ANNOTATION

For the dissertation for Doctor of Philosophy (PhD) degree
on the speciality "6D070100 – Biotechnology" of Makpal Torekhanova
on the theme «Bioremediation of fishery wastewater and obtaining feed additives for fish based on microalgae»

General description of the research: The dissertation is devoted to studying the possibility of purifying wastewater from fish farming using microalgae and using microalgae biomass as a feed additive for fish. The study revealed the effectiveness of microalgae in the decomposition of organic and inorganic substances in waste water, the level of water purification, as well as the biochemical composition and nutritional value of the obtained biomass. In addition, the possibilities of using microalgae biomass as a feed additive to improve fish growth and health indicators were evaluated. The results of the work suggest applied solutions aimed at ensuring environmental sustainability in fisheries and improving production efficiency.

The relevance of the research: Currently, aquaculture is considered one of the fastest growing sectors of the food industry worldwide. The development of aquaculture provides the population with an important source of food and reduces the pressure on natural resources caused by fishing. Artificial fish breeding not only ensures sustainable fish production but also helps maintain the biological balance of ecosystems, restore the population of valuable fish species. However, the rapid development of aquaculture, besides its economic efficiency, poses a number of environmental problems. One of the most significant issues is the pollution of the environment by components of fish farm effluents, consisting of nitrogen compounds such as ammonia, nitrite, nitrate, phosphorus, and organic carbon compounds. In this regard, one of the strategically important issues is considered to be the development of waste-free and low-waste technologies, the rational use of natural resources, and the protection of the environment through the application of an ecological systems approach in the development of fisheries.

Microalgae are a valuable asset with great potential in the field of waste-free processing technologies. The use of microalgae in aquaculture is determined by their broad metabolic capabilities and high productivity. Moreover, they are capable of purifying contaminated waters by using organic substances as nutrients found in polluted wastewater. Being photosynthetic organisms, microalgae enrich the aquatic environment with oxygen, accelerate the oxidation processes and mineralization of organic compounds. Additionally, there is a lot of information in the literature about the influence of microalgae on the microbiological characteristics of water due to their bacteriostatic or bactericidal action. Based on the use of microalgae in the bioremediation process of aquaculture water bodies, the possibility of obtaining inexpensive microalgae biomass with nutritional value is considered a promising source for use in fish farming. This is considered

relevant, especially in conditions of acute shortage in the feed industry of high-quality feedstock, in enhancing feed quality, enriching them with various natural biologically active substances. Thus, cultivating microalgae in contaminated wastewater of aquaculture water bodies provides an opportunity to obtain a cheap and economically efficient method of growing microalgae, not only for water purification but also for obtaining a valuable source of various natural products. This approach is also beneficial from an ecological point of view, because in addition to cleaning the aquatic environment, it contributes to the preservation of natural reserves and the sustainable development of Fisheries.

The purpose of the research: Biological treatment of fish farm wastewater based on microalgae and the production of feed additives for fish.

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

1. Studying the possibilities of bioremediation of fish farm wastewater using microalgae;
2. Cultivating microalgae in fish farm wastewater and analyzing the biochemical composition of the obtained biomass;
3. Exploring the potential of microalgae as a feed supplement in fish farming;
4. Determine the economic efficiency of using a biological additive based on microalgae as fish feed.

The research objects and materials: The objects of the study included the cultures of microalgae *Chlorella vulgaris* SP BB-2, *Parachlorella kessleri* Bh2, and *Chlamydomonas reinhardtii Dangeard* CC-124, as well as the fish tilapia (*Oreochromis niloticus*).

Research methods: The study employed biotechnological, microbiological, ichthyological, and physico-chemical methods.

The scientific novelty of the research: The possibility of using the microalgae *Chlorella vulgaris* SP BB-2 for the effective bioremediation of polluted wastewater from fish farms has been demonstrated.

A biomass of microalgae rich in proteins, carbohydrates, and lipids has been obtained for use as a feed additive for fish.

The economic efficiency of using a biological additive based on microalgae as a feed additive for tilapia fish (*Oreochromis niloticus*) has been determined.

Theoretical and practical significance of the research: The microalga *Chlorella vulgaris* SP BB-2 was selected as a culture with high purification potential for the purpose of treating wastewater from fish farms contaminated with various organic substances.

The optimal amount of the biological additive based on the microalga *Chlorella vulgaris* SP BB-2 to be used as a feed additive for tilapia fish (*Oreochromis niloticus*) has been identified.

The economic efficiency of using the biological additive based on the microalga *Chlorella vulgaris* SP BB-2 as a feed additive for tilapia fish (*Oreochromis niloticus*) has been determined.

As a result of this work, a patent was obtained for the invention «Microalgae

strain *Chlorella vulgaris* sp BB-2, perspective for production of biomass and purification of waste water from organo-mineral pollution» Patent number №35781 2021/0211.1.

The main provisions for the defense: The microalgae *Chlorella vulgaris* SP BB-2 possess a high cleansing ability for the purpose of treating wastewater from fish farms contaminated with various organic substances.

The biomass composition of *Chlorella vulgaris* SP BB-2, cultivated in fish farm wastewater, consists of 57.0% protein, 11.4% carbohydrates, and 16% lipids, with the main share of amino acids represented by alanine, arginine, aspartic acid, glutamic acid, lysine, and leucine.

Adding 25% of *Chlorella vulgaris* SP BB-2 biomass as a feed additive to fish diets increases fish farm productivity.

Adding 25% of *Chlorella vulgaris* SP BB-2 biomass as a feed additive to fish diets improves the external and internal quality indicators of tilapia (*Oreochromis niloticus*).

Key research findings and conclusion:

1. Among the studied microalgae *Chlorella vulgaris* SP BB-2, *Parachlorella kessleri* Bh2, and *Chlamydomonas reinhardtii Dangeard* CC-124, the microalgae *Chlorella vulgaris* SP BB-2 demonstrated high growth rates in fish farm wastewater.

2. In evaluating the effectiveness of microalgae strains in treating fish farm wastewater, it was found that the concentrations of COD, ammonium nitrogen, and phosphorus decreased in the water composition, and microbiological characteristics improved, particularly a 70-75% reduction in HPC and a decrease of 5 in the coliform index. The biomass composition of *Chlorella vulgaris* SP BB-2, cultivated in fish farm wastewater, consists of 57.0% protein, 11.4% carbohydrates, and 16% lipids, with the main share of amino acids represented by alanine, arginine, aspartic acid, glutamic acid, lysine, and leucine. The lipid extract of the *C. vulgaris* SP BB-2 strain consisted of 30.7% saturated and 69.3% polyunsaturated fatty acids, with a total fatty acid content of 90.2 mg/g.

3. The microalgae biomass added to tilapia feed in various concentrations, including a 25% microalgae-based feed additive of *Chlorella vulgaris* SP BB-2, positively influenced the growth and development of morphophysiological indicators of tilapia. It was found that adding 25% of *Chlorella vulgaris* SP BB-2 biomass as a dietary supplement for fish led to an increase in maximum weight gain by 102 g and average daily gain by 1.46 g compared to the control group by approximately 13%. The microalgae-based biological additive for tilapia (*Oreochromis niloticus*) had good external and internal indicators. It was proven that the experimental group fish had high commercial quality based on internal indicators such as the length of the intestine, stomach, heart, spleen, and liver. It was found that the microalgae-based biological additive increased the biochemical indicators of tilapia (*Oreochromis niloticus*) blood, with the protein content in the blood serum of the experimental group being 14% higher compared to the control.

This confirms that feed supplemented with microalgae biomass is a source of additional protein and other components.

4. According to histological studies, the control groups showed stromal swelling, epithelial desquamation, and changes in goblet cell sizes from 11.37 μm to 24.56 μm, whereas in the group receiving additional microalgae, no swelling was observed, which contributed to an increase in the cellular composition of the submucosal layer and the maintenance of the integrity of the intestinal crypts and epithelial cells. Furthermore, it was found that the proportionality of goblet cell sizes ranged from 10.15 to 10.55 μm.

5. It was shown that in the intestinal microflora of tilapia (*Oreochromis niloticus*) using a microalgae-based biological mixture, the percentage of *Lactobacillus* species increased, and the amount of *Aeromonas* and *Pseudomonas* species decreased compared to the control group.

6. The microalgae-based biological additive *C. vulgaris* SP BB-2 increases production profitability by 25%.

The contribution of author for the results described in the dissertation: The analysis of literature data on the researched problem, the setting of research goals and objectives, the conduct of experimental research, the analysis of the obtained results, and statistical processing, as well as the writing of the dissertation, were carried out independently by the author. In addition, in order to ensure the reliability of the methods used in the study and the results obtained, work was carried out in accordance with the standards of laboratory analyzes.

Research approbation: The research results and the main principles of the dissertation were presented and discussed at the following international scientific conferences and symposiums:

- At the international scientific conference for students and young scientists "Farabi Alemi," Almaty, Kazakhstan, 2019;
- At the international scientific conference for students and young scientists "Farabi Alemi," Almaty, Kazakhstan, 2020;

Publications: The main composition of the dissertation is reflected in 11 published works, including 2 articles in peer-reviewed foreign scientific journals indexed in Web of Science or Scopus databases with an impact factor of at least zero, 4 articles in republican scientific journals from the list of the Committee for Control in the Field of Education and Science of the Republic of Kazakhstan and 4 theses at international conferences. According to the results of the study, a patent for invention No.357812021/0211.1 was obtained on the topic «Microalgae strain *Chlorella vulgaris* sp BB-2, perspective for production of biomass and purification of waste water from organo-mineral pollution».

Dissertation structure: The dissertation consists of 92 pages of computer text, symbols and abbreviations, an introduction, a literature review, research materials and methods, research results and their discussion, a conclusion, and 170 sources used. The work includes 21 tables, 16 figures, and 2 appendices.