

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 speciality
"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	<p>1.1 Compliance with priority areas of science development or government programs:</p> <p>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)</p> <p>2) The thesis was completed within the framework of another state program (indicate the name of the program)</p> <p>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)</p>	<p>The microalgae are attracting a great deal of interest from researchers as well as industrialists, owing to potential for obtaining new and efficient products of biotechnological importance. The work is compliant with priority areas of science in Kazakhstan across the fields of biomedical science and technology, research in the field of renewable and bio-based energy, agricultural science, innovative technologies and advanced methods in the processing of bio-based materials; life sciences; and environmental protection.</p> <p>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.</p> <p>It is important to note the full compliance with the direction of science for industry, including under the program: Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020-2025.</p>
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 conducted preliminary study on biodiversity of microalgae in Almaty region including new strain. This work reviewed and evaluated some of the

			<p>prominent applications of native microalgae by focusing on such as bioenergy, antibiotic potential and bioindication for aquatic ecosystem. Moreover, the work strives to assesses the whole-cell as well as applications of microalgae extract including revealing the existence of valuable constituents, such as saturated fatty acids and terpenoids in microalgae strains, that also provide additional value other than obtaining biofuel (Cetane number more than 47) and contribute to interesting biological activities, including strong antibacterial activity. Limited amount of knowledge on the antibacterial and biotechnological potential of the studied strains is previously available. The research work showed microalgae as convenient material to be used in biomonitoring based on its comparatively high sensitivity to metal contamination at low concentration.</p>
3.	The principle of independence	<p>Self-reliance level: 1) High; 2) Medium; 3) Low; 4) No independence</p>	<p>The experimental work has been undertaken by the author independently. The degree of self-reliance is noted higher.</p>
4.	The principle of inner unity	<p>4.1 Justification of the relevance of the thesis: 1) Substantiated; 2) Partially justified; 3) Not substantiated.</p>	<p>The dissertation's relevance is high and importance emphasized in selection of topic. The dissertation elucidated that locally isolated microalgae from fresh water bodies could be the most prolific photosynthetic biomass as an alternative renewable resource with the added advantages from an ecological and biotechnological perspective.</p>
		<p>4.2 The content of the thesis reflects the topic of the thesis: 1) Reflects; 2) Partially reflects; 3) Does not reflect</p>	<p>The dissertation's overall content reflect the topic and thesis statement. The dissertation is presented with well-articulated introduction, a reasoned and critical review of previous research literature, comprehensive materials and methods, evidence-based research results and discussion with future implications of findings, conclusion and list of used sources. Each section and part tie back to thesis, support the statement of purpose with main results and details as the dissertation progress.</p>

	<p>4.3. The purpose and objectives correspond to the topic of the thesis:</p> <ol style="list-style-type: none"> 1) correspond; 2) partially comply; 3) do not match 	<p>The purpose and objectives are well thought out and clearly expressed, and corresponded to the topic of thesis, aiming at finding and accurately identifying the most promising microalgae strains with higher potential (i) to be used as raw-material source for biofuel, potentially replace and reduce unsustainable conventional raw material such as crops and (ii) to be used as natural product source for developing wide range effective antibiotics (iii) performing bioassays to evaluate its biochemical composition and molecular mechanisms against cytotoxic effect of Heavy Metals.</p>
	<p>4.4 All sections and provisions of the thesis are logically interconnected:</p> <ol style="list-style-type: none"> 1) fully interconnected; 2) the relationship is partial; 3) there is no relationship 	<p>The thesis' sections and provisions are all logically linked 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.</p>
	<p>4.5 New solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with known solutions:</p> <ol style="list-style-type: none"> 1) there is a critical analysis; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors 	<p>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.</p>
<p>5. Scientific novelty principle</p>	<p>5.1 Are the scientific results and provisions new?</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) 	<p>Scientific results and provisions are completely new. Results provide an important information of microalgal diversity and presence in Almaty region and also reflect the resourcefulness of the promising under-explored strains. Lipid analysis and fatty acids analysis depicted the potential of microalgae for number of metabolites with various application in bioenergy, environment</p>

		and pharmaceutical industry.
	<p>5.2 Are the dissertation findings new?</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) 	<p>All the conclusions of the thesis are completely new: the dissertation findings exhibited research work at molecular, cellular and organism level and applications of microalgae in different disciplines that complement each other. The dissertation encompasses whole range of activities from identification and sequencing of microalgae strains to factually exploring the pharmaceutical applications, biofuel applications, genetic and outlook the scope of the microalgal biotechnology industry.</p> <p>Completely new and across multi-disciplines.</p>
6.	<p>5.3 Technical, technological, economic or management decisions are new and reasonable:</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) <p>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)</p>	<p>All main findings and conclusion are based on scientifically significant credible results obtained from using computational tools and presented in a consistent, explicit, factual and objective manner. Evidence-based approach has been used for the interpretation of results which support the conclusions.</p>
7.	<p>It is necessary to answer the following questions for each provision separately:</p> <p>7.1 Is the position proven?</p> <ol style="list-style-type: none"> 1) proven; 2) rather proven; 3) rather not proven; 4) not proven <p>7.2 Is it trivial?</p> <ol style="list-style-type: none"> 1) yes; 2) no <p>7.3 Is it new?</p> <ol style="list-style-type: none"> 1) yes; 2) no <p>7.4 Application level:</p>	<p>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.</p> <p>i) – this provision is proved by the data presented on preliminary evaluation of biodiversity in freshwater bodies of Almaty region, characterization and molecular identification of selected isolates/strains;</p> <p>ii) This provision is not trivial, biodiversity preliminary assessment is fundamental to explore the relationship between freshwater biodiversity and ecosystem services, and better understand how biodiversity theoretically might influence the rate and stability of ecosystem functionality;</p> <p>iii) New: the native microalgal consortia have never been explored up to now at many fresh water reservoirs of Almaty region</p>

- 1) narrow;
- 2) medium;
- 3) wide

7.5 Is it proven in the article?

- 1) yes;
- 2) no

simultaneously. New isolates have been accurately identified and database has been added to NCBI BLAST.

iv) wide application level, knowledge of the microalgae species inhabiting the freshwater bodies is limited. The work has provided described and predicted taxonomic genera of microalgae to be found in Almaty region.

v) proven in the article: article has been published in this regard.

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.

i) proven: The results of the lipid content of the studied isolates, and the fatty acid methyl ester (FAME) composition investigated using GC/MS are given in provided chromatogram showing a high proportion of long-chain fatty acids.

ii) is not trivial: the work confirmed the potentiality and the beneficial utilization of microalgae for biodiesel production.

iii) new: unusual free fatty acids and composition identified in most of the studied isolates.

iv): the obtained free fatty acids have wide application level with implications in food, pharmaceutical, cosmetics industry, and biofuel production.

v) proven in the article: article for lipids analysis not cited.

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.

i) Proven: the results of disk diffusion and MIC test successfully screened crude extracts of microalgae isolates for antibacterial activity using different concentrations and significant results were reported.

ii) is not trivial: the results showed potential use of microalgae as antibacterial agents against gram negative and gram positive

bacterial pathogens of human, thus holds tremendous potential as solution to the problem of antibiotic resistance.

iii) New: the antibacterial activity of crude extract microalgae isolates against gram negative bacteria has not been much reported as the membrane structure of gram negative bacteria is complex. The ability of native microalgae to act upon the cell membrane shows the likely presence of novel metabolites.

iv) wide application level: the novel antibacterial compounds of freshwater microalgae origin generally possess antioxidants having application in various areas such as pharmaceutical, human or animal nutrition, and cosmetic industry.

v) proven in the article: article has been published in this regard.

4) Accumulation of heavy metals in cells of *Ankistrodesmus 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.

i) proven: this provision proof is based on the results of ultrastructural and physiological effects of exposure of cadmium (Cd) on cultured cells of *Ankistrodesmus sp.* B-11 revealing its high sensitivity at low concentration of heavy metal. The dissertation presented ultrastructure alteration (in thylakoid membrane) and molecular mechanism for reduced Chl and negatively effecting PSII and photosynthetic efficiency.

ii) is not trivial: Assessing the harmful effects of heavy metals on microalgae are of particular significance because microalgae play such an important role as primary producers and the first link in the food chain of aquatic environment.

iii) new: the strain *Ankistrodesmus sp.* B-11 has been reported for the first time as bioindicator along with details regarding the high sensitivity and response mechanism against heavy metal contamination.

iv) wide application level: heavy metal contamination lead to changes in biological balance of ecosystem. The timely

	<p>assessment of such imbalance using microalgae is of great importance for the ecosystem stability. v) proven in the article: article has been published in this regard.</p> <p>Appropriate research design and methods are adopted and described in detail providing a comprehensive overview of all the research process yielding maximum relevant information. The study utilized emerging approaches and analytical tools considering their advantages, relevance and applicability to identify and assess the microalgae strains, their biochemical composition and molecular mechanism.</p> <p>The dissertation research procedure for processing and interpreting data are sufficiently advanced, adequate described in sufficient detail to allow other researcher to repeat the research for further advancement, yielding results as objective as possible. Several computer software programs and algorithm have been used to conduct hierarchical cluster analysis, principal component analysis, library management & identification, and comparative quantification & polymorphism analysis.</p> <p>All relevant theoretical conclusions, patterns, and results have been reported concisely and objectively, and confirmed by experimental research conducted by integrating experimental data and computational results.</p> <p>All major statements based on the data obtained are supported and confirmed by primary and secondary scientific literature.</p>
<p>8.1 Choice of methodology - justified or methodology detailed in sufficient detail 1) yes; 2) no</p>	<p>The principle of reliability Reliability of sources and information provided</p>
<p>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</p>	<p>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</p>
<p>8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature</p>	<p>8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature</p>

9	<p>8.5 Used literature sources are sufficient / not sufficient for a literature review</p> <p>9.1 The thesis has theoretical value: 1) yes; 2) no</p> <p>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</p>	<p>Sufficient and recent literature source has been given which has analyzed and critically evaluated a clear picture of the state of knowledge on the topic of dissertation.</p> <p>The significance of theoretical framework of dissertation is high and complement the research. All theories and explanations provided more solid logic and demonstrated the importance of studying and exploring indigenous microalgae and their biotechnological potentialities for future industrial-scale valorization in biotechnological fields.</p>	<p>The work provided new practical suggestion and recommendations. The indigenous microalgae from Almaty region are a potential source of biomass, which may have great biodiversity and consequent variability in their biochemical composition. The work demonstrated microalgae as next-generation resources with the potential to present high-tech, low-cost, and environmentally friendly solutions to address urgent industrial and agricultural demands.</p> <p>By correlating the data of several fatty acids identified in the study obtained by using GC-MS, with transcriptome, it will serve as valuable resource for discovering novel genes responsible for the biosynthesis and metabolic engineering. The suitability of studied indigenous microalgae strains as biodiesel feedstock based on iodine value, oxidation stability, and Cetane number suggest great biofuel potential, promising for biofuel production economically viable and environmentally sustainable.</p> <p>The antibacterial activity of studied microalgae strains against wide range of pathogenic bacteria holds its practical significance as candidates in novel, antibacterial drug discovery. The work also established the isolates' sensitivity/resistance spectrum, and molecular response mechanisms against heavy metals Zn and Cd which emphasized additional research to identify, isolate, and characterize more indigenous strains with potential to have environmental</p>
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	1) yes; 2) no		applications.
	9.3 Are the suggestions for practice new? 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new)		Completely new
10.	The quality of writing and design Academic writing quality: 1) high; 2) average; 3) below average; 4) low.		High Flow of results and discussion is considerably high, however, the better use of tables to present data can be made.

Based on the above, I propose to award Huma Balouch the degree of Doctor of Philosophy (PhD).

Official Reviewer:

Acting Professor of the Department of Management and Engineering in the Field of Environmental Protection, Gumilyov Eurasian National University, Candidate of Biological Sciences, Associate Professor



Akbayeva Lyailya

