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## ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК  
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## STUDY OF PROMISING HETEROCYSTIC CYANOBACTERIAL STRAINS FOR BIOHYDROGEN PRODUCTION

**Abstract.** Nitrogen-fixing 3 strains of cyanobacteria as *Nostoc caldicola* RI-3, *Anabaena variabilis* R-I-5 and *Anabaena* sp. Z-1 were taken from collection for hydrogen researches. In order to select strains of cyanobacteria characterized with high hydrogen-producing activity, hydrogen evolution was studied by the collection three strains in the dark and under light conditions. According to the obtained results, from 3 collection strains of cyanobacteria, *Nostoc caldicola* RI-3 and *Anabaena* sp. Z-1 showed relatively high hydrogen-producing activity in the dark. It was established that the highest hydrogen productivity was in the strain *Nostoc caldicola* RI-3 from all. The maximum hydrogen accumulation in this culture was observed after 72 h of incubation which in this case amounted to 0,032  $\mu\text{mol H}_2$  mg/chl/h. While in the light, there was an active accumulation of hydrogen by the cells of the strain *Anabaena variabilis* R-I-5, the nitrogenase activity of which was also significantly higher in the light than in other strains. The highest rate of hydrogen accumulation was 0,012  $\mu\text{mol H}_2$ /mg chl/h.

**Key words:** biohydrogen, heterocystic cyanobacterial strains, gas chromatograph.

**Introduction.** The World energy problems and environmental changes are two main trends that are forcing humankind to seek new sources of energy. Microorganisms played a main role in shaping the fuel that we currently use: coal, oil, and gas. Millions of years ago, these types of fuels were cellular organic material formed as a result of biochemical reactions. Currently, in connection with the development of biotechnology, many living organisms can also be considered as raw materials for obtaining new, cheaper and at the same time environmentally friendly energy sources – various types of biofuels [1].

One alternative, as an environmentally friendly fuel, is the production of biohydrogen. Hydrogen is an environmentally friendly energy carrier for the upcoming energy industry. Based on the energy sources and electron donors used by microorganisms, the microbiological processes for producing hydrogen can be divided into dark anaerobic hydrogen evolution, light-dependent hydrogen evolution without oxygen evolution, and light-dependent hydrogen and oxygen evolution, which is called biophotolysis [2,3].

Compared to green microalgae, cyanobacteria attract more attention from researchers for hydrogen production. Among these species, special attention is paid to filament cyanobacteria, which use the enzyme nitrogenase to produce hydrogen under conditions of nitrogen deficiency. At the same time, hydrogen is produced as a by-product of nitrogen fixation and its conversion into urea, in addition, nitrogenase uses ATP as a substrate for this reaction. In cyanobacteria that use this enzyme, nitrogenase is located in heterocysts and thus it is protected from the inhibitory effect of oxygen, it has a very thick, weakly oxygen-permeable membrane, moreover, heterocysts have active respiration (absorption). These advantages of heterocystous cyanobacteria make them the only organisms capable of releasing hydrogen in the presence of molecular oxygen in the air [4].

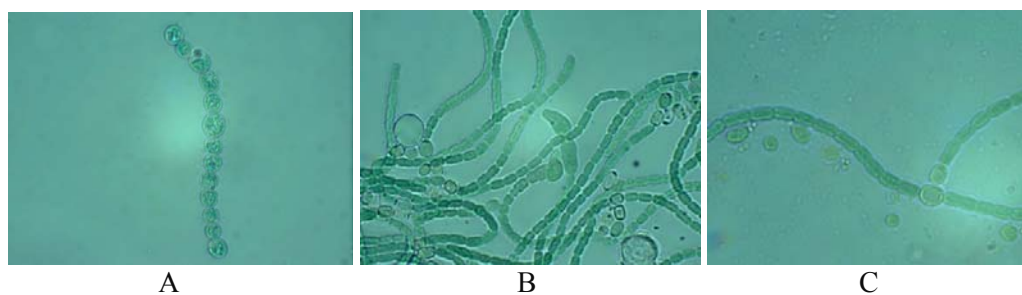
However, it should be noted that despite the literature data indicating the possibility of hydrogen production by cyanobacteria cultures with relatively high efficiency and in bright light, there are still problems limiting the use of cyanobacteria for converting solar energy. In addition to the sensitivity of this process to oxygen and the simultaneous release of oxygen and hydrogen, this is primarily due to the low efficiency and speed of the process. In this regard, scientific research aimed at increasing the rate of hydrogen evolution by photosynthetic microorganisms is currently very relevant. First, research in this area should be aimed at finding new, more productive strains of cyanobacteria that actively produce hydrogen and optimize the process of their cultivation in order to increase the efficiency of their conversion of the substrate into hydrogen [5].

In this regard, the aim of this work was to search for new strains of heterocystous cyanobacteria, active hydrogen producers.

The article presents the results of the 3 collection strains of cyanobacteria and their capabilities in the evolution of hydrogen in the dark and in the light are studied.

**Methods and materials.** The experiments were carried out with three strains of cyanobacteria from the collection of Biotechnology laboratory in KazNU (Almaty, Kazakhstan) – *Anabaeba* sp. Z-1 [10], *Anabaena variabilis* R-I-5 [11], *Nostoc caldicola* RI-3 [11]. The optical density of the cultures was recorded on a PD-303 UV spectrophotometer with a wavelength of 720 nm. The cultures were grown at 25°C under lighting with an intensity of 250  $\mu\text{mol}/\text{m}^2/\text{s}$  in 250 ml conical flasks. The growth dynamics of the cultures was determined spectrophotometrically on a PD-303 spectrophotometer at a wavelength of 750 nm, measurements were carried out every 24 hours [10]. To obtain biomass in order to determine the hydrogen-producing ability, the strains were cultured under artificial light (45  $\mu\text{mol}/\text{m}^2/\text{s}$ ) supplied from three sides of glass tubes containing 70 ml of BG-11 liquid nutrient medium [11]. Nitrogenase activity was determined by the level of acetylene-reducing activity of cyanobacteria by the acetylene method. The accumulation of  $\text{H}_2$  was measured using GC 3210 (GL Sciences, Japan). Methanol (100%) was used to measure chlorophyll concentrations, and the absorbance of the supernatant was measured spectrophotometrically at 665,2 and 750 nm. Determinations were carried out in five replicates. The figures show the arithmetic means of 3-5 biological replicates and their standard errors.

**Results and discussion.** The most studies focus on hydrogen producing by nitrogenase enzymes of cyanobacteria containing heterocysts. In order to find more productive strains of phototrophic microorganisms actively producing hydrogen, three strains of cyanobacteria from Biotechnology laboratory collection that with heterocysts were studied: *Nostoc caldicola* RI-3, *Anabaena variabilis* R-I-5 and *Anabaeba* sp. Z-1. The study of pure cultures of cyanobacteria characterized by high productivity and the determination of the hydrogen-producing activity of the isolated microalgae strains. Below are their microphotographs and a brief description obtained on the basis of a study of their cultural, morphological, physiological and biochemical properties (figure 1).



A – *Nostoc caldicola* RI-3; B – *Anabaena variabilis* R-I-5; C – *Anabaeba* sp. Z-1.

Figure 1 – Micrographs of isolated cyanobacteria (stole. 100x)

In order to determine the ability of the collection cyanobacteria strains to grow on a nitrogen-free medium, the cultures of cyanobacteria were selected for productivity on the media BG<sub>0</sub>-11 (experiment) and BG-11 (control). The productivity of biomass growth was determined by the coefficient of growth rate and yield of dry biomass of cyanobacteria. To do this, the studied strains were cultured on nutrient media for 9 days, the initial cell density for all the studied cultures was 0,5. After 9 days, the biomass of

cyanobacteria was separated from the culture fluid, dried, and the yield of dry biomass was determined. The results are shown in figure 2.

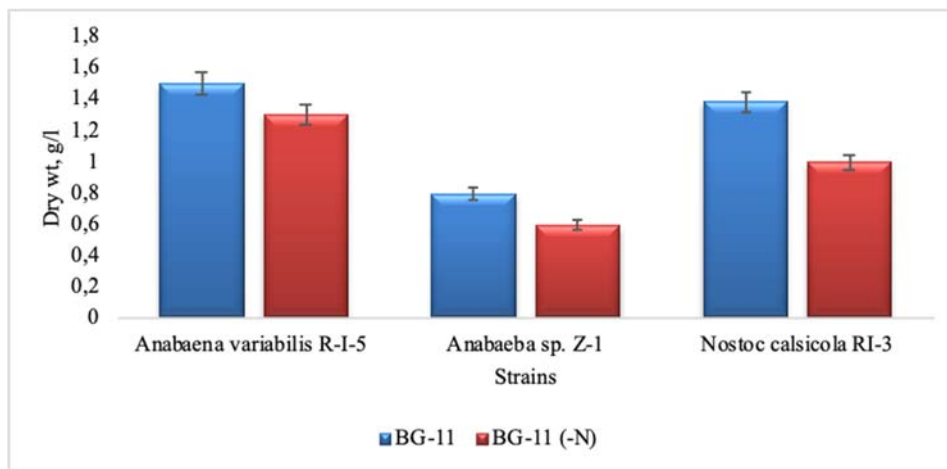


Figure 2 – The results of determining the productivity of the isolated microalgae strains

The obtained results indicate a slight difference in the productivity of the collection strains on a nitrogen-free medium, however, the strains *Anabaena variabilis* R-I-5 and *Nostoc caldicola* RI-3 showed high values. These results were confirmed by the results of determination of the nitrogenase activity of the studied strains by the acetylene method. Nitrogenase activity of three was detected in all 3 strains by GC after creating anaerobic conditions after culturing for 24 hours in the light. Comparison of nitrogenase activity, measured as the formation of ethylene, with specific  $H_2$ . According to the results, among the studied isolated strains of *Anabaeba* sp. Z-1 showed significantly low results for the production of ethylene (nitrogenase), while the strain *Anabaena variabilis* R-I-5 produces 3,57  $\mu\text{mol}$  ethylene/mg DW/h, for strain *Anabaeba* sp. Z-1 this value was 1,82  $\mu\text{mol}$  ethylene/mg DW/h. Thus, the strain *Anabaena variabilis* R-I-5 showed comparable high level of ethylene production (figure 3).

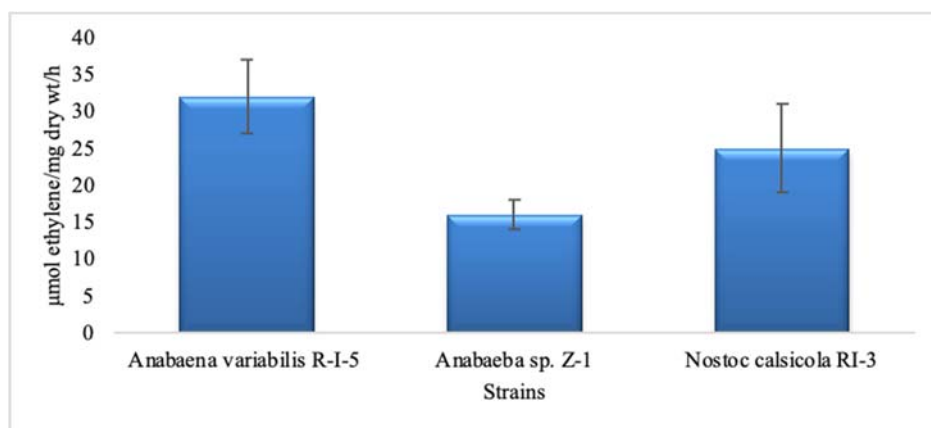


Figure 3 – Rate of acetylene reduction, which measures nitrogenase activity of the isolated strains

At the next stage, in order to select strains of cyanobacteria characterized by high hydrogen-producing activity, the evolution of hydrogen was studied by the isolated three new strains in the dark and under light conditions.

It is known that the use of cyanobacteria, which contain heterocysts and are used for hydrogen evolution with the help of nitrogenase and hydrogenase, as in the case of nitrogen-fixing cultures, is more promising. In them, the enzyme nitrogenase is localized mainly in specialized cells – heterocysts, which are formed under conditions of a lack of bound forms of nitrogen. In this case, oxygen is formed only in vegetative cells. And the presence of a thick membrane that weakly transmits oxygen, which provides

spatial protection of nitrogenase from its inhibitory effect, and besides, they are characterized by active breathing (with oxygen uptake) makes heterocystic cyanobacteria the only organisms that can release hydrogen in the presence of molecular oxygen in the air [11].

According to the results obtained, hydrogen evolution in the dark was observed in all studied cultures. The highest hydrogen productivity was in the strain *Nostoc caldicola* RI-3, whose cells began to produce hydrogen 24 hours after degassing in the dark. The hydrogen yield at this time was 0,005  $\mu\text{mol H}_2/\text{mg chl/h}$ . The maximum hydrogen accumulation in this culture was observed after 72 h of incubation, which at the same time amounted to 0,032  $\mu\text{mol H}_2/\text{mg chl/h}$ ; in the next hours of the experiment, a slow decrease in hydrogen evolution is observed.

The remaining strains had less hydrogen-producing activity in the dark compared to *Nostoc caldicola* RI-3. Moreover, a relatively higher level of  $\text{H}_2$  production was observed in the strain *Anabaena variabilis* R-I-5. These two strains were characterized by different values of the hydrogen yield, and differed from each other by the time of its maximum accumulation. Thus, the accumulation of  $\text{H}_2$  by *Anabaena variabilis* R-I-5 cells after 24 hours was 0,0008  $\mu\text{mol H}_2/\text{mg chl/h}$ , and after 120 hours, the maximum  $\text{H}_2$  production equal to 0,025  $\mu\text{mol H}_2/\text{mg chl/h}$ . Among the studied strains of cyanobacteria, a significantly low ability to liberate hydrogen in the dark was revealed in the strain *Anabaeba* sp. Z-1. A slight hydrogen evolution by this strain was noted after 24 hours of incubation, this indicator by this time amounted to 0,004  $\mu\text{mol H}_2/\text{mg chl/h}$ , after which further decrease in this indicator is observed, and after 72 hours the production of hydrogen is not observed at all (figure 4).

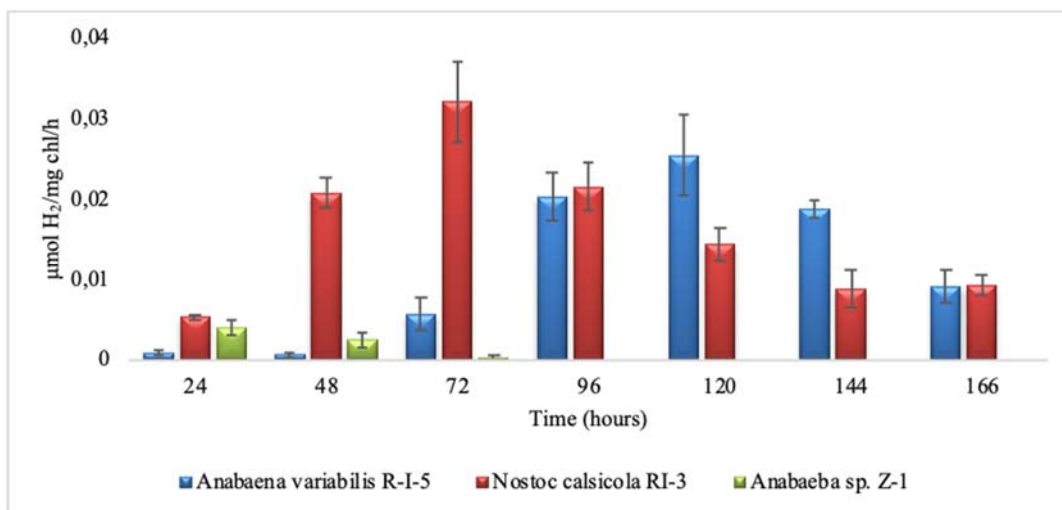


Figure 4 – Hydrogen evolution by the studied strains of cyanobacteria under anaerobic conditions in the dark

At the next stage of work, the accumulation of hydrogen by the studied strains of cyanobacteria was studied under lighting conditions. It is known that light energy is important for hydrogen evolution and acts as an electron donor for direct biophotolysis [2]. During photochemical reactions in the thylakoid membranes of cyanobacteria due to the energy of sunlight, under certain conditions, molecular hydrogen is released. Under normal conditions, microscopic cyanobacteria do not form hydrogen. The activity of PSI is not a prerequisite for the photodetection of hydrogen, although the electrons entering the thylakoid electron transport chain (ETC) during photodegradation of water can be accepted by hydrogenase. This leads to the fact that both oxygen and hydrogen are formed in cyanobacteria cells for a short time [12,13].

In this experiment, the studied cultures of cyanobacteria were cultivated similarly to the previous experiment, the conditions for cell incubation in the study of hydrogen productivity were the same and differed only in the presence of light. The initial optical density of the suspension for each culture was 1,5 at 730 nm. Hydrogen evolution by strains of cyanobacteria was observed during their incubation in an argon atmosphere for 190 h at an illumination of 30  $\mu\text{mol}/\text{m}^2/\text{s}$ .

It was found that the most active producer of hydrogen in the light was the strain *Anabaena variabilis* R-I-5. The production of hydrogen by *Anabaena variabilis* R-I-5 cells is observed on the first day after the establishment of anaerobic conditions. Active hydrogen evolution persists for six days, and then began to decrease. The highest rate of hydrogen accumulation was observed after 144 h, which amounted to



0,012  $\mu\text{mol H}_2/\text{mg chl/h}$ . It should be noted that the *Nostoc caldicola* RI-3 strain, which actively produces hydrogen in the dark, did not show similar activity under lighting conditions. Hydrogen evolution under illumination conditions after 24 hours was 0,002  $\mu\text{mol H}_2/\text{mg chl/h}$ , and after 120 hours, the maximum production of  $\text{H}_2$  was equal to 0,008  $\mu\text{mol H}_2/\text{mg chl/h}$ , then a gradual decrease in its evolution was observed. For the *Anabaena* sp. Z-1 strain, a similar pattern was observed in the evolution of hydrogen as in the first experiment, a slight hydrogen production of 0,002-0,0003  $\mu\text{mol H}_2/\text{mg chl/h}$  was noted after 24 h and 48 h (figure 5).

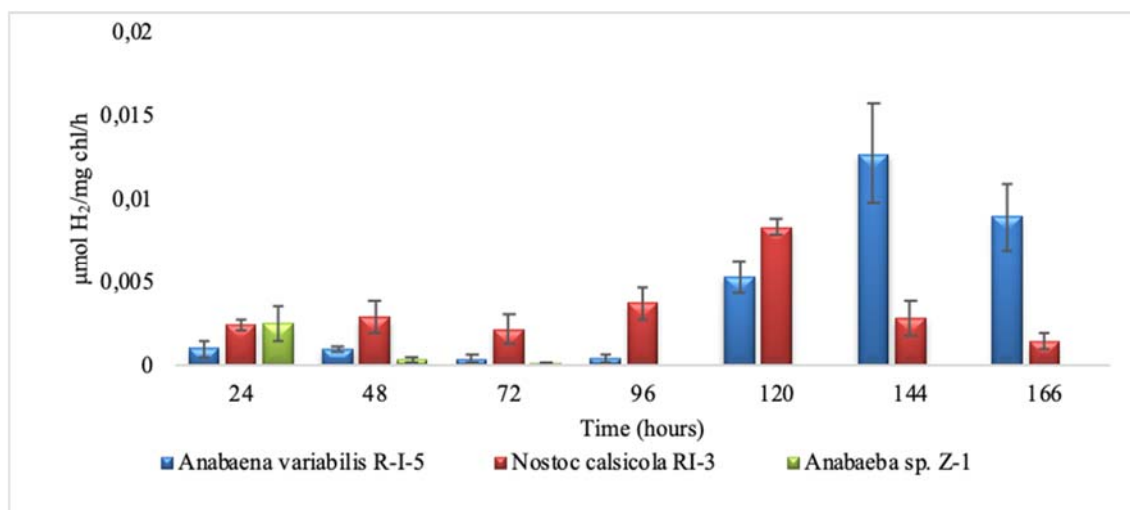


Figure 5 – Hydrogen evolution by the studied strains of cyanobacteria under anaerobic conditions under lighting

Thus, as a result of the studies, a high hydrogen-producing ability was established in the dark for the strain of cyanobacteria *Nostoc caldicola* RI-3 and under lighting conditions for the strain *Anabaena variabilis* R-I-5. In this case, the maximum hydrogen output by the cells of the strain of cyanobacteria *Nostoc caldicola* RI-3 in the dark amounted to 0,032  $\mu\text{mol H}_2/\text{mg chl/h}$ , which is almost 2,5 times higher than the hydrogen production by the strain *Anabaena variabilis* R-I-5 under lighting conditions. Our results are generally consistent with published data. There is information in the literature on the evolution of hydrogen by the *Spirulina platensis* Geitl. strain under anaerobic conditions in the light and in the dark, according to which this process optimally occurs at 32 °C, complete anaerobiosis in the dark [14]. There is also evidence of the active release of hydrogen by *Synechococcus* Nag. PCC 7942 cells in the dark under anaerobic conditions [15].

According to our experimental data, as expected, the production of hydrogen by isolated new strains strictly depends on the presence of lighting. The optimal condition for the evolution of hydrogen by *Nostoc caldicola* RI-3 and *Anabaena variabilis* R-I-5 cells was the absence of illumination, the presence of illumination led to a sharp decrease in  $\text{H}_2$  production. A likely reason for this may be too high activation of PSII, which contributes to the appearance of oxygen concentrations that inhibit the process of hydrogen evolution due to the inactivation of hydrogenase enzymes that catalyze the reduction of protons to molecular hydrogen due to the directed photosynthetic electron flow.

**Conclusion.** The biological production of molecular hydrogen through photosynthesis, which has several advantages over other methods for producing  $\text{H}_2$ , is increasingly attracting researchers as a possible alternative to modern non-renewable energy technologies. This technology can be put into practice if you choose the right path for the efficient use of sunlight by phototrophic microorganisms that can potentially turn solar energy into hydrogen energy. The future of such technologies depends on such scientific achievements as the search for active strains with the required characteristics and the selection of appropriate strategies for improving their strains for the photobiological production of hydrogen. Our studies are aimed primarily at finding promising producers among nitrogen-fixing cyanobacteria and understanding the mechanisms of this process. The article presents the results of the isolation of new strains of heterocystous cyanobacteria from natural sources and the study of their capabilities in the production of hydrogen. According to the results obtained, from the collection three strains of heterocystic cyanobacteria, the strains *Nostoc caldicola* RI-3 and *Anabaena* sp. Z-1 showed a relatively high hydrogen-

producing activity in the dark. The obtained scientific results testify to the promise, practical significance and the need for further study of the isolated cyanobacteria as biosystems capable of efficiently converting light energy into chemical energy of hydrogen – an alternative and environmentally friendly fuel. This scientific information, after additional research, can be applied comprehensively in the development of methods for producing biological hydrogen by cells of heterocystic cyanobacteria.

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### **БИОСУТЕГІ ӨНДІРУГЕ ПЕРСПЕКТИВТІ ГЕТЕРОЦИСТАЛЫ ЦИАНОБАКТЕРИЯЛАРДЫҢ ШТАММДАРЫН ЗЕРТТЕУ**

**Аннотация.** Цианобактериялар – метаболиттік ерекшеліктері негізінде табиғатта кең таралған және топырақ пен тұщы сулардан мұхитқа дейінгі әртүрлі экологиялық аймақтарда өмір сүретін микроорганизмдердің үлкен тобы. Қазіргі таңда цианобактериялардың активті штаммдары қалпына келетін энергия көздерін (биодизель, биосутек, биоэтанол, т.б.) алуға белсенді түрде қолданылады. Цианобактериялар биожанармай алу үшін Күн сәулесінің негізінде жүзеге асатын фотосинтез процесін қолданады және нәтижесінде пайда болған энергияны АТФ және НАДФ түрінде қорға жинап, клеткалардың тіршілігіне қолданады. Солардың ішінде цианобактериялармен биосутек өндіру процесі – осы күнге дейін қалыптасқан әдістердің ең маңыздысы болып табылады. Соңғы таңдағы зерттеулер көрсеткендей, цианобактериялардың барлық түрлері сутек бөлуге қабілетті. Дегенмен, олардың тіршілік ету ерекшеліктеріне сай қалыптасқан морфология-генетикалық айырмашылықтары негізінде сутек бөліну мөлшері мен уақыты әртүрлі болып келеді. Цианобактериялар  $H_2$  молекулаларын екі фермент – гидрогеназа және нитрогеназа белсенділігі арқылы бөліп шығарады. Нитрогеназа – азот фиксациясына жауап беретін, күрделі фермент және олар көбіне гетероцисталарда орналасып, сутек бөлуге қабілеттілік танытады. Ал, гидрогеназа тек қана вегетативті клеткаларда шоғырланып, қарапайым химиялық реакцияны, яғни, протондар мен электрондардан сутек түзілуін катализдейді.

Клеткалардың сутек бөліп шығаруы тікелей биофотоллиз процесіне байланысты болып келеді. Биофотоллиз – бұл суды молекулалық оттегі мен сутекке бөлу үшін биологиялық жүйелерде жарық энергиясы қолданылатын процесс. Ал биофотоллиз процесі пайдаланылатын энергия көзіне байланысты тікелей және жанама деп екі бөлінеді. Тікелей фотоллиз - цианобактериялар клеткалары арқылы фотосинтез негізінде алынған энергияны суды оттегі мен протондарға ыдырату процесі болып табылады. Ал, жанама биофотоллиз – сутегі алу процесі, онда электрондар цианобактериядағы гликоген сияқты сақталған органикалық қосылыстардан алынады. Қазіргі таңда жүргізілген зерттеулер дәлелдегендей, екі түрлі процесс те цианобактериялар үшін маңызды болып табылады, себебі клеткалардың тіршілік етуіне байланысты олардың қабілеттіліктері әр түрлі болып келеді. Сондықтан, жанама биофотоллиз процесі көбіне қараңғы ортада жүзеге асады. Осы тұрғыда, біздің зерттеуіміздің мақсаты – коллекциядан алынған үш цианобактерия штаммдарының сутек бөлу қабілетін тікелей және жанама биофотоллиз процесстері арқылы зерттеу болды.

Бізбен жүргізілген жұмыста сутек бөлу қарқындылығын зерттеу үшін *Nostoc caldicola* RI-3, *Anabaena variabilis* R-I-5 және *Anabaena* sp. Z-1 цианобактерия штаммдары коллекциядан алынды. Сутегі өндірудің жоғары белсенділігімен сипатталатын цианобактериялардың штамдарын таңдау үшін қараңғы және жарық жағдайларда коллекциялық үш штаммның сутегі бөлу қабілеті зерттелінді. Алынған нәтижелерге сәйкес, цианобактериялардың 3 коллекциялық штамдарынан, *Nostoc caldicola* RI-3 және *Anabaena* sp. Z-1 қараңғыда салыстырмалы түрде жоғары сутегі өндіретін белсенділік көрсетті. Қараңғыда сутегінің ең жоғары өнімділігі *Nostoc caldicola* RI-3 штаммымен тіркелді. Бұл штаммда сутектің жоғары жинақталуы 72 сағат инкубациядан кейін байқалды, 0,032 мкмоль  $H_2$ /мг хл/сағ құрады. Ал, жарық жағдайында *Anabaena variabilis* R-I-5 штаммының клеткаларымен сутектің белсенді жинақталуы байқалды, оның нитрогеназа белсенділігі басқа штамдарға қарағанда едәуір жоғары болды. Осы штамммен сутек бөлінуінің ең жоғары жылдамдығы 0,012 мкмоль  $H_2$ /мг хл/сағ құрады. Біздің эксперименттік мәліметтерімізге сәйкес, бөлініп коллекциялық штаммдармен сутегі өндірісіне жарық теріс әсерін тигізді. *Nostoc caldicola* RI-3 және *Anabaena* sp. Z-1 клеткалары арқылы сутектің эволюциясы үшін оңтайлы жағдай қараңғы орта болды және жарықтандырудың болуы  $H_2$  өндірісінің күрт төмендеуіне әкелді. Себебі, ФС2 хлорфилдерінің тым жоғары активтенуі болуы

мүмкін, ол фотосинтетикалық электрондардың бағытталған ағынының әсерінен протондардың молекулалық сутегіге төмендеуін катализдейтін гидрогеназа ферменттерінің инактивациясы тудырып, сутегі эволюциясы процесін тежейтін оттегі концентрациясының пайда болуына ықпал етуі мүмкін.

Біздің зерттеулеріміз гетероцисталы цианобактериялар арасында перспективті өндірушілерді табуға және осы процестің маңызын түсінуге бағытталды.

**Түйін сөздер:** биосутек, гетероцист цианобактерия штамдары, газ хроматографиясы.

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### **ИЗУЧЕНИЕ ШТАММОВ ГЕТЕРОЦИСТНЫХ ЦИАНОБАКТЕРИЙ, ПЕРСПЕКТИВНЫХ ДЛЯ ПОЛУЧЕНИЯ БИОВОДОРОДА**

**Аннотация.** Цианобактерии представляют собой большую группу микроорганизмов, которые широко распространены в природе благодаря своим метаболическим свойствам и живут в различных экологических зонах от почвы и пресной воды до океана. В настоящее время активные штаммы цианобактерий активно используются в производстве возобновляемых источников энергии (биодизель, биоводород, биоэтанол и др.). Цианобактерии используют процесс фотосинтеза, основанный на солнечном свете, для производства биотоплива и хранения полученной энергии в форме АТФ и НАДФ, которые используются для жизнедеятельности клеток. На сегодняшний день среди этих процессов производство биоводорода цианобактериями является одним из наиболее важных методов. Недавние исследования показали, что все типы цианобактерий способны выделять водород. Однако количество и сроки выделения водорода варьируются в зависимости от морфологических и генетических различий, сформированных в соответствии с особенностями их существования. Цианобактерии секретируют молекулы  $H_2$  благодаря активности двух ферментов – гидрогеназов и нитрогеназов. Нитрогеназа – это комплексный фермент, ответственный за фиксацию азота, который часто находится в гетероцистах и способен выделять водород. Гидрогеназа, с другой стороны, концентрируется только в вегетативных клетках и катализирует простую химическую реакцию, то есть образование водорода из протонов и электронов.

Выделение водорода клетками напрямую связано с процессом биофотолитиза. Биофотолитиз – это процесс, с помощью которого световая энергия используется в биологических системах для разделения воды на молекулярный кислород и водород. А процесс биофотолитиза делится на прямой и косвенный, в зависимости от используемого источника энергии. Прямой фотолитиз – это процесс расщепления воды на кислород и протоны из энергии, получаемой в результате фотосинтеза цианобактериальными клетками. Косвенный биофотолитиз – это процесс получения водорода, при котором электроны получают из хранимых органических соединений, таких как гликоген, цианобактерии. Недавние исследования показали, что оба процесса важны для цианобактерий, так как их способности варьируются в зависимости от выживания клеток. Поэтому процесс непрямого биофотолитиза часто проводится в темноте. Таким образом, целью нашего исследования было изучение способности выделения водорода у трех штаммов цианобактерий из коллекции посредством процессов прямого и непрямого биофотолитиза.

Для исследований водорода из коллекции были взяты 3 штамма азотфиксирующих цианобактерий, таких как: *Nostoc caldicola* RI-3, *Anabaena variabilis* R-I-5 и *Anabaena* sp. Z-1. Для отбора штаммов цианобактерий, характеризующихся высокой водородпродуцирующей активностью, было проведено исследование по производству водорода выделенными тремя новыми штаммами в темных и светлых условиях. Согласно полученным результатам, из 3 коллекционных штаммов цианобактерий *Nostoc caldicola* RI-3 и *Anabaena* sp. Z-1 показали относительно высокую водородообразующую активность в темноте. Установлено, что наибольшее выделение водорода была у штамма *Nostoc caldicola* RI-3. Максимальное накопление водорода в этой культуре наблюдалось после 72 ч инкубации, которое в данном случае составило 0,032 мкмоль  $H_2$ /мг хл/ч. В то время как на свету происходило активное накопление водорода клетками штамма *Anabaena variabilis* R-I-5, активность нитрогеназы которого также была значительно выше на свету, чем у других штаммов. Самая высокая скорость накопления водорода составила 0,012 мкмоль  $H_2$ /мг хл/ч. Максимальная скорость выделения водорода этим штаммом составляла 0,012 мкмоль  $H_2$ /мг хл/ч. Согласно нашим экспериментальным данным, свет оказывал негативное влияние на выработку водорода коллекционными штаммами. Оптимальными условиями для эволюции водорода через клетки *Nostoc caldicola* RI-3 и *Anabaena* sp. Z-1 стали темнота и наличие освещения, которые и привели к резкому снижению производства  $H_2$ . Хлофиллы ФС2 могут иметь слишком высокую активность, которая вызывает инактивацию ферментов гидрогеназы, катализирующую снижение протонов на молекулярный водород под

влиянием направленного потока фотосинтетических электронов и способствовать возникновению концентрации кислорода, сдерживающего процесс эволюции водорода.

Наше исследование было направлено на поиск перспективных продуцентов водорода гетероцистных цианобактерий и понимание важности этого процесса. Полученные результаты указывают на перспективы, практическую значимость и необходимость дальнейшего изучения цианобактерий как биосистем, способных эффективно преобразовывать световую энергию в молекулярный водород и экологически чистое топливо.

**Ключевые слова:** биоводород, гетероцистные цианобактериальные штаммы, газовая хроматография.

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