**ABSTRACT**

for the dissertation for Doctor of Philosophy (PhD) degree
on the speciality "8D05105 – Biotechnology" of Ardak Kakimova
on the theme «Screening of cyanobacterial strains - hydrogen producers and optimization of their cultivation conditions»

**General description of the research:** The research focuses on the isolation and investigation of active strains of cyanobacteria with potential applications in bioenergy production from various aquatic ecosystems.

**The relevance of the research:** The current scarcity of fossil fuels and the consequences of climate change are driving humanity to seek alternative energy sources. Biofuel, with its numerous advantages, is emerging as a primary energy source capable of replacing traditional fuels. By expanding the conversion of biological resources into biofuels, humanity can reduce the ecological burden on nature, decrease pollution of terrestrial and aquatic environments, including carbon dioxide emissions into the atmosphere. Hydrogen is considered the most promising candidate for achieving an environmentally clean and renewable energy source in the future. This fact has intensified global scientific interest in studying hydrogen as a biofuel. Hydrogen has the potential to become the most important energy source in the near future and contribute to addressing the global air pollution problem.

One of the modern directions in bioenergy research is the search for agents capable of producing hydrogen without polluting the environment, as well as the development of technologies that ensure high hydrogen yields. Photosynthetic microorganisms, including cyanobacteria with high metabolism, are of particular interest in the utilization of biological methods for hydrogen production. The use of cyanobacteria as potential hydrogen producers is important and effective because they are the only bacteria capable of performing oxygenic photosynthesis, utilizing solar energy, and converting photosynthetic products into chemical energy, namely carbohydrates.

However, not all strains of cyanobacteria are equally efficient in hydrogen production. Cultivation conditions have a significant influence on their productivity. Therefore, it is necessary to identify and select potential strains of cyanobacteria that are efficient hydrogen producers and optimize the conditions for their cultivation.

This research aims to expand the repertoire of microorganism strains with high potential in bioenergy. Transitioning to various renewable and environmentally friendly energy sources based on microorganisms can help reduce the adverse consequences of climate change and facilitate the shift to a sustainable energy system. The research topic is relevant as it addresses an important scientific and social problem and contributes to the acquisition of new fundamental knowledge with subsequent practical applications.

**The purpose of the research:** To search for, isolate, identify, and study new strains of cyanobacteria obtained from various ecological systems, also optimizing their cultivation conditions and developing laboratory protocols for obtaining biohydrogen based on these strains

**The main tasks of the research to accomplish purpose are as following**:

1. Isolate and identify axenic cultures of cyanobacteria from various ecosystems.
2. Determine the nitrogenase activity of new and collection strains of cyanobacteria.

3. Assess the potential of the newly isolated and collection strains of cyanobacteria in biohydrogen production.
4. Optimize the cultivation conditions of active strains of cyanobacteria to increase hydrogen yield.
5. Develop a laboratory protocol for biohydrogen production based on active strains of cyanobacteria.

**The research objects and materials:** The research objects included the collection strain *Desertifilum* sp. IPPAS B-1220 (CCMKazNU) and the cyanobacterial cultures *Anabaena variabilis* A-1, *Anabaena variabilis* A-2, *Synechocystis sp* S-1, *Oscillatoria sp* O-1, *Phormidium tenue* P-1, *Nostoc commune* N-1, *Nostoc calcicola* N-2, *Oscillatoria sp* O-2, which were isolated from the ecosystems of Kyzylorda, Turkistan, and Almaty regions in the Republic of Kazakhstan.

**Research methods:** The research utilized microbiological, algological, biotechnological, molecular genetic, physical, chemical, and statistical methods of investigation.

**The scientific novelty of the research:** The algal composition of Lake Kyzylkol, the Aris and Ok rivers in the Turkistan region of the Republic of Kazakhstan, was studied for the first time.

Eight axenic strains of cyanobacteria were isolated and identified for the first time from various ecosystems in the Kyzylorda, Turkistan, and Almaty regions, and their morphological and cultural properties were examined.

High nitrogenase enzyme activity was demonstrated in the heterocyst strain of cyanobacteria *Anabaena variabilis* A-1.

It was observed for the first time that the heterocyst strain *Anabaena variabilis* A-1 exhibited a high capacity for hydrogen evolution in the dark, surpassing other strains of cyanobacteria.

The addition of 25 mmol HEPES and 50 mmol sodium bicarbonate to the medium was shown to enhance the release of biohydrogen ($H_2$) in the heterocyst strain of cyanobacteria *Anabaena variabilis* A-1. Furthermore, it was found that the optimal pH for hydrogen evolution in this strain was neutral (pH 7).

Hydrogen photoproduction by heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 was slightly increased under combined nitrogen and sulfur deficiency. In the $BG_0$-11-S medium, the maximum hydrogen productivity and average hydrogen production level reached 9.82 μmol $H_2$/mg chl/h.

A laboratory protocol for hydrogen production based on the selected heterocyst strain of cyanobacteria, *Anabaena variabilis* A-1, was developed.

**Theoretical and practical significance of the research:** An assessment of nitrogenase and hydrogenase enzyme activity was conducted for several strains of cyanobacteria isolated from various ecosystems and selected from the "CCMKazNU" collection of the Photobiotechnology Laboratory. Their ability to produce biohydrogen was determined.

A heterocyst strain of cyanobacteria, *Anabaena variabilis* A-1, was obtained, which is a producer of biohydrogen, and its biomass can be utilized in bioenergy applications.

The isolated strains of cyanobacteria, *Anabaena variabilis* A-2, *Oscillatoria* sp. O-1, *Synechocystis* sp. S-1, and *Phormidium tenue* P-1, were introduced into the collection of phototrophic microorganisms for further use in biotechnology.

The heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 has been deposited in the Republican Collection of Microorganisms (Astana, Kazakhstan) under the number RKM0960 as of October 20, 2021.

A utility model patent with the number №8167 dated 28.02.2023 has been obtained for the "Heterocystous strain of cyanobacteria *Anabaena variabilis* A-1 as a raw material for biofuel production" with the aim of expanding the arsenal of microbial strains used as raw materials for biofuel production.

**The main provisions for the defense:**

Five axenic cyanobacteria strains were isolated from different ecosystems in the Kyzylorda, Turkistan, and Almaty regions. These strains were identified as *Anabaena variabilis* A-1, *Anabaena variabilis* A-2, *Oscillatoria* sp. O-1, *Synechocystis* sp. S-1, and *Phormidium tenue* P-1.

The heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 exhibited a high level of ethylene production, reaching 15.2 μmol ethylene/mg dry weight/h, indicating high nitrogenase enzyme activity in this culture.

The heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 strain showed the ability to produce 3.7 times more hydrogen in the dark compared to light, and 43 times more hydrogen compared to the *Phormidium tenue* P-1 strain.

The cyanobacteria strain *Synechocystis* sp. S-1 demonstrated the highest hydrogen production activity under light conditions.

The addition of 50 mmol NaHCO3 + 25 mmol HEPES to a neutral medium (pH 7) resulted in a increase in hydrogen evolution by the heterocyst strain of cyanobacteria *Anabaena variabilis* A-1.

The combination of nitrogen and sulfur deficiency enhanced hydrogen production by the cells of the heterocyst strain of cyanobacteria *Anabaena variabilis* A-1. The hydrogen productivity in $BG_0$-11-S medium was three times higher compared to BG-11-S medium.

**Key research findings and conclusion:**

1. The algal flora composition of water sources of the Turkestan and Almaty regions was studied, 15 cyanobacteria species were identified from Uygur district, 31 species of cyanobacteria from Lake Kyzykol and the Ok and Arys rivers, 19 species of cyanobacteria from rice fields of Almaty and Kyzylorda regions.
2. 8 axenic cyanobacterial cultures were isolated from 17 accumulated cultures and 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 based on cultural-morphological and physiological characteristics. Through molecular genetic analysis of the 16S rRNA gene, the strains of the newly isolated cyanobacteria were identified and designated as *Anabaena variabilis* A-2, *Anabaena variabilis* A-1, *Oscillatoria sp.* O-1, *Synechococus sp.* S-1 and *Phormidium tenue* P-1.
3. The heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 showed a high level of ethylene production, reaching 15.2 μmol ethylene/mg dry weight/h, indicating high nitrogenase enzyme activity in this culture.
4. During screening, the productivity of the selected heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 in the dark was found to be 8.67 μmol $H_2$/mg chl/h, which was 17.2 times higher than its hydrogen evolution capacity under light conditions. It was established that among the newly isolated cyanobacteria strains, heterocyst strain *Anabaena variabilis* A-1 actively produces hydrogen and can be used as a source for biofuel production. The heterocyst strain of cyanobacteria *Anabaena*

*variabilis* A-1 has been deposited in the Republican Collection of Microorganisms (Astana, Kazakhstan) under the number RKM0960 as of October 20, 2021.

5. *Synechococcus* S-1 was found to be the most active hydrogen producer under light conditions, with a rate of 2.35 μmol $H_2$/mg chl/h, which is 3 times lower than the dark hydrogen production rate of the heterocyst strain *Anabaena variabilis* A-1.

6. The photoproduction of hydrogen by the heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 was enhanced with the combination of nitrogen and sulfur deficiency. It was found that the highest hydrogen productivity and the average hydrogen production rate were achieved in the $BG_0$-11-S medium, making it the most suitable optimization compared to other modified media. Hydrogen productivity in $BG_0$-11-S medium was three times higher than in BG-11-S medium.

7. A laboratory protocol for producing biohydrogen based on the selected strain of cyanobacteria has been developed. Based on the obtained results, a utility model patent with number №8167 dated 28.02.2023 has been obtained for the "Heterocystous strain of cyanobacteria *Anabaena variabilis* A-1 as a raw material for biofuel production" with the aim of expanding the arsenal of microbial strains used as raw materials for biofuel production.

**The contribution of author for the results described in the dissertation:**

The analysis of literature data related to the research problem, formulation of research goals and objectives, conducting experimental studies, analysis of the obtained results, statistical processing, and presentation of the dissertation work have been carried out by the author independently.

**Relationship of the research with the scientific project.**

The dissertation work was conducted within the framework of the projects AP08052481 "Development of biodiesel production technology based on active strains of microalgae" (2020-2022) and AP09260785 "Development of hydrogen production technology based on promising strains of cyanobacteria for biofuel production" (2021-2023).

**Research approbation:**

The results of the research and the main findings of the dissertation work were presented and discussed at the following international scientific conferences and symposiums:

1. International Scientific Conference of Students and Young Scientists "Farabi alemi" April 6-9, 2020, Almaty, Kazakhstan.
2. 11th European Workshop on the Biology of Cyanobacteria, September 7-9, 2020, Porto, Portugal.
3. International Scientific and Practical Conference "Aspects and Innovations of Environmental Biotechnology and Bioenergy," February 12-13, 2021, Almaty, Kazakhstan.
4. 5th Symposium on EuroAsian Biodiversity (SEAB-2021), July 1-3, 2021, Almaty, Kazakhstan, Mugla, Turkey.
5. 11th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainable Development" (ICPRS 2023), July 3-9, 2023, Istanbul, Turkey.
6. **Publications:**

The main results of the dissertation are presented in 13 published works: among them, 4 articles in republican scientific journals from the list of the Committee for Control in the Sphere of Education and Science of the Republic of Kazakhstan, 2 scientific articles in the 1st quartile, and 6 conference abstracts at international conferences. Based on the obtained results, a patent for a utility model with the patent number №8167 from 28.02.2023 was obtained for the "Heterocystous strain of cyanobacterium *Anabaena variabilis* A-1 as a raw material for biofuel production" with the aim of expanding the arsenal of microorganism strains used as raw materials for biofuel production.

**Dissertation structure:**

The dissertation consists of 117 pages of computer text, including symbols and abbreviations, introduction, literature review, materials and methods, research results and their discussion, conclusion, and a bibliography of 241 cited sources. The work includes 5 tables, 43 figures, and 1 appendix.

The dissertation is dedicated to the isolation of active strains of cyanobacteria from various ecosystems for application in bioenergy. The research involved the isolation of active strains of cyanobacteria, studying their physiological and biochemical properties, and their identification.

From different aquatic ecosystems, bacteriologically pure cultures of cyanobacteria were isolated, including *Anabaena variabilis* A-1, *Anabaena variabilis* A-2, *Synechocystis sp* S-1, *Oscillatoria sp* O-1, *Phormidium tenue* P-1, *Nostoc commune* N-1, *Nostoc calcicola* N-2, *Oscillatoria sp* O-2.

The heterocyst strain of cyanobacteria *Anabaena variabilis* A-1 was selected as an active hydrogen producer, which can be used as a raw material for biofuel production in bioenergy. Optimization was conducted to increase the hydrogen yield.