2.2 Research project description template

EURIZON FELLOWSHIP PROGRAMME

"Remote Research Grants"

Valid applications need to be submitted through the website: https://indico.desy.de/event/38700/ by the Principal Investigator of the Ukrainian team before May 8th 2023 at 12:00 Pm (noon) CEST time. Before applying please read carefully the Terms of Reference (ToR).

Title[1] of the research project: *Biofuel Cells Based on Microbial Enzymes and Nanocomposite Materials for Powering Microelectronic Devices*

submitted by

PRINCIPAL INVESTIGATOR (PI)[2]:

First name and Family name (English)	*Mykhailo Gonchar*

Table of Contents:

[1] Please verify that this information corresponds to the one reported in section 1 of the online application form.
[2] Please verify this information corresponds to the one reported in the section 4 of the online application form.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871072.

2.2 Research project´s full description* *(Max 1200 words)*

The search for new renewable energy technologies and approaches for the utilization of technogenic by-products (often toxic) are the main problems of modern science. In this regard, the development of biofuel cells (BFCs) based on microbial enzymes and cells capable of utilizing the by-products as substrates with the simultaneous generation of electricity can help to solve these two actual problems – cheap renewable energetics and environmental protection [1]. BFCs are novel bioelectrochemical devices that use living microorganisms or their enzymes to generate electricity through the enzymatic oxidation of organic substrates [2-3]. This innovative technology has a number of potential applications: generation of renewable energy, especially for powering electronic mini- and microdevices [4-6], production of bio-hydrogen, wastewater treatment, water desalination, etc. [7]. Notably, compared to fuel cells based on metal catalysts, the BFCs are capable of converting chemical energy into electricity using biological catalysts. This technology offers several advantages over traditional batteries, including the use of renewable and clean catalysts, the selectivity of the reaction, fuel flexibility, and the ability to operate at a milder temperature [8]. All these advantages would lead to an economically viable process in the future. BFCs are highly promising due to a combination of desirable technological features, such as efficiency, selectivity and biocompatibility of the enzymes. Therefore, they could power implantable medical devices [9-10].

The main aim of the project is the development of new BFSs based on purified microbial enzymes immobilized onto electroconductive nanocomposite materials. As substrates of enzymatic reactions and primary energy sources, it is planned to use low-cost and often toxic technogenic by-products for which there is a problem with their utilization, in particular: methanol and formaldehyde – for the yeast alcohol oxidase (AO) and phenol derivates – for the fungal laccase. Therefore, the project intends to combine two scientific goals – cheap renewable energy and environmental protection. As the sources of the enzymes, mutant yeast strains and classically selected fungal producers will be used, constructed/selected at the Department of Analytical Biotechnology (DAP), Institute of Cell Biology, NAS of Ukraine. The mentioned above enzymes have useful biotechnological characteristics (high thermostability, high affinity for target substrates) and are easily isolated by simple and cheap procedures developed at DAP. The designed biofuel cells, after their characterization, will be tested for powering microelectronic sensor devices. For this purpose, we will specifically design small-size (a few mm^2) biofuel cells compatible by their size with electronic chips. The application of nanoparticles in combination with the enzymes (AO or laccase) at BFCs construction will allow us to significantly improve the catalysis rate, both of the anode and cathode reactions, as well as will increase the operating time of the constructed BFCs up to several months. So, the expected specific power densities of constructed enzymatic BFCs will be in the range from 1.2 to 5 $mW \cdot cm^{-2}$.

The research program consists of the following tasks:

Milestones

Task 1. Cultivation of yeast and fungal cells; isolation and purification of oxidoreductases (alcohol oxidase and laccase) – promising biocatalysts for the construction of biofuel cells (BFCs)

1.1 Screening of mushroom fungi by their ability to overproduce extracellular laccase in liquid cultures. Cultivation of mutant yeast cells and selected mushrooms, overproducing the target

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oxidoreductases, alcohol oxidase and laccase.
The optimal conditions for the cultivation of the producers will be determined, ensuring the maximum yield of the target enzymes (composition of the culture medium, intensity of aeration, temperature, time of cultivation, etc.).
1.2 Isolation, purification, enzymatic and electrochemical characterization of the enzymes: alcohol oxidase (AO) and laccase.
Enzymes isolated from the mutant yeast cells and the culture medium of the selected fungi will be concentrated and/or purified using ion exchange and/or affinity chromatography. Purified preparations of AO and laccase will be investigated using different approaches: electrophoretic (to test their purity), spectrophotometric (to determine kinetic parameters – KM and k_{cat}) and electrochemical (activity toward electron-transfer mediators, determination of redox potential).

Duration: 01.07.2023 to 31.08.2023.
Task 2. Design of laboratory prototypes of microbial BFCs by the use of nanomaterials and study of their functional characteristics
2.1. Synthesis of nanocomposite materials with a high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron-mediator activity.
To improve communication between the biocatalyst and the electrode, screening and additional modification of the synthesized nanomaterials by inorganic electron transfer mediators (synthetic dyes, electrically conductive films, ferrocene, cyanoferrate derivatives, etc.) will be carried out.
2.2. Design of BFC laboratory models based on mutant yeast and selected fungal cells producing oxidoreductases.
The anode of microbial BFC will be modified with selected nanocomposite materials with the best electron-mediator activity. Carbon electrodes modified with different types of Pt (electrodeposited Pt, Pt nanoparticles, platinum black) are planned to be used as the cathode material. The electrodes with immobilized laccase or nanozymes will also be used as cathodes. To provide more efficient communication between cellular redox systems and the electrode surface, yeast and fungal cells will be used in a permeabilized state.
2.3. Investigation of the functional characteristics of microbial BFCs.
The functional characteristics of engineered microbial BFCs will be evaluated by the use of inexpensive organic substrates and some by-products for which there is a problem of remediation, in particular, methanol and formaldehyde – for the AO-overproducing mutant yeast strain. The possible advantages of the constructed laboratory models of new microbial BFCs based on nanocomposites and enzyme-producing cells will be investigated (high current and power density, long operating time).
Duration: 01.09.2023 to 31.12.2023.

Task 3. Design of laboratory prototypes of enzyme BFCs on the basis of purified oxidoreductases and nanocomposites and study of their operational parameters
3.1. Investigation of the possibility of using selected nanomaterials as an immobilization matrix for covalent or physical binding of biocatalysts on the electrode surface.
In order to ensure a high local concentration of the BFC biocatalyst and to increase its stability, screening for covalent and physical immobilization of the purified preparations of AO and laccase on the electrode surface will be carried out.

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3.2. Investigation of the basic operational parameters of enzyme-based BFCs.
The primary operational parameters will be investigated for the designed laboratory prototypes of enzymatic BFCs, based on AO and laccase: current density, power density and maximum operating time. The most effective BFC variants will be selected and compared with existing enzyme-based analogues.
Duration: 01.01.2024 to 30.04.2024.

Task 4. Testing of the best-selected BFCs for the powering of microelectronic chips.
The developed enzymatic as well as the cell-based BFCs will be connected to the microelectronic sensor and the sensor will be operated autonomously powered only from the BFC. Autonomously operating microelectronic devices continuously powered by small BFC will be beneficial for various environment-monitoring functions, including biosensors designated specifically for homeland security. Further miniaturization of the sensor device and connected BFC would allow their operation as implantable biomedical devices. The microelectronic device can accommodate the biocatalytic electrodes directly in the structure of the device, thus further reducing the device size and eliminating unnecessary wiring connecting the BFC and the electronic device.
Duration: 01.05.2024 to 31.07.2024.

2.3 COLLABORATION WITH THE EUROPEAN PARTNERS, IMPACT, DISSEMINATION

2.3.1 Description of the collaboration with the European partner(s)[3]*. *(Max 500 words)*

The Institute of Physical Chemistry, Polish Academy of Sciences (IChF PAS) has research, administrative and innovation management competence in thematic of the planned project proposal. The Head of the Nanoelectrochemistry Laboratory, Dr. Wojciech Nogala (PL-PI), is a high level professional in different fields of Chemistry, Nanotechnology and Biotechnology. He has great experience in developing small biofuel cells. The current project represents the combination of two approaches, namely, genetic engineering and nanotechnology and their application to create novel fuel cells. The project deals with Analytical and Physical Chemistry, Biotechnology and Bioelectrochemistry. The topic of the project proposal is crossed with the thematic of the projects recently carried out by the PL-PI, namely, MNiSW Iuventus Plus IP2012 048872 "Nanoelectrodes for single biomolecules and ultrafast electrode processes studies" and NCN Opus 2016/23/B/ST4/02868 "Mechanisms of interaction between silver nanoparticles and biological cells" and is closely related to the investigation of the impact of nanoparticles on the living cells.
In the Department of Electrode Processes, IChF PAS (hereinafter referred as PL), there is modern high-tech equipment for the design and operation of biofuel cells (Potentiostat/Galvanostat Metrohm Autolab PGSTAT30). To characterize the size and morphology of NPs: HPC, SLS, DLS, particle size analyzers, high resolution SEM, TEM, NMR, Perkin Elmer LS 50B Fluorescence spectrometer, dynamic light scattering instrument is available in the Institute labs.
The Department of Analytical Biotechnology, Institute of Cell Biology, National Academy of

[3] The name and contact details of the European partners are to be mentioned in the Application form (Section 3).

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Sciences of Ukraine (further referred as UA) has the material and hardware support for the cultivation of yeast and fungal cells (culture media, sterile boxes, autoclaves, thermostats, thermostatic shakers, disintegrators, centrifuges, lyophilizers; the equipment for isolation, purification of enzymes and study of their physicochemical characteristics and apparatus for conducting electrochemical studies (potentiostats "CHI 1200A" and "PGstat16").

Realization of the current project will make it possible to join the ideas, technical approaches and experimental skills of UA and PL scientists. For the realization of the Project's tasks, it is planned to carry out a number of experiments with the combined efforts of two scientific teams: UA and PL teams.

The implementation of the current project will take place through performing part of the planned tasks by the UA team members at the facilities existing in the PL Institute. In its turn, the PL partner also provides the travel and accommodation expenses for Ukrainian team members during the period of the planned research. It should be mentioned that most of the planned tasks will be performed by the UA team, however, PL partner, in turn, will support the analysis, monitoring and dissemination of the obtained results. Therefore, visiting the Laboratory of Nanoelectrochemistry, IChF PAS in the frame of the project will help to solve UA scientific needs and promote expertise exchange which will be useful in carrying out the planned research focused on the construction of biofuel cells based on enzymes, yeast cells and nanomaterials. Therefore, the expected results of the planned project are beyond the current level of technology and therefore are innovative and realistic for the performance of Ukrainian team members).

2.3.2 Expected outcomes of the research project*. *(Max 300 words)*

In the current project, the new bio-catalytic nano-biomaterials will be used for the construction of enzymatic and microbial biofuel cells. BFCs do not need conventional fuels such as oil or gas and can therefore reduce economic dependence on oil-producing countries. The proposed BFC, being the devices for energy accumulation/transformation, at the same time will be promising for environment bioremediation, in particular, for removing/detoxifying dangerous formaldehyde and methanol from waster-waters.

As a result of scientific cooperation, Polish colleagues will have access to the unique, commercially unavailable, recombinant enzymes obtained by Ukrainian Partner from genetically modified microorganisms. In its turn, the Ukrainian team will take advantage of the Polish Partner's achievements in the field of obtaining novel nanomaterials and will get access to the unique equipment.

As a result of the implementation of the project, new laboratory prototypes of biofuel cells with improved analytical parameters will be created, which are potentially patentable.

Expected results of international cooperation within the Project:

- integration of scientists from two European countries with common intra- and interdisciplinary research;

- methodological enrichment of scientists of two parties;

- preparation of joint projects within European scientific and scientific-technical programs;

- publications in prestigious scientific journals;

- significant educational influence due to the participation of young scientists/post-graduates and students in planned research;

- Career growth of young scientists as a result of publications in prestigious international journals.

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The realization of the current project will support the operations and long-term sustainability of Ukrainian scientists. The obtained results will boost future collaboration and partnership opportunities for Institutes as well as will be the basis for submitting new joint projects.

2.3.3 Dissemination of the results*. *(Max 200 words)*

Cooperation between the Ukrainian and the Polish laboratories will take the form of joint research activities and evaluation of the results that will be displayed in joint publications and/or patenting. The results will be presented at International conferences, symposia and workshops. The realization of the scientific tasks of the project will provide excellent scope for the transfer of knowledge through well-connected partnership between two countries and will provide the knowledge sharing the promising benefit of both partners and nano-science in general.

2.3.4 Possible references related to the research proposal (optional) . *(Max 300 words)*

[1] G. Beretta, M. Daghio, T.A. Espinoza, A. Franzetti, A.F. Mastorgio, S. Saponaro and E. Sezenna, Progress towards bioelectrochemical remediation of hexavalent chromium, Water 11, 2336 (2019). https://doi.org/10.3390/w11112336.

[2] E. Andriukonis, R. Celiesiute-Germaniene, S. Ramanavicius, R. Viter and A. Ramanavicius, From microorganism-based amperometric biosensors towards microbial fuel cells, Sensors 21, 7, 2442 (2021). https://doi.org/10.3390/s21072442.

[3] J. Huang, Y. Zhang, J. Li, X. Deng, P. Zhao, X. Jin and X. Zhu, Rational, Optimization of tether binding length between the redox groups and the polymer backbone in electroactive redox enzyme nanocapsules for high-performance enzymatic biofuel cell, ACS Appl. Energy Mater. 4, 5, 5034–5042 (2021). https://doi.org/10.1021/acsaem.1c00604.

[4] P. Bollella, I. Lee, D. Blaauw and E. Katz, A microelectronic sensor device powered by a small implantable biofuel cell, Chem. Phys. Chem. 21, 120-128, (2020). https://doi.org/10.1002/cphc.201900700.

[5] M. Grossi, Energy harvesting strategies for wireless sensor networks and mobile devices: A Review, Electronics 10, 6, 661 (2021). https://doi.org/10.3390/electronics10060661.

[6] M. Zhu, Z. Yi, B. Yang and C. Lee, Making use of nanoenergy from human – Nanogenerator and self-powered sensor enabled sustainable wireless IoT sensory systems, Nano Today 36, 101016 (2021) https://doi.org/10.1016/j.nantod.2020.101016.

[7] J.C. Ruth and A.M. Spormann, Enzyme electrochemistry for industrial energy applications—a perspective on future areas of focus, ACS Catal. 11, 10, 5951–5967 (2021). https://doi.org/10.1021/acscatal.1c00708.

[8] C. Ganzales–Solino and M. Di Lorenzo, Enzymatic fuel cells: towards self-powered implantable and wearable diagnostics, Biosensors (Basel) 8, 1 (2018). https://doi.org/10.3390/bios8010011.

[9] S. Choi, Biofuel cells and biobatteries: misconceptions, opportunities, and challenges, batteries, 9, 2, 119 (2023). https://doi.org/10.3390/batteries9020119.

[10] K. Kižys, A. Zinovičius, B. Jakštys, I. Bružaitė, E. Balčiūnas, M. Petrulevičienė, A. Ramanavičius and I. Morkvėnaitė-Vilkončienė, Microbial biofuel cells: Fundamental principles, development and recent obstacles, Biosensors 13, 2, 221 (2023). https://doi.org/10.3390/bios13020221.

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2.4 RESEARCH TEAM DESCRIPTION and FINANCIAL PLAN

2.4.1 Description of the roles within the research team*. *(Max 500 words)*

Mykhailo Gonchar, DrSc, Head of the Department of Analytical Biotechnology. Principal Investigator, supervision of the team research, preparation of scientific reports and international publications. Close collaboration with European principal investigator (Dr. Wojciech Nogala) and synergies in conducted research. Representation of the results at international conferences and forums.

Nataliya Stasyuk, Ph.D., Research Scientist. Synthesis of nanomaterials with a high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron-mediator activity; Design of BFC laboratory models based on mutant yeast and selected fungal cells producing oxidoreductases; Investigation of the functional characteristics of microbial BFCs; Testing of the best-selected BFCs for the powering of microelectronic chips.

Olha Demkiv, Ph.D., Research Scientist. Design of laboratory prototypes of enzyme BFCs on the basis of purified oxidoreductases and nanocomposites and study of their operational parameters. Investigation of the possibility of using selected nanomaterials as an immobilization matrix for covalent or physical binding of biocatalysts on the electrode surface; Investigation of the basic operational parameters of enzyme-based BFCs.

Anna Moroz. PhD Student, Engineer. Screening, cultivation, and stabilization of mutant yeast and selected fungi cells – overproducers of laccase; participation in the synthesis of nanocomposite materials for application in the construction of biofuel cells; investigation of the functional characteristics of microbial BFCs.

Oksana Zakalska, MSc., Engineer. Cultivation of mutant yeast cells overproducing of alcohol oxidase . Participation in the construction of laboratory prototypes of enzymatic biofuel cells and studying their functional characteristics.

2.4.2 Financial plan*. *(Max 500 words)*

Please indicate for your research team what are the wished estimated monthly grants per each member *(within the maximum and minimum amounts described in the ToR):*

Role	*Name and Surname (English)*	*Euro per Month*
PI: Principal Investigator, *DrSc, Head of the Department of Analytical Biotechnology. 45 years of experience. Supervision of the team research, preparation of scientific reports and international publications. Close collaboration with European principal investigator (Dr. Wojciech Nogala) and*	**Mykhailo Gonchar**	1,600

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synergies in conducted research. Representation of the results at international conferences and forums.		
Team Member 2: Ph.D., Research Scientist. *15 years of experience. Synthesis of nanomaterials with a high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron-mediator activity; Design of BFC laboratory models based on mutant yeast and selected fungal cells producing oxidoreductases; Investigation of the functional characteristics of microbial BFCs; Testing of the best-selected BFCs for the powering of microelectronic chips.*	**Nataliya Stasyuk**	**1,550**
Team Member 3: Ph.D., Research Scientist. 20 *years of experience. Design of laboratory prototypes of enzyme BFCs on the basis of purified oxidoreductases and nanocomposites and study of their operational parameters. Investigation of the possibility of using selected nanomaterials as an immobilization matrix for covalent or physical binding of biocatalysts on the electrode surface; Investigation of the basic operational parameters of enzyme-based BFCs.*	**Olha Demkiv**	**1,550**
Team Member 4: *MSc., Engineer. 35 years of experience. Cultivation of mutant yeast cells overproducing of alcohol*	**Oksana Zakalska**	**1,100**

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oxidase. Participation in the construction of laboratory prototypes of enzymatic biofuel cells and studying their functional characteristics.		
Team Member 5: *PhD Student, Engineer. 3 years of experience. Screening, cultivation, and stabilization of mutant yeast and selected fungi cells – overproducers of laccase; participation in the synthesis of nanocomposite materials for application in the construction of biofuel cells; investigation of the functional characteristics of microbial BFCs.*	**Anna Moroz**	**700**

If your team is composed by more than 5 Members please add new table lines.

2.4.3 Research team members information

Team Member 2 – information

First name ENG[4]*	NATALIYA
Family name ENG*	STASYUK
Date of Birth*	*07/11/1987*
Gender*	*Female*
Phone number(s)	*+380978084622*
E-mail address(es)*	*stasuk_natalia@ukr.net*
Institute of affiliation[5]*	*Institute of Cell Biology, National Academy of Sciences of Ukraine*
Affiliation Institute address	*Drahomanov Str. 14/16, Lviv, 79005, Ukraine*
Current position[6]*	*Research Scientist*
Country of permanent residence*	*Ukraine*
Country of current residence*	*Ukraine*
Citizenship	*Ukrainian*
Knowledge of English *	*Good*
Highest level of instruction achieved*	*PhD*

Team Member 3 – information

First name ENG[7]*	OLHA

[4] As mentioned in the ID document, please use English alphabet;
[5] Please mention if you are still affiliated to that institute or until when you were affiliated;
[6] Please mention if you are still in the same position or until when you held it;

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Family name ENG*	DEMKIV
Date of Birth*	*20/07/1981*
Gender*	*Female*
Phone number(s)	*+380676723695*
E-mail address(es)*	*demkiv@yahoo.com*
Institute of affiliation[8]*	*Institute of Cell Biology, National Academy of Sciences of Ukraine*
Affiliation Institute address	*Drahomanov Str. 14/16, Lviv, 79005, Ukraine*
Current position[9]*	*Research Scientist*
Country of permanent residence*	*Ukraine*
Country of current residence*	*Ukraine*
Citizenship	*Ukrainian*
Knowledge of English *	*Good*
Highest level of instruction achieved*	*PhD*

Team Member 4 – information

First name ENG[10]*	OKSANA
Family name ENG*	ZAKALSKA
Date of birth*	*25/04/1963*
Gender*	*Female*
Phone number(s)	*+380322612144*
E-mail address(es)*	*zakalska@yahoo.com*
Institute of affiliation[11]*	*Institute of Cell Biology, National Academy of Sciences of Ukraine*
Affiliation Institute address	*Drahomanov Str. 14/16, Lviv, 79005, Ukraine*
Current position[12]*	*Engineer*
Country of permanent residence*	*Ukraine*
Country of current residence*	*Ukraine*
Citizenship	*Ukrainian*
Knowledge of English *	*Good*
Highest level of instruction achieved*	*MSc*

Team Member 5 – information

First name ENG[13]*	ANNA
Family name ENG*	MOROZ
Date of birth*	*22/12/1998*
Gender*	*Female*

[7] As mentioned in the ID document, please use English alphabet;
[8] Please mention if you are still affiliated to that institute or until when you were affiliated;
[9] Please mention if you are still in the same position or until when you held it;
[10] As mentioned in the ID document, please use English alphabet;
[11] Please mention if you are still affiliated to that institute or until when you were affiliated;
[12] Please mention if you are still in the same position or until when you held it;
[13] As mentioned in the ID document, please use English alphabet;

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Phone number(s)	*+380969849262*
E-mail address(es)*	*morozanna1998@gmail.com*
Institute of affiliation[14]*	*Institute of Cell Biology, National Academy of Sciences of Ukraine*
Affiliation Institute address	*Drahomanov Str. 14/16, Lviv, 79005, Ukraine*
Current position[15]*	*PhD Student, Engineer*
Country of permanent residence*	*Ukraine*
Country of current residence*	*Ukraine*
Citizenship	*Ukrainian*
Knowledge of English *	*Good*
Highest level of instruction achieved*	*MSc*

[14] Please mention if you are still affiliated to that institute or until when you were affiliated;
[15] Please mention if you are still in the same position or until when you held it;

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2.5 SIGNATURES

After completing all the chapters of this form, it shall be signed by the PI and by all research team members, then the PI should upload it together with all other relevant documents (indicated in the Terms of Reference of the call) in the online application form.
Please remember that the limit in the number of words per each section of the application form constitutes one of the eligibility criteria, so make sure that all requirements are respected.

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Signature of the PI:
Mykhailo Gonchar
_____

Date: 28/04/2023

Signatures of all other team members:

Name, Family name(English)
Nataliya Stasyuk
_____

Signature
_____

Name, Family name(English)
Olha Demkiv
_____

Signature
_____

Name, Family name(English)
Oksana Zakalska
_____

Signature
_____

Name, Family name(English)
Anna Moroz
_____

Signature
_____

Please note that this document must be signed by all team members, so if your team is composed by more than 5 members, please add their signatures here below.

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