of the work was to study the influence of glycine, as an exogenously introduced organic source of carbon and nitrogen, on the growth and intensity of the photosynthetic processes of spirulina.

Materials and Methods

For research, the culture of cyanobacteria *Spirulina platensis* (Gom.) Geitl. strain LGU-603 was used, which is taken from the collection of cultures of Kholodny Institute of Botany of the National Academy of Sciences of Ukraine.

The cultivation process was carried out in Zaruk nutrient medium in a vertical tubular apparatus of 8 cm in diameter and of volume 2 dm^3 in a constant mixing of the culture medium with air.

The illumination of the culture on the surface of the apparatus was maintained at the level of 8 Klux, the duration of the photoperiod was 12 hours a day. Mercury lamps DRL-400 were used as a source of illumination. The temperature of the culture medium was maintained in the range of 30–32 °C. The initial density of the culture was approximately 0.15 g of absolutely dry biomass (ADB)/dm^3, which corresponds to an optical density of 0.45–0.47 at a wavelength of 750 nm. The evaporation of the liquid from the cultivator was compensated every day by the addition of distilled water. Distilled water was added before sampling.

The administration of various concentrations of glycine (50, 100, 150, 200, 250 and 300 mg/dm^3) was carried out in two, four, seven and ten days from the beginning of cultivation. Taking into account the stability of cultivation conditions by illumination, photoperiod, temperature and mixing intensity of suspension with air, the introduction of glycine occurred at a certain density of culture (Table).

Cultivation of spirulina was carried out in a cumulative mode for 14 days. Spirulina, which was cultured in Zaruk medium without the addition of glycine, was used as a control. The results presented in tables and graphs are the arithmetic mean of three repeated experiments.

Each day the photomicroscopy of the culture was carried out for the purpose of detecting the reaction of spirulina to the administration of glycine.

The growth of biomass was determined by photometric method by changing the optical density of the suspension at a wavelength of 750 nm. The conversion of optical density units into dry biomass value was carried out according to the gauge curve. Sampling for determining the density of the culture was carried out every day at the time of turning on the light, after the dark phase of cultivation.

During the study, the ADB and moisture of the product by weight method [12], the mass fraction of protein in spirulina

Conditions for glycine introduction

The growth phase	Time of glycine introduction, days from the beginning of cultivation	Optical density of culture medium at the time of glycine introduction, $\lambda = 750$ nm	Density of culture at the time of glycine introduction, g of ADB/dm^3
Exponential growth phase	2	1.1	0.4
Linear growth phase	4	2.2	0.7
Growth declining phase	7	3.4	1.0
Stationary growth phase	10	3.7	1.2

biomass — by biuret test [13], the phycocyanin and chlorophyll content — using spectrophotometric methods were determined [14, 15].

Statistical processing of experimental data was performed using Microsoft Excel 2010. All experiments were performed in triple repetition. Results that do not have a statistically significant difference with the control (0% of glycine, $P > 0.05$) in the figures are indicated by ≠.

Results and Discussion

According to the results of studies of the effect of glycine various concentrations, introduced at different growth stages, on spirulina biomass accumulation (Fig. 1), it was determined that an increase in glycine concentration to a certain value leads to an intensification of culture growth. In all cases, this concentration depended on the culture density when glycine was added.

The most intense growth of spirulina occurs when glycine was introduced 7 days after the beginning of cultivation, i.e. at a culture density of 1.0 g of ADB/dm^3· At the same time, the highest accumulation of spirulina biomass was achieved — 2,11 g of ADB/dm^3, when glycine was introduced at a concentration of 150 mg/dm^3, which is by 53% more than in the control.

As can be seen from Fig. 1, the higher the density of the culture, the greater the concentration of glycine should be introduced in order to obtain the highest biomass productivity of culture. It should be noted that high concentrations of glycine, introduced in the early phases of spirulina growth, lead to a decrease in the productivity of the culture.

The results of photomicroscopy showed that after glycine introduction there is an intense fragmentation of cyanobacteria trichome, a certain number of individual fragments of trichome named hormogonia, consisting of a small number of cells, appear.

Previously, we have shown [16, 17] that the greater glycine concentration was introduced into the culture medium, the more trichome with a small number of cells were formed, and, accordingly, more intensive fragmentation occurred. The intense trichome fragmentation resulted in the intensification of the reproduction process of the culture and thus in increase of its growth rate and productivity.

After glycine introduction, spirulina growth is inhibited for a while, it moves from a more intense phase of growth to a less intense (lag phase), whose duration depends on both the concentration of the added glycine and the growth phase on which the glycine was introduced.

After some time after glycine introduction, the intensity of spirulina growth gradually

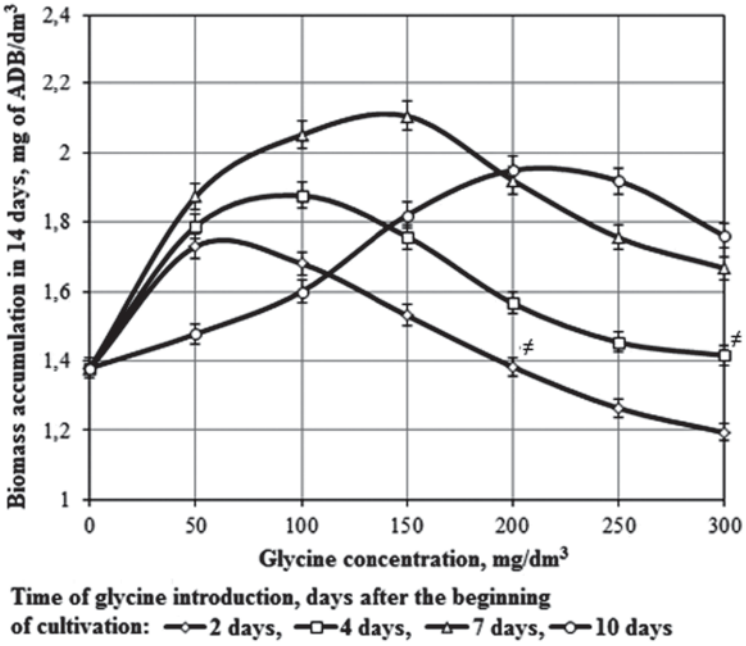

Fig. 1. **Glycine influence on spirulina biomass accumulation depending on the time of its introduction:**
here and after **r**esults that do not have a statistically significant difference with the control (0% of glycine, $P > 0.05$) in the figures are indicated by ≠. Between nearby points there are insignificant distinctions that statistically not reliable

increased, spirulina continued to develop in accordance with the following phases of typical development.

Investigating the effect of glycine on the intensity of phycocyanin accumulation in spirulina biomass, we have found (Fig. 2) that glycine introduction into the culture medium make it possible to intensify significantly the phycocyanin biosynthesis.

When glycine was introduced 4 days after the beginning of cultivation (in the phase of linear growth at culture density of 0.7 g of ADB/dm^3), the most intense phycocyanin accumulation occurred with an increase in the concentration of glycine up to 200 mg/dm^3 and reached a value of 19% of the ADB, which was by 56% more than in control.

As can be seen from Fig. 2, the introduction of glycine into the culture medium 7 days after the beginning of cultivation (in declining growth phase at a culture density of 1.0 g of ADB/dm^3) contributed to the most intense accumulation of phycocyanin in the biomass of spirulina. Thus, the introduction of glycine at a concentration of 200 mg/dm^3 allowed us to increase the content of phycocyanin in biomass by 77% compared with the control — up to 21.8% of the ADB.

The introduction of glycine 10 days after the beginning of cultivation (in the stationary phase of growth at culture density of 1.2 g of ADB/dm^3) in all investigated concentrations (from 50 to 300 mg/dm^3) also resulted in the intense phycocyanin accumulation in spirulina biomass.

Thus, with an increase in glycine concentration in the culture medium, an increase in the content of phycocyanin in the spirulina biomass occurs irrespective of the growth phase at which the glycine is introduced. The greatest accumulation of phycocyanin in spirulina biomass (up to 22.5% of ADB) occurs when glycine is introduced in the culture medium in concentrations of 150–300 mg/dm^3 in declining growth phase (when the culture density is approximately 1.0 g of ADB/dm^3). This allows us to increase the content of phycocyanin in spirulina biomass by 77–83%, compared with the content of phycocyanin in biomass obtained without the addition of glycine.

Investigating the effect of glycine on the intensity of protein biosynthesis, we have found (Fig. 3) that the addition of 100–150 mg/dm^3 of glycine 4 days after the beginning of cultivation (in the phase of linear growth at culture density of approximately 0.7 g of ADB/dm^3) led to an increase of protein content in spirulina biomass up to 66–67% of ADB (which exceeded the control by 29–31%).

The introduction of glycine 7 days after the beginning of cultivation (in declining growth phase at culture density of approximately 1.0 g of ADB/dm^3) at a concentration of up to 150 mg/dm^3 resulted in the largest accumulation of protein in spirulina biomass — almost up to 72% of ADB, which is by 41% more than in control.

Thus, the highest protein content in spirulina biomass (up to 72% of ADB) can

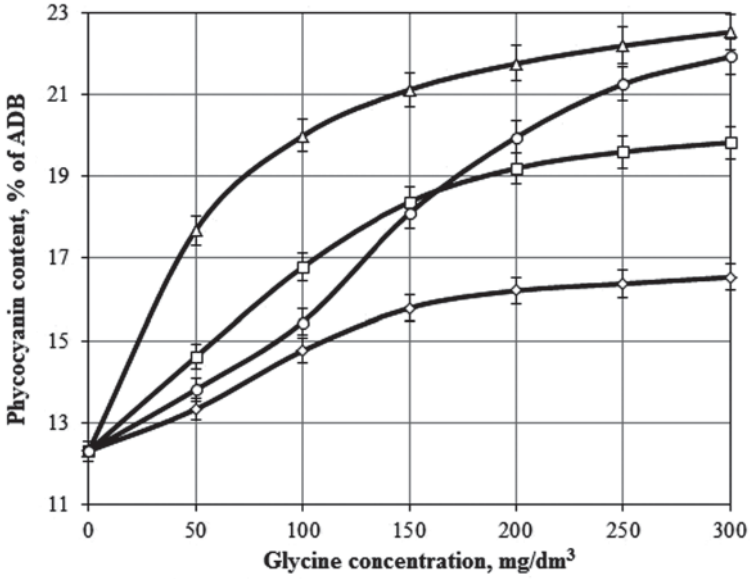

Fig. 2. **Effect of glycine various concentrations on phycocyanin content**

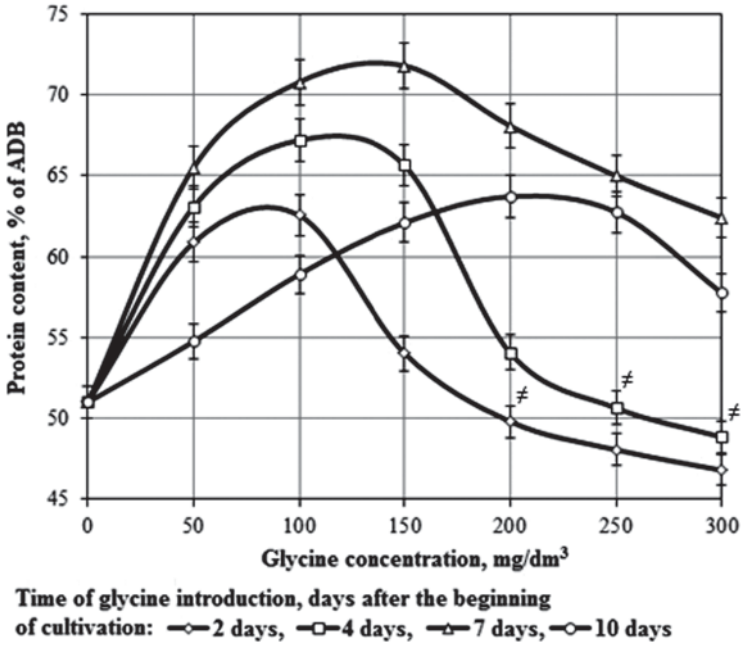

Fig. 3. **Effect of glycine various concentrations on protein content**

be obtained by glycine introduction into the culture medium in concentrations of 100–150 mg/dm^3 in declining growth phase (at culture density of about 1.0 g of ADB/dm^3). In this case, the protein content is increased by about 40%, compared with its content in biomass, obtained without the addition of glycine.

The study of glycine effect on chlorophyll accumulation in spirulina biomass shows (Fig. 4) that the greatest effect on chlorophyll biosynthesis is caused by glycine, introduced 7 and 10 days after the beginning of cultivation, in all investigated concentrations.

The introduction of glycine 7 days after the beginning of cultivation (at a density of about 1.0 g of ADB/dm^3) contributed to the most intense chlorophyll biosynthesis compared to chlorophyll accumulation when glycine was added in other studied growth phases. Thus, when glycine was added in culture medium in a concentration of 50 mg/dm^3, the chlorophyll content in biomass increased by 10% compared with the control; when glycine was added in a concentration of 100 mg/dm^3 — by 16.5%; in a concentration of 150 mg/dm^3 — by 21,5%, and then practically was not changed.

When glycine was introduced in all investigated concentrations10 days after the beginning of cultivation (in the stationary phase of growth at culture density of approximately 1.2 g of ADB/dm^3), an increase in chlorophyll content in spirulina biomass occurred.

Statistically significant increase in chlorophyll content in spirulina biomass occurred when glycine was introduced in culture medium in concentrations greater than 100 mg/dm^3. At concentrations of glycine added of 200 mg/dm^3 or more, the most intense chlorophyll content was observed and its content in biomass was higher compared with the content in biomass obtained when glycine was introduced at the same concentrations but 2 and 4 days after the beginning of cultivation. The largest chlorophyll accumulation was at a concentration of glycine of 300 mg/dm^3, which is by 25% more than in the control and was approximately 2.08% of ADB.

Thus, with an increase in glycine concentration in the culture medium, an increase in chlorophyll content is most intense when glycine is introduced in growth declining phase and stationary phase, i. e., at a culture density of approximately 1.0–1.2 g of ADB/dm^3. The greatest chlorophyll accumulation in spirulina biomass (up to 2.13% of ADB) can be achieved by glycine introduction in the culture medium at a concentration of 250–300 mg/dm^3 in the growth declining phase (at culture density of about 1.0 g of ADB/dm^3). In this case, the chlorophyll content increases by about 27–28%, as compared to the chlorophyll content in biomass obtained without the addition of glycine.

Thus, the studies have shown that the exogenous glycine introduction in the culture medium leads to an intensification

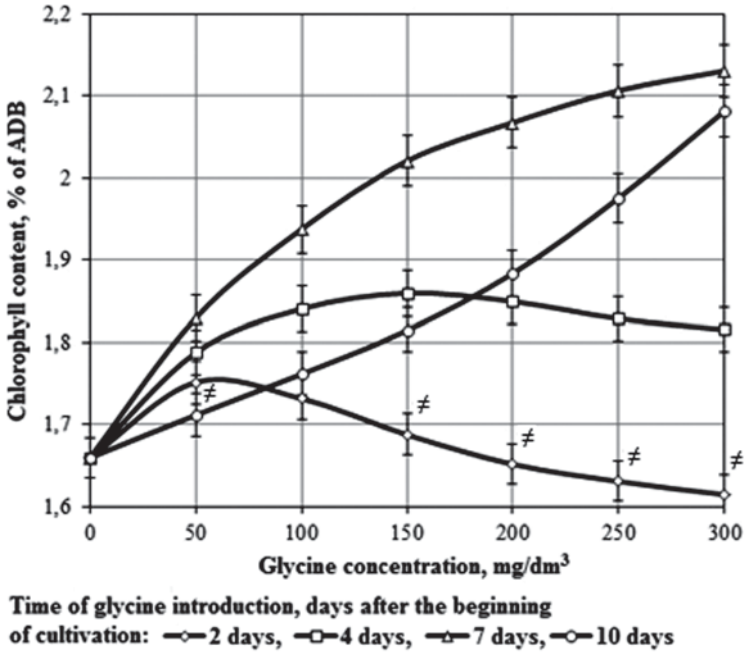

Fig. 4. **Effect of glycine various concentrations on chlorophyll content**

of cyanobacteria growth rate. By varying the concentration of glycine, and its application at different stages of growth the increase in content of protein and pigments (phycocyanin and chlorophyll), which will greatly increase the biological value of biomass in general, may be achieved. This makes it possible to set up the production on an industrial scale of such an important pigment for medicine as phycocyanin.

In our opinion, the most effective way to increase the biological value of spirulina biomass is the introduction of glycine at concentration of 150 mg/dm^3 into the culture medium 7 days after the beginning of cultivation, that is, at the stage of growth declining at a culture density of 1.0 g of ADB/dm^3. Under these conditions, spirulina culture is characterized by the highest biomass and important biologically active compounds productivity.

Comparing the results obtained with the action of the coordination compound Zn (II) containing glycine and serine [8] at a concentration of 5 mg/dm^3, which increases the spirulina biomass productivity by 26%, the content of phycobiliproteins — by 32%, the content of protein — by 17%, the introduction of glycine in concentrations of 150 mg/dm^3 or more in the growth declining phase (with culture density of about 1.0 g of ADB/dm^3) allows us to increase the yield of biomass by 50%, the content of phycocyanin — by 80%, the content of protein — by 40%.

This effect of glycine may be explained by the fact that it is the precursor of protoporphyrin [18, 19], from which chlorophyll and phycocyanin are formed, thereby, possibly, glycine increases the photosynthetic activity of spirulina.

In addition, glycine is the simplest aliphatic amino acid, acts as a complexing agent (chelate) with trace elements, which may contribute to more efficient use by spirulina cells. Since glycine is a component of structural proteins that are the part of cell walls, so it is possible that it is embedded in spirulina tissue together with the trace elements.

Thus, it has been determined that the exogenous introduction of glycine into the culture medium leads to the intensification of growth and photosynthetic processes of spirulina, which is expressed in increase of spirulina productivity, the content of main photosynthetic pigments, namely, phycocyanin, chlorophyll, and the protein as the main component of biomass.

It has been established that exogenously introduced glycine leads to fragmentation of microalgae trichome, the intensity degree of which depends on both the concentration of introduced glycine and the stage of culture development. Trichome fragmentation leads to the intensification of spirulina growth and, correspondingly, to the increase in its productivity, because of an increase in the number of trichome with a small number of

rapidly growing cells. The introduction of glycine permits to increase the growth rate of spirulina and achieve a high density of culture due to an increase in the duration of the log-phase.

It has been found that the greatest spirulina biomass productivity (up to 2.1 g of ADB/dm^3) and protein productivity (up to 72% of ADB) can be achieved as a result of glycine introduction into the medium at concentrations of 100–150 mg/dm^3 in the growth declining phase (at culture density of approximately 1.0 g of ADB/dm^3). At the same time, the productivity of culture compared with the productivity at cultivation without glycine increases by an average of 50%, and the protein content — by 40%.

It has been determined that the most intense phycocyanin accumulation (up to 22.5% of ADB) and chlorophyll (up to 2.13% of ADB) in spirulina biomass can be achieved by glycine introduction into the culture medium at concentrations of 150 mg/dm^3 or more in growth declining phase (at culture density of about 1.0 g of ADB/dm^3). This allows us to increase the content of phycocyanin in spirulina biomass by 80–83%, and chlorophyll content — by 27% compared to the content of these pigments in biomass obtained during cultivation without glycine.

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ВПЛИВ ЕКЗОГЕННОГО ГЛІЦИНУ НА РІСТ І ФОТОСИНТЕТИЧНІ ПРОЦЕСИ ЦІАНОБАКТЕРІЇ *Spirulina platensis* (Gom.) Geitl

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Метою роботи було вивчити вплив екзогенно внесеного гліцину на ріст та інтенсивність фотосинтетичних процесів спіруліни. Для дослідження використовували культуру ціанобактерії *Spirulina platensis* (Gom.) Geitl. штам LGU-603 з колекції Інституту ботаніки ім. М. Г. Холодного НАНУ. Процес культивування проводили на живильному середовищі Zaruk у вертикальному тубулярному апараті діаметром 8 см і об'ємом 2 дм^3 за контактного змішування культурального середовища з повітрям. Внесення гліцину в культуральне середовище сприяло збільшенню продуктивності спіруліни, вмісту основних фотосинтетичних пігментів та протеїну, а також давало змогу підвищити швидкість росту спіруліни і досягати високої густини культури внаслідок збільшення тривалості log-фази.

Інтенсифікація росту *Spirulina platensis* та зростання її продуктивності відбувалося внаслідок фрагментації трихом ціанобактерії, тобто збільшення кількості трихом з невеликою кількістю клітин, які швидко ростуть. Ступінь фрагментації залежала від концентрації внесеного гліцину і стадії розвитку культури.

Ключові слова: гліцин, спіруліна, ріст та інтенсивність, фотосинтетична активність.

ВЛИЯНИЕ ЭКЗОГЕННОГО ГЛИЦИНА НА РОСТ И ФОТОСИНТЕТИЧЕСКИЕ ПРОЦЕССЫ ЦИАНОБАКТЕРИИ *Spirulina platensis* (Gom.) Geitl

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Целью работы было изучить влияние экзогенно внесенного глицина на рост и интенсивность фотосинтетических процессов спирулины. Для исследования использовали культуру цианобактерии *Spirulina platensis* (Gom.) Geitl. штамм LGU-603 из коллекции Института ботаники им. Н. Г. Холодного НАНУ. Процесс культивирования проводили на питательной среде Zaruk в вертикальном тубулярном аппарате диаметром 8 см и объемом 2 дм^3 при контактном смешивании культуральной среды с воздухом. Внесение глицина в культуральную среду способствовало увеличению продуктивности спирулины, содержания основных фотосинтетических пигментов и протеина, а также позволяло повышать скорость роста спирулины и достигать высокой плотности культуры вследствие увеличения продолжительности log-фазы.

Интенсификация роста *Spirulina platensis* и повышение ее продуктивности происходило вследствие фрагментации трихом цианобактерии, т. е. увеличения количества трихом с небольшим количеством быстрорастущих клеток. Степень фрагментации зависела от концентрации внесенного глицина и стадии развития культуры.

Ключевые слова: глицин, спирулина, рост и интенсивность, фотосинтетическая активность.