ABSTRACT

of the dissertation for the philosophical doctor degree (PhD) on specialty 6D070100 - "Biotechnology"

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Bioremediation of polluted aquatic ecosystems based on microalgae and water plants consortia

General characteristics of the work. The dissertation work is devoted to the creation of the association of higher aquatic plants and microalgae and to study the possibility of its use in bioremediation of polluted aquatic ecosystems.

Relevance of the research topic

Insufficient treatment of domestic and industrial wastewater is an urgent environmental problem in many regions of the world. Moreover, despite all the measures and methods used for wastewater treatment, a lot of untreated pollutants are directly discharged to natural water bodies. In recent years the problems of dynamics and conservation of biological diversity have gained increasing attention due to the growing anthropogenic impact on various ecosystems. In the conditions of extremely tense ecological situation developing in many regions of the world, geochemical cycles of heavy metals in the biosphere seem to be determined less by natural redistribution but more by anthropogenic activity. The problem of environmental pollution by various ecotoxicants is exacerbated by the urbanization and industrialization within the country.

One of the most dangerous pollutants are heavy metal ions (HM). It is known that heavy metals can form extremely toxic compounds, interacting with other substances, and accumulate in the food chain "water – plants – animals – people" in quantities many times exceeding their content in water bodies, which can be the reason of various diseases of the nervous system and a number of others, including cancer.

In recent years many environmental scientists are increasingly paying attention to the study of the "destiny" of pollutants caught in the natural environment, their subsequent transformation and the relationship with living organisms that directly affect the assessment of environmental pollution. To accelerate the processes of purification and restoration of disturbed aquatic ecosystems, it is necessary to use biological reserves not only of bacteria, but also of other communities, including organisms with different biochemical capabilities. Natural associations have a much richer set of restorative functions, as they always include photosynthetic organisms-higher plants, eukaryotic algae and cyanobacteria. The use in artificial water purification systems of organisms' consortia of different taxonomic groups, the use of microorganisms' active strains-destructors, the obtaining and use water-resistant microalgae, as well as the introduction into the cleansing consortium of higher aquatic plants, allows to create a new integrated biotechnology for cleaning and restoration of water bodies contaminated with various pollutants including heavy metals. In this regard, the search and selection of active biological objects to create a consortium, the identification of the main types of relationships between them in artificially formed associations and the creation on this basis of a consortium with a

wide range of heavy metal sorption, its wide application for bioremediation of natural and wastewater have especially relevance.

Aim of research: Creation the association of higher aquatic plants and microalgae and its use in conducting bioremediation of contaminated aquatic ecosystem.

To achieve this goal, the following **tasks** were formulated:

1. To search and isolate algologically and bacteriologically pure microalgae cultures from various contaminated aquatic ecosystems;
2. To select the most productive species of microalgae for further use in bioremediation;
3. To select species of microalgae with high sorption heavy metals capacity;
4. To select species of higher aquatic plants with high sorption heavy metals capacity;
5. To create association of higher aquatic plants and microalgae for bioremediation of contaminated aquatic ecosystems;
6. To study the possibility of using the association of higher aquatic plants and microalgae in bioremediation of contaminated wastewater.

Objects of research. As objects of research were used microalgae strains of *Chlorella vulgaris* BB-2, *Chlorella vulgaris* BB-1, *Chlorella vulgaris* B-12, *Scenedesmus obliquus* B-3, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, *Ankistrodesmus* sp. BI-1 isolated from Elek river of Aktobe region and Bilikol lake of Zhambyl region, also higher plants *Pistia stratiotes, Elodea canadensis* and *Lemna minor* selected from the reservoir "Pervomaysky" in Iliysky district of Almaty region.

Methods of research

In the dissertation work biotechnological, microbiological, genetic and physical-chemical methods were used.

Scientific novelty of research

For the first time the species composition of algal flora of Bilikol lake and Elek river were studied. Further 7 algologically and bacteriologically pure strains of microalgae *Chlorella vulgaris* BB-2, *Chlorella vulgaris* BB-1, *Chlorella vulgaris* B-12, *Scenedesmus obliquus* B-3, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, *Ankistrodesmus* sp. BI-1 were isolated from the studied objects.

Moreover, for the first time the association was established on the basis of selected strains of microalgae and higher aquatic plants, characterized by high sorption capacity to various heavy metals, and studied the possibility of its use in bioremediation of polluted aquatic ecosystems.

Scientific and practical significance of the work

Based on microalgae *Ankistrodesmus* sp. B-1 and the highest aquatic plant *Pistia stratiotes* created an effective consortium for use in the purification of aquatic ecosystems polluted with with high concentrations of nutrients and heavy metals.

Isolated microalgae *Chlorella vulgaris* BB-2, *Chlorella vulgaris* BB-1, *Chlorella vulgaris* B-12, *Scenedesmus obliquus* B-3, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, *Ankistrodesmus* sp. B-1 were included in the

collection of phototrophic microalgae of al-Farabi KazNU for their further use in solving biotechnological problems.

Strains of *Chlorella vulgaris* BB-2, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, *Ankistrodesmus* sp. B-1 deposited in RSE "Republican collection of microorganisms" of the Committee of science of the Ministry of education and science of the Republic of Kazakhstan under the numbers RKM 0713, RKM 0709, RKM 0710, RKM 0714 respectively.

The patent of RK for useful model "Method of biological treatment of domestic and industrial wastewater" № 2551 from 12.12.2017 was received.

The data obtained in the course of scientific research are introduced into the educational process at the Biotechnology Department of al-Farabi Kazakh National University, for the 3rd year undergraduate specialty "6B070100-Biotechnology" in the discipline "Environmental biotechnology".

Basic provisions for the defence:

Algologically and bacteriologically pure cultures of microalgae isolated from aquatic ecosystems are identified as *Chlorella vulgaris* BB-2, *Chlorella vulgaris* BB-1, *Chlorella vulgaris* B-12, *Scenedesmus obliquus* B-3, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, *Ankistrodesmus sp.* BI-1;

Strains of microalgae *Chlorella vulgaris* BB-2, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4 and *Ankistrodesmus* sp. BI-1 characterized by high resistance and sorption capacity against heavy metals: cadmium, lead, zinc and copper to a concentration of 10 MPC.

High resistance and sorption capacity of higher aquatic plants *Elodea canadensis* and *Pistia stratiotes* against heavy metals: cadmium, lead, zinc and copper to a concentration of 10 MPC;

Obtaining association of higher aquatic plant *Pistia stratiotes* and microalgae *Ankistrodesmus* sp. BI-1, which has a high bioremediation capacity for heavy metals and biogenic elements.

Main research results and conclusions:

1. In the result of species composition of samples collected from Bilikol Lake were identified 96 species belonging to 4 divisions (*Cyanophyta* - 31, *Bacillariophyta* - 20, *Euglenophyta* - 19, *Chlorophyta* – 26), 9 classes, 9 orders, 17 families and 32 genera. There are 181 species were determined in Yelek river belonging to 4 divisions (*Cyanophyta*- 58, *Bacillariophyta*- 54, *Euglenophyta*- 6, *Chlorophyta*– 63), 12 classes, 20 orders, 49 families and 76 genera. 10 algologically pure cultures of green microalgae were isolated from Bilikol Lake and Elek River, 7 of them were bacteriologically pure. Based on the study of cultural and morphological properties and analysis of the nucleotide sequence of 18S rRNA genes, the bacteriologically pure strains were identified as *Chlorella vulgaris* BB-2, *Chlorella vulgaris* BB-1, *Chlorella vulgaris* B-12, *Scenedesmus obliquus*B-3, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4 and *Ankistrodesmus* sp. BI-1.

2. It was determined that the isolated strains of *Chlorella vulgaris* sp BB-2, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4 and

Ankistrodesmus sp BI-1 microalgae had a high productivity at a cultivation temperature 26-28°C with a lighting intensity 4000 lx.

3. It was determined that from studied microalgae cultures are more effective bioaccumulators of copper - *Chlorella vulgaris* BB-2, cadmium – *Ankistrodesmus* sp.BI-1 and *Chlamydomonas reinhardtii* B-4, lead – *Ankistrodesmus* sp.BI-1 and *Chlorella vulgaris* BB-2. Zinc was accumulated in all investigated cultures of microalgae. It was revealed that heavy metals from the medium weree selectively absorbed in the sequence Zn^{2+}> Cu^{2+}> Cd^{2+}> Pb^{2+}.

4. It was revealed that higher aquatic plants *Elodea canadensis* and *Pistia stratiotes* are characterized by high stability and sorption capacity for heavy metals: cadmium, lead, zinc and copper at concentrations up to 10 MPC, it should be noted that *Pistia stratiotes* and *Elodea canadensis* are macroconcentrators in respect of zinc, lead and copper, and microconcentrators for cadmium. While *Lemna minor* is microconcentration in relation to all the studied metals.

5. It was found that the green microalgae *Chlorella vulgaris* BB-2 and higher aquatic plants *Pistia stratiotes* and *Elodea canadensis* were characterized by a neutral type of relationship, whereas the green microalgae *Ankistrodesmus* sp. BI-1 and *Pistia stratiotes* formed a symbiotic type of relationship. On the basis of the obtained data, the association of the higher aquatic plant *Pistia stratiotes* and microalgae *Ankistrodesmus* sp. BI-1 for use in bioremediation of water contaminated with heavy metals.

6. In the results of application the association of higher aquatic plant *Pistia stratiotes* and microalgae *Ankistrodesmus* sp. BI-1 in wastewater treatment, effectiveness of biogenic element deletion on the 8th day was 98%, heavy metal ions 89-93% during consortium addition: *Ankistrodesmus* sp. BI-1 – 40.0×10^6 cell/ml and *Pistia stratiotes* – 600 g/m^3 (60±2 unit).

Personal contribution of the author

The analysis of the literature data concerning the problem under study, the definition of the purpose and objectives of the study, conducting the experimental studies, the analysis of the results and statistical processing, the presentation of the dissertation work performed personally by the author.

Connection of work with the plan of state programs

The dissertation work was performed under project No. 4256/GF4 "Bioremediation of various wastewaters from heavy metals through the application of phyto-algal-cyanobacterial consortia", № state registration 0115RK00395 (2015-2017); No. 0477/GF4 "Replenishment, preservation, certification and creation of a data Bank collection cultures of phototrophic microorganisms promising for the production of biofuels, bio-fertilizers and biologically active substances" № state registration 015RK00290 (2015-2017); AR05131743 "Development of scientific and methodical bases of technology of biomonitoring and forecasting of the state of polluted aquatic ecosystems with the use of phototrophic microorganisms", № 0118RK00086 state registration (2018-2020);

Approbation of scientific work

The materials of the dissertation are reported and discussed at the following international conferences and symposia:

- International conference of students and young scientists "Farabi Alemi", Almaty, Kazakhstan, 2015-2018
- "Symposium 5th all-Russian Symposium with international participation of autotrophic microorganisms", Russia, Moscow, 2015.
- International scientific and practical conference "Modern problems of biotechnology: from laboratory research to production" within the III international Farabi readings, KazNU, Almaty, 2016
- Materials of the international scientific-practical conference "Actual problems of biotechnology, ecology and physical-chemical biology", KazNU, Almaty, 6-7 April, 2017
- 36th International Conference of the Polish phycological society "Mass development of algae: prospects for biodiversity, ecology and management", Poland, Lublin-lower Kazimierz, 24-27 May 2017
- 37th International conference of the Polish phycological society "Green future: algae-applications and prospects", Poland, Krakow, 21-25 may 2018

Publications

The materials of the dissertation were published in 22 scientific works, including 1 article in peer-reviewed foreign scientific journals indexed in the databases of Web of Science and Scopus with non-zero impact factor; 9 articles in national scientific journals from the list of the Committee for control in the field of education and science of the Republic of Kazakhstan; 12 abstracts in the materials of international conferences and symposia. As a result of research 1 patent for useful model of the Republic of Kazakhstan was received.

The structure of the dissertation

The dissertation work is presented on 134 pages of computer text and consists of symbols and abbreviations, introduction, review of literature, materials and methods of research, results and their discussion, conclusion, list of sources from 239 titles. The scope of work includes 13 tables, 38 figures and 3 annexes.