Brief information about the project

Name of the project	AP09260785 «Development of technology for producing biohydrogen based on promising strains of cyanobacteria for the production of biofuels» (0123PK00131)
Relevance	Expanding the conversion of biological raw materials into biofuels, humanity simultaneously reduces the environmental burden on nature, decreases pollution of land and water bodies, as well as emissions of CO2 into the atmosphere. In the field of biofuels, biohydrogen is the cleanest and most valuable type of fuel produced and can be the most promising candidate for the role of an environmentally friendly and renewable energy carrier of the future. A modern direction in bioenergy is the search for objects capable of producing biohydrogen that does not pollute the environment, as well as the development of high-performance technologies. Phototrophic microorganisms, including cyanobacteria, which possess high metabolic potential, are of particular interest in this regard. The use of cyanobacteria as potential producers of biohydrogen is especially relevant and advantageous, as they produce hydrogen through the conversion of solar energy and do not require complex or costly nutrient media for in vitro cultivation. The main idea: Within the framework of the project, extensive work will be carried out to replenish the collection of phototrophic microorganisms with new strains isolated from various extreme ecosystems of our republic, their study, and identification. In order to select the most productive microalgae, they will be screened for cell growth rate, and their ability to produce hydrogen will be studied. The cultivation conditions of the selected cyanobacterial cultures - potential hydrogen producers, including such parameters as light intensity, composition of nutrient media, pH value of the environment, etc., will be optimized. The influence of oxygen stress, nitrogen, sulfide, and phosphorus starvation on the physiological-biochemical properties of the cyanobacterial cell - the hydrogen producer will also be studied. Additionally, a technology for cultivating strains of cyanobacteria - hydrogen producers, harvesting their biomass, and obtaining biohydrogen based on cyanobacteria under laboratory conditions will be developed.
Purpose	The goal of the project is to develop a technology for producing biohydrogen based on promising cyanobacteria strains for the biofuel production.
Objectives	1. Isolation of active cyanobacteria cultures from water and soil samples of various extreme ecosystems of the Republic of Kazakhstan in order to search for cyanobacteria cultures that actively produce hydrogen. To achieve this goal, accumulative cultures of cyanobacteria will be obtained from water and soil samples, and individual species will be cleared of accompanying microflora.

	2. Study of morphological,cultural and physiological properties of isolated cyanobacteria cultures. To achieve this goal, we will study the morphological properties of isolated axenic cyanobacteria cultures and characterize their cultural and physiological properties 3. Screening of isolated and collection strains of cyanobacteria by their biomass productivity and ability to release hydrogen. To achieve this goal, the growth rate and biomass growth of isolated and collected cyanobacteria strains will be studied, and their ability to produce hydrogen will be determined. 4. The identification of obtained new cultures of cyanobacteria with a high potential in the production of biohydrogen. To implement this task, the active strains of cyanobacteria selected during screening will be identified. 5. Optimization of cultivation conditions for cyanobacteria strains – potential producers of hydrogen to increase the total biomass. To implement this task, will be searched optimal conditions for mass cultivation of selected cultures of cyanobacteria - potential producers of hydrogen, including such parameters as temperature, light intensity, composition of nutrient media, pH value of the medium, etc. 6. Perform metabolic modulation of cyanobacteria - producer of biofuels in order to increase the active production of hydrogen. To achieve this goal, the effect of oxygen stress, nitrogen, sulfide, and phosphorus starvation on the physiological and biochemical properties of a cyanobacterium cell - a hydrogen producer - will be studied. 7. Based on the experimental data obtained, develop regulations for obtaining biohydrogen based on cyanobacteria - hydrogen producers in the laboratory. To implement this task, the technology of cultivation strains of cyanobacteria – producers of biohydrogen, collecting their biomass, and producing biohydrogen based on cyanobacteria in the laboratory will be developed.
Expected and achieved results	As a result of the implementation of this project, the following scientific research has been conducted: a search and isolation of cyanobacterial cultures - hydrogen producers from water and soil samples of various extreme ecosystems of the Republic of Kazakhstan, their axenic cultures have been obtained, and their cultural-morphological and some biochemical and physiological properties have been studied; Screening of isolated and collection cyanobacterial cultures for productivity of their biomass and ability to produce hydrogen has been conducted.

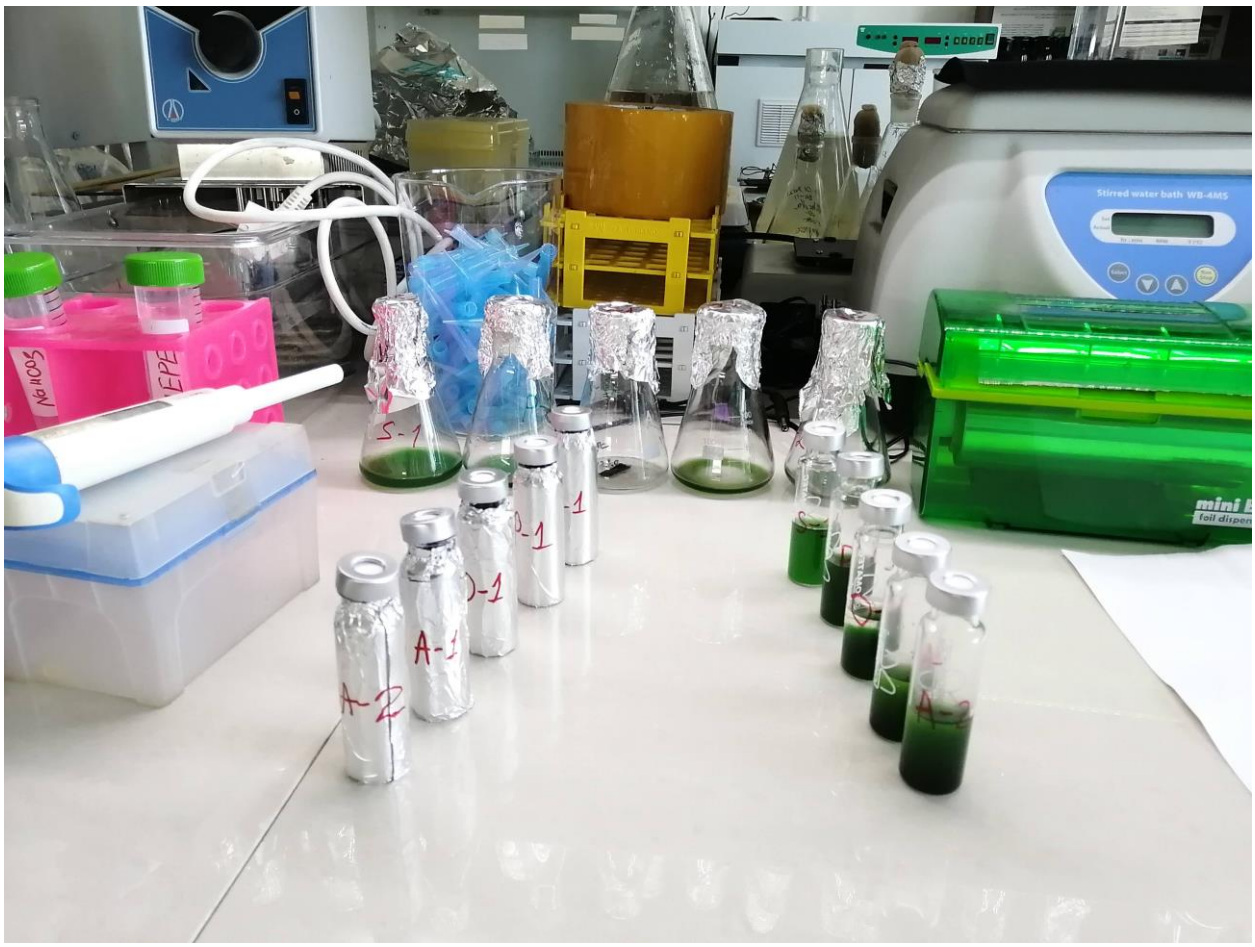
	The following results have been obtained in the course of the work: 1. 15 species and varieties of cyanobacteria were discovered from the hot spring of the Uygur district, 31 from Lake Kyzylkol, as well as from the Aris and Ok rivers, and 19 from rice fields in the Almaty and Kyzylorda regions. 2. 8 axenic cultures of cyanobacteria were isolated from 17 isolates of accumulative cultures, and based on cultural-morphological and physiological characteristics, they were identified as *Nostoc N-1, Oscillatoria O-2, Synechococcus S-1, Phormidium P-1, Nostoc N-2, Anabaena A-1, Oscillatoria* O-1, and *Anabaena* A-2. 3. It was determined that the cyanobacterial cultures *Anabaena A-2, Anabaena A-1, Oscillatoria O-1, Synechococcus S-1*, and *Phormidium* P-1 have the highest rates of cell growth and biomass yield, determining their high productivity, and were identified using molecular-genetic analysis of 16S rRNA genes as *Anabaena variabilis A-2, Anabaena variabilis A-1, Oscillatoria sp. O-1, Synechococcus sp. S-1*, and *Phormidium tenue* P-1. 4. A high level of ethylene production was revealed in the heterocystous strain of cyanobacterium *Anabaena variabilis* A-1, reaching 15.2 μmol ethylene/mg dry weight/h, indicating high activity of the nitrogenase enzyme in this culture. 5. As a result of screening for hydrogen production capability, the heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 was selected, with hydrogen yield in darkness reaching 8.67 μmol H_2/mg Chl/h. This figure was almost 17.2 times higher than under illumination conditions for the same strain. 6. It was established that *Synechococcus* sp. S-1 is the most active hydrogen producer in light, yielding 2.35 μmol H_2/mg Chl/h, which is 3 times lower than *Anabaena variabilis* A-1 in darkness. 7. It was found that the addition of 25 mM HEPES and 50 mM sodium bicarbonate to the BG-11 medium increases the yield of biohydrogen (H_2) in the heterocystous strain of cyanobacterium *Anabaena variabilis* A-1. 8. It was shown that the photoproduction of hydrogen by the heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 using a combination of N and S deficiency (BG_0-11-S) was 9.82 μmol H_2/mg Chl/h, demonstrating results 3 times higher than in the BG-11-S medium. In the course of optimizing maximum hydrogen production, the BG_0-11-S medium was selected as the most suitable compared to other modified media. 9. A laboratory procedure for obtaining biohydrogen based on the selected heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 has been developed. Based on the obtained results, a patent for a useful model No. 8167,

	dated 28.02.2023, "Heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 for biofuel production as a raw material" has been obtained in order to expand the arsenal of microorganism strains used as raw materials for biofuel production.
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles	1. Bolatkhan Kenzhegul, Doctor of Philosophy (PhD), Associate Professor, Hirsch Index – 11 ResearcherID: AAZ-8890-2020 ORCID https://orcid.org/0000-0001-7133-6546 **Scopus author ID:** 55977615700 2. Zayadan Bolatkhan, Doctor of Biological Sciences, Professor, Academician of the National Academy of Sciences of the Republic of Kazakhstan, Hirsch Index – 16, ResearcherID: B-1664-2015, ORCID https://orcid.org/0000-0002-4572-2416**, Scopus author ID:** 6504770922. 3. Sarsekeeva Fariza Kudaybergenovna, Doctor of Philosophy (PhD), Hirsch Index -3, ResearcherID: E-4491-2015, ORCID https://orcid.org/0000-0001-9119-2279, **Scopus author ID:** 56524602300 4. Kakimova Ardak Bolatovna, Doctor of Philosophy (PhD), Hirsch Index -4ResearcherID: ABD-5813-2021, ORCID: https://orcid.org/0000-0001-5612-1002, **Scopus author ID:** 57219604772 5. Sandybaeva Sandugash Kalzhanovna, PhD candidate, Hirsch Index -2, Researcher ID: AGO-0562-2022, ORCID https://orcid.org/0000-0002-4340-8749, Scopus author ID: 57560350900.
List of publications with links to them	I. Scientific and Educational Manuals: 1 B.K. Zayadan, F.S. Sarsekeeva, K. Bolatkhan. Phototrophs of microorganisms in bioenergetics 2023: Monograph - Almaty: "Tanba" Publishing House, 2023. - 215 p. II. Monographs: 1 B.D. Kosalbayev, A.K. Sadvakasova, B.K. Zayadan. Phototrophic microorganisms in bioenergetics 2022: Monograph - Almaty: "Polytech" Publishing House, 2022. - 320 p. III. Articles in peer-reviewed foreign scientific journals indexed in the Web of Science or Scopus databases with a non-zero impact factor: 6 1.Bekzhan D. Kossalbayev, Ardak B. Kakimova, Kenzhegul Bolatkhan, Bolatkhan K. Zayadan, Sandugash K. Sandybayeva, Asemgul K.Sadvakasova, Suleyman I. Allakhverdiev. Biohydrogen production by novel cyanobacterial strains isolated from rice paddies in Kazakhstan//International Journal of Hydrogen. Energy. Volume 47, Issue 37, 30 April 2022, Pages 16440-16453 https://doi.org/10.1016/j.ijhydene.2022.03.126 2. Gulzhanay K.Kamshybayeva, Bekzhan D.Kossalbayev, Asemgul K.Sadvakasova, Bolatkhan K.Zayadan, Ayshat M.Bozieva, DmitryDunikov, Saleh Alwasel Suleyman I.Allakhverdiev. Strategies and

	economic feasibilities in cyanobacterial hydrogen production// International Journal of Hydrogen Energy, ISSN: 0360-3199, Vol: 47, Issue: 69, Page: 29661-29684. Q-1,процентиль – 90. https://doi.org/10.1016/j.ijhydene.2022.06.277 3. Gulzhanay K. Kamshybayeva, Bekzhan D. Kossalbayev, Asemgul K. Sadvakasova, Ardak B. Kakimova, Meruyert O. Bauenova, Bolatkhan K. Zayadan, Chi-Wei Lan, Saleh Alwasel, Tatsuya Tomo, Jo-Shu Chang, Suleyman I. Allakhverdiev. Genetic engineering contribution to developing cyanobacteria-based hydrogen energy to reduce carbon emissions and establish a hydrogen economy. Int J Hydrogen Energy. Available online 25 January 2023 In Press, Corrected Proof. https://doi.org/10.1016/j.ijhydene.2022.12.342 4. Gulzhanay K. Kamshybayeva, Bekzhan D. Kossalbayev, Asemgul K. Sadvakasova, Meruyert O. Bauenova, Bolatkhan K. Zayadan, Ayshat M. Bozieva, Hesham F. Alharby, Tatsuya Tomo, Suleyman I. Allakhverdiev. Screening and optimisation of hydrogen production by newly isolated nitrogen-fixing cyanobacterial strains, International Journal of Hydrogen Energy, Volume 48, Issue 44, 2023, Pages 16649-16662, ISSN 0360-3199, https://doi.org/10.1016/j.ijhydene.2023.01.163. 5. Bekzhan D. Kossalbayev, Girayhan Yilmaz, Asemgul K. Sadvakasova, Bolatkhan K. Zayadan, Ayaz M. Belkozhayev, Gulzhanay K. Kamshybayeva, Gaukhar A. Sainova, Ayshat M. Bozieva, Hesham F. Alharby, Tatsuya Tomo, Suleyman I. Allakhverdiev. Biotechnological production of hydrogen: Design features of photobioreactors and improvement of conditions for cultivating cyanobacteria, International Journal of Hydrogen Energy, 2023, In Press, Corrected Proof. ISSN 0360-3199, https://doi.org/10.1016/j.ijhydene.2023.09.001. 6. Huma Balouch, Bolatkhan K. Zayadan, Asemgul K. Sadvakasova, Bekzhan D. Kossalbayev, Kenzhegul Bolatkhan, Donus Gencer, Dilek Civelek, Zihni Demirbag, Hesham F. Alharby, Suleyman I. Allakhverdiev. Prospecting the biofuel potential of new microalgae isolates, International Journal of Hydrogen Energy, Volume 48, Issue 50, 2023, P. 19060-19073, ISSN 0360-3199, https://doi.org/10.1016/j.ijhydene.2023.02.028 IV. Articles in journals recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan: 3 1.B. K. Zayadan, A.B. Kakimova, K. Bolatkhan, S.K. Sandybayeva, B.D. Kosalbayev, D.B. Nurabayeva. Production of Bio-hydrogen from Cyanobacteria:

	Challenges and Opportunities. International Journal of Biology and Chemistry. Vol.14, No 1, 4 (2021) https://doi.org/10.26577/ijbch.2021.v14.i1.01\ 2. A.I. Token, Zh.A. Ramazanova,K. Bolatkhan, R. Mammadov, A.K. Sadvakasova, D.K. Kirbaeva, F.K. Sarsekeyeva. Search and isolation of cyanobacteria cultures from the soils of rice fields of the republic of Kazakhstan. Экология сериясы. №2 (67). 2021. с.41-48. 3. S.K. Sandybayeva, K. Bolatkhan, A.B. Kakimova, A.K. Toktybay, G.A. Akhmetova, B.K. Zayadan. Isolation and study of morphological and cultural properties of cyanobacterial community from hot springs in Almaty region. Bulletin of KazNU, Environmental series. – 2023. -№2 (75) - C. 112-125. V. Abstracts in the proceedings of international conferences: 61. Zayadan B.K., Tatsuya Tomo, Kakimova A.B., Kossalbayev B.D. «Prospects of heterocystic cyanobacteria in the production of biohydrogen». Collection of the International scientific and practical conference «Aspects and innovations of environmental biotechnology and bioenergy» 12-13 February, 2021 y., Almaty, Kazakhstan, P. 266-269. 2. Zayadan B.K., Kakimova A.B., Bolatkhan.K. «Study of the Ability to Release Hydrogen of New Cyanobacteria Cultures to Produce Biofuel». Collection of the «5th Symposium on EuroAsian Biodiversity (SEAB-2021)» 1-3 July, 2021 y., Almaty Kazakhstan, Mugla Turkey. ISBN: 978-625-409-945-8. P 292. 3. Zayadan B.K., Los' D.A., Sadvakasova A.K., Bolatkhan K., Sarsekeeva F.K. Prospects of biotechnology for biofuel production based on photosynthetic microorganisms. International scientific and practical conference “Aspects and innovations of environmental biotechnology and bioenergy”. – 12-13 February 2021. – P.281-286. 4. Ardak Kakimova, Bolatkan Zayadan, Kenzhegul Bolatkhan, Asemgul Sadvakasova, Nurzia Akmukhanova, Fariza Sarsekeeva, Bekzhan Kossalbayev, Suleyman Allakhverdiev. «Potential cultures of cyanobacteria as feedstock for biohydrogen production». 11th International conference on Photosynthesis and Hydrogen Energy Research for sustainability, 3-9 July, 2023 y., Istanbul, Turkey. 5. Bekzhan Kossalbayev, Asemgul Sadvakasova, Bolatkan Zayadan, Meruert Bauenova, Gulzhanay Kamshybayeva, Suleyman Allakhverdiev. «Investigation of oxygen, carbon dioxide, and nitrogen gases influence on hydrogen production of cyanobacteria». 11th International conference on Photosynthesis and Hydrogen Energy Research for sustainability, 3-9 July, 2023 y., Istanbul, Turkey.
Patents	Patent of Republic Kazakhstan for a Utility Model "Heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 as a raw material for biofuel production" No. 8167 dated February 28, 2023. Authors: Kakimova

	A.B., Zayadan B.K., Bolatkhan K., Sadvakasova A.K., Sandybaeva S.K.


Stirred water bath WB-4MS
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