REVIEW
the official reviewer for the dissertation work Huma Balouch
on the topic "Study of biodiversity of microalgae of Almaty region and prospecting for biotechnological valuable strains", submitted for the degree of Doctor of Philosophy (PhD) in the specialty "6D070100-Biotechnology".

№	Criteria	Compliance with the criteria (one of the answer options must be checked)	Justification of the position of the official reviewer
1.	The topic of the thesis (as of the date of its approval) corresponds to the directions of development of science and / or state programs	1.1 Compliance with priority areas of science development or government programs:	The dissertation research corresponds with the following priority areas of science development: **5.1 Biotechnology in agriculture and environment protection** The dissertation demonstrated a detailed examination of the biosorption interactions between microalgae cell organelles and heavy metals, providing future perspectives of physicochemical and genetic approaches that could be used to explore the bioindication as well as phytoremediation process for microalgae in terms of removal efficiency or selectivity for a targeted metal contaminant. **5.2 Biotechnology in medicine:** The study reported antibacterial activity in microalgae against wide range of pathogenic bacteria (gram positive as well as negative) that makes them of great choice for drug discovery and drug development. Lipid and GC/MS analysis reported in this dissertation revealed the high-value bioactive substances in microalgae strains such as carotenoids, phenolics, polyunsaturated fatty acids, terpenes, polyacetylenes, sterols, aromatic organic acids, and aldehydes that are closely related to a range of pharmacological activities including, antimicrobial, antioxidant, antiviral, antitumor, anti-inflammatory, and anti-allergy effects. Thus the research falls in following sub-categories of biotechnology in medicine. 5.2.2 Molecular, genomic, cellular and bioinformation

			technologies for the development of applied biology of personalized medicine; 5.2.4 New technologies and biologically active substances for solving the problems of ante- and postnatal development, aging, prolongation of human life; 5.2.6 Study the prevalence and mechanisms of occurrence of infectious diseases dangerous and relevant for Kazakhstan, the development of effective means of their control, the development of alternative means of combating antibiotic-resistant microorganisms and drug-resistant viruses. **5.3 Development of domestic pharmaceutical science and industrial biotechnology:** 5.3.1 Creation of new domestic, original, medicinal, diagnostic and prophylactic drugs and treatment methods for import substitution and development of the pharmaceutical industry in Kazakhstan; 5.3.2 Technologies for obtaining valuable components from plant, animal and mineral raw materials using biotechnological methods.
		1) The dissertation was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program) 2) The thesis was completed within the framework of another state program (indicate the name of the program) 3) The thesis corresponds to the priority direction of the development of science, approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction)	The dissertation research work was undertaken under the framework of three projects including 'AP08052402- Development of technology for obtaining bio-fertilizers based on nitrogen-fixing cyanobacteria'; AP08052481- Development of a technology for producing biodiesel based on active strains of microalgae'; 'AP05131743- Development of scientific and methodological foundations for biomonitoring technology and forecasting the state of polluted aquatic ecosystems using phototrophic microorganisms. Corresponds to the priority direction of development of science "Rational use of natural resources, processing of raw materials and products."
2.	Importance for science	The work **makes** / does not make a significant contribution to science, and its importance is **well disclosed** / not disclosed	The work makes a significant contribution to science, its importance is well disclosed. The work explored multifaceted role of microalgae – potential utilization for use as feedstock for biofuel, potential effectiveness as the antibacterial agent and bio-indicator. The widespread applications of studied microalgae

			strains have not been previously extensively studied. The author used the right analytical tools combining classical as well as modern approach, isolated promising microalgae strain with high lipid productivity and relatively high content precursor for biofuel. Moreover, study exhibited effective inhibitory activity of microalgae strains against wide range of pathogenic bacteria, and also revealed the sensitivity of microalgae to metal contamination. The reliable and efficient use of microalgae in the biomonitoring of the aquatic environment has been proven.
3.	The principle of independence	Self-reliance level: 1) **High**; 2) Medium; 3) Low; 4) No independence	The author carried out all of the experiments herself. The doctoral student demonstrated a great level of independence in work. Balouch H. obtained novel and significant outcomes and scientific provisions submitted for defence were independently substantiated and proven, justifying the relevance of the research topic.
4.	The principle of inner unity	4.1 Justification of the relevance of the thesis: 1) **Substantiated**; 2) Partially justified; 3) Not substantiated.	The dissertation's relevance is fully established. The maximum potential of diverse strains of microalgae has been tapped with applications ranging from biofuel production; production of novel bio-active compounds, antibiotics; and metal contamination detection.
		4.2 The content of the thesis reflects the topic of the thesis: 1) **Reflects**; 2) Partially reflects; 3) Does not reflect	The dissertation's content accurately reflects the topics, reported and discussed the findings on initial biodiversity study on native microalgal strains, pure culture isolation and accurate identification, biomass analysis for biofuel potential, evaluation of antibacterial activity of microalgae's crude extract and isolates' susceptibility to heavy metal.
		4.3. The purpose and objectives correspond to the topic of the thesis: 1) **correspond**; 2) partially comply; 3) do not match	The thesis's goal and objectives are in line with the topic. All steps of achieving the objectives are covered by tasks, finding promising strains of microalgae with potential biotechnological applications with a particular focus on high-value added lipids and biodiesel, healthcare applications, and ecological assessment.
		4.4 All sections and provisions of the thesis are logically	The thesis' sections and provisions are all logically linked

		interconnected: 1) **fully interconnected**; 2) the relationship is partial; 3) there is no relationship	together. The direction and the current state of research in this field is thoroughly discussed. The methods are discussed in detail. The research findings are organised in a logical order and accurately depict the complete goal-achieving process, step by step.: promising microalgae strains were isolated and cultured, isolates represented valuable resource for precursor fatty acids for biofuel production, significant antibacterial activity was displayed by four strains, high sensitivity of the microalgae strain to metal contamination (low concentrations of cadmium) were investigated and proved. The main findings and a list of cited literature are well presented.
		4.5 New solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with known solutions: 1) **there is a critical analysis**; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors	At every level, the author's methodologies and proposed new solutions are substantiated and evaluated in contrast to existing ones: obtaining pure axenic cultures of microalgae isolates, lipids analysis and FAMEs profiling, evaluating the biofuel antibacterial properties and ability in bio-indication against heavy metal, effectiveness of the methods used and the materials obtained.
5.	Scientific novelty principle	5.1 Are the scientific results and provisions new? 1) **completely new;** 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The research results put forward as novelty are substantiated and new both in theoretical and practical terms. FAMEs profile of two strains *Parachlorella kessleri* ZBD-04 and *Ankistrodesmus falcatus* ZBD-03 resulted in Cetane number more than 47, which is an ideal component and number to be considered for biodiesel. Effective antimicrobial activities of four strains, including few lesser-known, against both gram positive and gram negative bacteria have been shown.
		5.2 Are the dissertation findings new? 1) completely new; 2) **partially new (25-75% are new);** 3) not new (less than 25% are new)	The conclusions regarding the assessment of the diversity of the algal flora should be formulated in more detail. The predominance of Bacillariophytes, Cyanobacteria and Chlorophytes is common in normally functioning water bodies. The use of specific methods for assessing biodiversity, such as the models of Shannon-Weaver, Jacquard and others, would make it possible to reveal in more detail the degree of taxonomic diversity of the algal flora. The conclusions regarding biotechnology research are new: the studied isolates have new compounds with biofuels and antimicrobial properties, which may lead to the discovery of completely new

			classes of metabolites and, therefore, are considered more valuable.
		5.3 Technical, technological, economic or management decisions are new and reasonable: 1) **completely new;** 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The technological solutions to the given aims and objectives of dissertation are innovative and well-supported. The advanced methodological approaches employed allowed for the creation of unique results.
6.	The validity of the main findings	All main findings **are based** / are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities)	The dissertation include evidence-based conclusion supported by reliable scientific data and clear interpretation of results and highly researched information from several credible sources highlighting concise significance of the study.
7.	The main provisions for the defense	All main findings are / are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities) It is necessary to answer the following questions for each provision separately: 7.1 Is the position proven? 1) **proven;** 2) rather proven; 3) rather not proven; 4) not proven	1 The majority of species, making up the phytoplankton community in freshwater bodies of Almaty region, were diverse and showed significant variations among different taxa.: The findings for this provision revealed that Bacillariophytes, Cyanophytes, and Chlorophytes comprising 283 genera dominated practically all freshwater bodies, with the greatest diversity seen in the lakes Balkhash and Alakol with higher saprobic index and anthropogenic activities. Results of identification and isolation of seven axenic monoculture of microalgae isolates also presented in the dissertation. 2) Six microalgal species, based on parameters for fast growth, were cultivated and their total lipid and FAME profiles indicated the high potential of two strains *Parachlorella kessleri* ZBD-04, and *Ankistrodesmus falcatus* ZBD-03 for use as feedstock for biofuel based on their calculated biodiesel properties. This provision is proved on the basis of the data and chromatogram obtained for isolated strains *P. kessleri* showing the highest biomass production, lipid productivity and the high content of fatty acids C16 - C18, followed by *A. falcatus* and *M. griffithi*. 3) High potential of microalgal isolated strains for biotechnological application in different aspects and their utilization as raw-material for the production of high-value

			products such as antibiotics. This provision is proved based on the results of significant antibacterial activity noted of the methanol extract from the biomass of the isolated strains *Parachlorella kessleri* against *B. subtilis, S. aureus* and *K. pneumonia; Nephrochlamys subsolitaria* against *B. subtilis, P. aeruginosa* and *E. coli; Monorafidium sp.* against *K. pneumonia; Monoraphidium griffithii* and *Ankistrodesmus falcatus* against *K. pneumoniae* and *E. coli.* 4) Accumulation of heavy metals in cells of *Ankistrodemus sp.* and the observed ultrastructural changes indicated their sensitivity to the presence of potentially toxic heavy elements in the environment, thus indicative of water pollution. This provision is proved based on results of the influence of cadmium ions on the growth, photosynthesis and ultrastructure of cells of the microalga *Ankistrodesmus sp. B-11*. The abundance of *Ankistrodesmus sp. B-11* was significantly reduced when cadmium was added to the nutrient medium at a dose of 0.005–0.02 mg / l. The addition of cadmium at a concentration of >0.05 mg/L inhibited cell development completely.
		7.2 Is it trivial? 1) yes; 2) **no**	Dissertation provisions are not trivial. All provisions were based on a 'finding solutions' approach and each aspects of the research process are viewed from the perspective of modern knowledge in the most dynamic areas of modern biotechnology.
		7.3 Is it new? 1) **yes;** 2) no	The provisions and results based on data generated from research advance the current knowledge and serves to increase utilization of the potential of microalgae in several orders of magnitude.
		7.4 Application level: 1) narrow; 2) medium; 3) **wide**	The application level of dissertation is wide. The dissertation recognized the potential of microalgae for exploitation in different fields including pharmaceuticals, ecology and renewable energies.
		7.5 Is it proven in the article? 1) **yes;** 2) no	The proof of all provisions is confirmed by the publication of research results in 1 research article with impact factor, indexed in Web of Science (WoS) and SCOPUS, 4 articles in scientific journals recommended by Education and Scientific Monitoring

			Committee of Ministry of Education and Science of the Republic of Kazakhstan (CCESF MES RK), 4 abstract in the materials of international conferences. Two articles are under process of publication in reputable international peer reviewed scientific journals.
8.	The principle of reliability Reliability of sources and information provided	8.1 Choice of methodology - justified or methodology detailed in sufficient detail 1) **yes;** 2) no	The best possible methodological approach was used relating acuurately to the central purpose of dissertation and described in detail.
		8.2 The results of the dissertation work were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies: 1) **yes;** 2) no	The outcomes of the dissertation research were produced through the use of modern scientific research methods including computer-aided pattern recognition model, computer-assisted database construction, software-based search and identification modules that facilitated obtaining the most efficient identification, characterization and quantification analysis.
		8.3 Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by experimental research (for areas of training in pedagogical sciences, the results have been proven on the basis of a pedagogical experiment): 1) **yes;** 2) no	Experimental research and the obtained results based on reliable and highly standardized experimental parameters have fully established and corroborated the identified relationship and patterns, as well as the anticipated theoretical conclusions.
		8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature	All important statements are evidence-based supported and confirmed by citations to current and credible scientific literature.
		8.5 Used literature sources are sufficient / not sufficient for a literature review	A vast number of contemporary literary sources were used to completely cover current research linked to the dissertation's topic, substantiating the research's direction, practical value, and novelty.

9	Practical value principle	9.1 The thesis has theoretical value: 1) **yes**; 2) no	The dissertation is of high theoretical importance, considering the importance of microalgae in environmental and ecological processes and the potential wide range uses of its biomass. Therefore, research on the biodiversity study and prospecting for biotechnological valuable strains of microalgae is extremely relevant.
		9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice: 1) **yes**; 2) no	This dissertation is extremely important in terms of its practical implications. The research findings revealed microalgae as viable solution to many scientific, environmental and economic challenges and can be implemented through the creation of new microalgae-based products. From a practical perspective, locally isolated microalgal strains have been investigated to reflect the potential as renewable biodiesel feedstock, antibacterial agent and bioindication candidate. These isolates yield multipurpose biomass with a high lipid and unique FAME content including carotenoids, sterols, PUFA which have nutritional and medicinal applications as well. Further focusing on the efficacy of growing these microalgal isolates, and assessing the conditions under which their growth and lipid production can be optimized, these isolates bode well for renewable, sustainable and economical sources of biofuels, bioactive medicinal products.
		9.3 Are the practice suggestions new? 1) **completely new;** 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The study identified and preserved new isolates of microalgae, and successfully explored their biotechnological potentials. Remarkable diversity of extracellular metabolites found in the local isolates are worthy to be recovered as bioactive substances using high-throughput bioassays for further use at commercial level.
10.	The quality of writing and design	Academic writing quality: 1) **high;** 2) average;	The conducted research is well-documented in concise, balanced, and logically structured dissertation that is easy to understand for even non-familiar reader. The dissertation demonstrates the

		3) below average; 4) low.	understanding of doctoral student in the different sub-disciplines and dissertation in systematic way, flowing from general to specific and from theory to practice.

Based on the above mentioned review for dissertation titled "Study of biodiversity of microalgae of Almaty region and prospecting for biotechnological valuable strains", I agree to award Huma Balouch the degree of Doctor of Philosophy (PhD) in the specialty "6D070100-Biotechnology".

Official Reviewer:
Deputy Director for Science of the Astana Botanical Garden,
a branch of the "Institute of Botany and Phytointroduction"
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