REVIEW

by Assoc. Prof., Dr. Zlatka Miltcheva Alexieva
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Regarding: The scientific production of Assoc. Prof., Dr. Lyudmila Vladimirova Kabaivanova for participation in a competition for awarding the academic position "Professor" in professional direction 4.3. Biological Sciences (Microbiology), announced in State Gazette no. 43/10.06.2022 for the needs of the Laboratory "Bioremediation and biofuels", Department of "Biotechnology", Institute of Microbiology – Bulgarian Academy of Sciences

Assoc. Prof. Dr. Ludmila Kabaivanova is the only candidate who submitted documents for participation in the announced competition. All necessary documents are submitted according to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations of the BAS and the Regulations of the Institute of Microbiology - BAS for its implementation.

PROFESSIONAL BIOGRAPHY

In 1992, Prof. Dr. Lyudmila Vladimirova Kabivanova received her higher education (Master's degree) at Sofia University "St. Cl. Ohridski", Faculty of Biology, Specialty - Biochemistry and Microbiology. In 1993, she began working at the Institute of Microbiology - BAS as a specialist. She has over 29 years of work experience in the specialty with a minimum experience of 5 years required by regulations. In 2006 she defended her PhD-thesis on the topic "Degradation of nitrile compounds with cells immobilized in different carriers by Bacillus sp. UG-5B, producer of thermostable nitrilase" at Stefan Angelov Institute of Microbiology, BAS.

In the same year, she was appointed as "Chief Assistant Professor". Since 2011, she has been elected and holds the academic position of "Associate professor" in the Department of Biotechnology of the Institute of Microbiology - BAS for 12 years, with the required two years. From 2016 to the present, after a competition at the Institute, he held the position of Head of the Department of "Applied Microbiology", currently the Department of "Biotechnology" and headed the "Bioremediation and Biofuels" Laboratory.

She held the position of Scientific Secretary of the Institute from 2016 to 2020, and is currently the Vice - Director. She was the Vice-Chairman of the General Assembly of Scientists at IMikB (2013), a member of the scientific council of the Institute of Microbiology - BAS, a representative at the General Assembly of Scientists at BAS (2013 - 2020). In the same period, she was a member of the permanent Attestation commission of the Institute and the Chairman of the Commission for conducting attestation of scientists and support staff, as well as of a number of other operational commissions. She has been repeatedly elected as a

member of Scientific juries and examination commissions for the implementation of the the Law on the Development of the Academic Staff in the Republic of Bulgaria.

Assoc. Prof. Dr. Ludmila Kabivanova has been a member of the Bioencapsulation Research Group, France – from 1996 to 2013 and a member of the National Research Center "Ecological Engineering and Environmental Protection" from 2019 until now. She was a reviewer of scientific articles and projects, a member of organizational and program committees of scientific forums and of 2 editorial boards.

Assoc. Prof. Dr. Ludmila Kabivanova taught in the master's program of the "Technology of silicates" department at University of Chemical Technology, Sofia - 2008-2010. She is a participant, as a teacher, in the "Student Practices" Program and in the Doctoral course at the Educational Center - BAS on the topic: Technological foundations of the immobilization of microbial cells and application to increase the efficiency of anabolic and catabolite processes. She supervised 3 doctoral students and 6 graduate students.

The main scientific interests of Associate Professor Dr. Kabivanova are in the field of microbial biotechnology, creation of immobilization systems related to aerobic and anaerobic biodegradation processes involved in detoxification and cleaning of the environment, obtaining renewable energy carriers, obtaining new biologically active substances.

MAIN SCIENTIFIC-METRIC INDICATORS RELATED TO THE COMPETITION

The analysis of the data relating to the indicators under the National Minimum Requirements from the Regulations for the Application of the Law on the Development of the Academic Staff in the Republic of Bulgaria shows the following: 50 points are set for the possession of the PhD. According to indicators from group B, 6 publications are presented: 3 - with Q2; 2 - with Q3 and 1 - with Q4, which gives a total of 102 points. According to indicators from group D, within indicator 7, 15 publications are presented, of which 1 - with Q1; 11 publications - with Q3; and 3 publications - with Q4. These data give a total of 226 items.

From the indicator for citations of publications in world-famous databases with scientific information (Web of Science and Scopus) or in general for indicators D, 100 citations are presented without self-citations, with which 200 points are collected. Regarding indicators from group E: Assoc. Prof. Kabaivanova managed 1 national (20 items) and 1 international project (50 items), participated in 5 national projects (50 items) and 1 international project (20 items). The funds raised for projects managed by the candidate are equal to 46.6 points. Assoc. Prof. Kabaivanova is the supervisor of 1 successfully defended

doctoral student (50 points), 1 doctoral student dismissed with the right of defense and 1 in the process of training. The total number of points according to indicators E is equal to 236.6 points.

The total number of points from the data filled in the Table for the minimum national requirements is 814.6 items with a minimum required of 640 items.

The conclusion is that Associate Professor L. Kabaivanova exceeds the requirements for each of the groups of indicators necessary for occupying the scientific position of "Professor" and in general scores 174.6 points more than the mandatory minimum.

Regarding "Additional criteria for the growth of the academic staff at the Institute of Microbiology - BAS", it can also be noted that the criteria have been exceeded. Assoc. Prof. Kabaivanova has presented 21 publications in journals referenced in WoS/Scopus in 19 of which she is the first or corresponding author. The total IF is 55,554. The list of citations contains 450 sources, H-index = 12. He also fulfilled the requirement for supervision of scientific research projects – 3 and he is the supervisor of two doctoral students who have been dismissed with the right to defend, one of whom has already defended.

GENERAL CHARACTERISTICS OF THE CANDIDATE'S SCIENTIFIC ACTIVITY

The treatment of wastewater contaminated with phenol and phenolic compounds has attracted much attention due to their toxicity and low biodegradability. Phenol and its derivatives are one of the largest groups of environmental pollutants due to their presence in many industrial wastewaters due to their widespread use. Phenol is on the US Environmental Protection Agency (EPA) priority pollutant list and has toxic, carcinogenic and mutagenic effects on humans, animals and aquatic organisms. Hexadecane is a toxic aliphatic compound, a component of petroleum fractions, which is used as a model molecule for hydrocarbon biodegradation studies.

In connection with these globally significant and never-losing relevance questions, a significant amount of research in the field of Aerobic biodegradation processes has been presented.

Original results were obtained on the degradation of significant amounts of phenol by immobilized cells of strains of *Aspergillus awamori* and *Rhodococcus wratislawiensis*. The possibility of the second strain to degrade separately and simultaneously representatives of aromatic and aliphatic compounds, in this case - phenol and hexadecane, was revealed. Scanning electron microscopy studies showed well-preserved cells entrapped within the heterogeneous super-macroporous structure of the cryogel, allowing unimpeded mass transfer

of xenobiotics. The immobilized strain can be used under real conditions to treat polluted industrial wastewater. The effective degradation of hexadecane by *Pseudomonas aeruginosa* cells immobilized in the SiO2-chitosan - polyethylene glycol system (V - 2, V- 3, G - 3, G - 4) is also proven.

One of the most interesting original results was demonstrated by research on the biodegradation of oil, which is a mixture of gaseous, liquid and solid hydrocarbons, the composition of which varies depending on the location of the deposits.

A new strain, molecularly taxonomically identified as *Bacillus cereus*, has been isolated that synthesizes biosurfactant and degrades crude oil. The experiments were performed with free and immobilized cells in two types of cryogel. Chromatographic analysis demonstrated that the strain degraded 93% of the aliphatic fraction in 48 hours. Reuse tests revealed that the oil-degrading ability of the immobilized cells was stable and maintained its rate after 47 days (up to the 20th duty cycle), under semi-continuous operation compared to only three cycles performed by free cells (G - 6).

Separately, the results of experiments with an immobilized strain of *Bacillus* sp., capable of producing a nitrilase enzyme and degrading a wide range of toxic nitrile substrates, as well as the established ability of a biosorbent (based on tetraethylorthosilicate) with included microalgae can be highly appreciated. species Porphyridium cruentum to remove ions of heavy metals - Cu(II), Cd(II) and Ni(II) from aqueous solutions (B - 6, G - 5).

Anaerobic digestion (AD) is a widespread biological process for treating organic waste to produce green energy. Assoc. Prof. Kabaivanova's main research in Anaerobic biodegradation processes includes the degradation of agricultural lignocelluloses' waste to obtain biogas and biomethane, as renewable energy carriers.

In the era of energy crisis, biomass-based bioenergy is receiving special attention. In general, a commonly used feedstock for bioenergy production is lignocellulosic biomass, as the most economical renewable source. Lignocelluloses' biomass refers to solid household waste, forest and agricultural products, algae, etc.

The conditions for obtaining biogas and biomethane during effective anaerobic degradation of agricultural waste - the corn stalks pre-treated with ultrasound - have been studied.

A circular, closed system has been implemented, following the basic principles of the circular economy. In the beginning, microbial anaerobic degradation of lignocellulose substrates takes place. An original approach was applied to cultivate microalgae based on the residual biomass from the initial process. Photosynthetic microalgae have industrial and

economic prospects, so their inexpensive cultivation has great potential for many applications. The resulting microalgae biomass is fed back into the bioreactor as a co-substrate and the process starts again. An improvement in yields was reported when treating the substrate with ultrasound (from 1116 cm3/L to 1350.5 cm3/L), as well as the possibility of algae biomass accumulation. When adding biomass from algae, as a co-substrate (4 g/L biomass), an increase in biogas yield was reported compared to experiments with a single substrate - corn stalks (G - 12).

In a laboratory bioreactor, a study was conducted on the production of biogas/biomethane during the anaerobic digestion of a mixture of wheat straw and corn stalks, used as a renewable source. The obtained results show that the highest substrate load - 45 g/L and cumulative volume of biogas produced - 8.22 L was observed at a temperature of 55 °C for 12 days. A degradation rate of 72% was established. A fast and efficient process for producing biogas from wheat straw was also developed, in which between the 2nd and 5th day, the highest amount of daily biogas production with a concentration of biomethane in it - 60% - was tracked.

Based on a sequencing library with primers for the V3–V4 region of 16S RNA and primers for archaeal RNA, a metagenomic analysis of the bacterial and archaeal diversity in the microbial consortium carrying out the anaerobic degradation of the used lignocellulose's substrates was carried out. Bacteria occupy 76.7%, and most of them belong to the Clostridia class (32.9%). Among the genera of bacteria, the presence of Pseudomonas should be noted. The share of archaea in the consortium is 1.37%, but the identified genera such as Methanocorpusculum, Methanobacterium, Methanomassiliicoccus, Methanoculleus and Methanosarcina deserve separate attention (B - 1). The study of the microbial diversity in the anaerobic bioreactor is crucial for the management of the biogas production process and should be continued and deepened.

Original research is devoted to the biodegradation of cellulose products that can be used in spaceflight by suitable cellulose-degrading non-pathogenic bacteria. A 72% biodegradation rate was achieved under anaerobic, mesophilic conditions in a bioreactor. The profile of volatile fatty acids accompanying the biodegradation process was monitored. The decomposition process is accompanied by the release of biogas, 45% of which is hydrogen. Biological production of hydrogen is a significant goal for environmental protection. Hydrogen is known as a clean energy resource, and its production by fermentation of various substrates of biological origin has been studied for a long time, with most studies being carried out with pure microbial cultures. In the presented study, cellulose degradation was

realized by a mixed microbial consortium through mixed acid fermentation. The performed metagenomic analysis shows that *Bacteroides oleiciplenus*, *Clostridium butyricum* and *Ruminiclostridium papyrosolvens* species predominate in the composition of the bacterial community carrying out the process, which is probably responsible for the degradation of cellulose (G - 13).

A number of mathematical models have been created, characterizing the optimal conditions for the implementation of a two-stage cascade process of anaerobic biodegradation, consisting of a hydrogen stage, followed by a methanogenic stage. A Kalman filter based on Newtonian control and extremum search was used to increase the maximum rate of hydrogen and methane production (G – 11, G – 15).

An important direction in organic agriculture is the replacement of fertilizers with a purely synthetic chemical composition with organic mass, achieving an increase in the development and yields of agricultural crops. Compost plays a major role in this process. It is a mixture of decomposing plant and food waste and natural fertilizer, which with proper aeration and amount of water, provide nutrients and improve the quality of the soil. This effect is due to a significant extent to the presence of many microorganisms that manage the chemical processes of decomposition, support the enrichment of the soil with carbon dioxide and ammonium ions, the release of heat, as well as the suppression of the development of pathogens. Composting is also an important part of waste management, as food and other compostable materials make up about 20% of waste in landfills and landfills.

The competition materials also present original research results on the inclusion of waste products and plant residues from agricultural practice and the use of the activities of bacteria and fungi in biodegradation processes. The results of the conducted experiments convincingly show that the application of compost increases the activity levels of key enzymes (cellulase and endoxylanase) involved in the decomposition of plant waste, as well as leads to a significant increase in the populations of all physiological groups of microorganisms, with the exception of spore-forming bacteria and mineral nitrogen-utilizing bacteria, which is a good indicator of increased soil microbial activity in the respective treatments. Applied organic matter and fertilizers, supplied with mineral and foliar nutrition, have a statistically proven positive effect on the growth parameters of potatoes. A collection of microorganisms was created, assimilating plant waste only as a source of carbon and nitrogen and suitable for addition to compost. [G – 10, G – 14].

One of the priority areas of biotechnology development is the search and research of new, innovative sources of biologically active substances. Recent studies suggest that algae

can be used for targeted biosynthesis of a number of compounds. Microalgae are a unique group of numerous types of unicellular organisms with a huge potential of applications in various fields of science and technology.

Polysaccharides are characteristic secondary metabolites of many algae. A number of studies have been done on red algae and the human health benefits of their polysaccharides. In the presented results, a cell-specific antitumor effect of a newly isolated extracellular polysaccharide from the red microalga *Porphyridium sordidum* was demonstrated in MCF-7 and MDA-MB231 breast cancer tumor cells. Its sugar composition was determined by HPLC analysis (Xyl:Glc and Gal:Man:Rha in a molar ratio of 1:0.52:0.44:0.31). An adjuvant therapeutic effect has been established when exposed to electrical impulses. Application of 75 μg/mL polysaccharide in combination with 200 V/cm electroporation caused a 40% decrease in MDA-MB231 cell viability and changes in cell morphology [V - 4]. Immunofluorescence analysis revealed morphological changes in polysaccharide-treated red microalgae *Rhodella reticulata* cancer cells [V - 5]. Administration of polysaccharides from *Porphyridium cruentum* also showed an antitumor effect in the highly metastatic cell line MDA-MB 231-47% [D – 1]. When studying the influence of changes in temperature and light intensity on the red microalgae *Rhodella reticulata*, changes in its metabolic profile were found. Regardless of light intensity, protein content was highest at lower temperatures (28°C) and higher temperature (28°C) showed the highest percentage of polyunsaturated eicosapentaenoic acid (EPA) content (46% of total fatty acid content) and in general for fatty acids, an important characteristic for this strain. This is a prerequisite for using EPA as an additive in the food industry [G - 8].

Physiological and biochemical changes in the green microalga *Chlorella vulgaris* under conditions of oxidative stress were monitored. Changes in the photosynthetic apparatus and antioxidant enzymes were found [G – 7].

Obtaining new biologically active substances from bacteria is a main direction in modern microbiological and biotechnological developments.

From a biomedical point of view, trehalose lipid biosurfactants have been shown to exert an impressive number of physiological and pathophysiologically related properties such as antimicrobial, antiviral, antiadhesive, anticancer or immunomodulatory properties. Trehalose lipid biosurfactant from *Nocardia farcinica* strain is a naturally occurring substance with potent anticancer activity. Considerable evidence has been accumulated on this trehalose lipid biosurfactant as a promising anticancer agent, but scientific data on possible side effects or its toxicity are almost completely lacking. In this regard, its effect on the isometric

contraction of isolated rat mesenteric arteries was investigated and it was shown that no effect was observed on the contractility of rat mesenteric arteries in vitro, which, together with the detected reduced viability of cancer cells, makes it suitable for potential medical appendix [G – 9].

The effects of isolated and purified trehalose lipid from *Rhodococcus wratislaviensis* strain on cell viability and migration of two human cancer cell lines (MCF7, MDA-MB231) and one normal cell line (MCF10A) were investigated. The obtained data suggest a mechanism of antitumor action and activity of the purified trehalose lipid and show its potential for biomedical application [G – 2].

MAIN SCIENTIFIC CONTRIBUTIONS OF THE CANDIDATE

Original and effective immobilization systems have been created based on a hybrid sol-gel matrix with the participation of chitosan, polyethylene oxide (PEO) and polyacrylamide (PAAm) cryogels. The production of rhamnolipids in cryogel-immobilized cells of *Pseudomonas aeruginosa* was investigated for the first time, and the process was shown to be non-toxic, cheap, rapid and could serve as a versatile tool to increase the yield of rhamnolipids, which support the biodegradation of poorly soluble pollutants of environment. The yield of rhamnolipids in the immobilized system exceeded that of free bacterial cells, distinguishing an efficient bioprocess. Scanning electron microscopy (SEM) revealed preservation of cell shape and proper distribution of cells below the matrix surface. Polymer matrices have chemical and biological stability and very good physical and mechanical characteristics, which are a prerequisite for a long life of these materials. A new strain, molecularly taxonomically identified as *Bacillus cereus*, has been isolated that synthesizes biosurfactants and degrades crude oil.

An original technological scheme for increasing the yield of biogas from agricultural waste is proposed, which uses a cheap, accessible and renewable plant lignocellulose substrate combined with microalgae growing in wastewater. Effective degradation of cellulosic materials was achieved by a mixed microbial consortium, accompanied by the production of biogas with high hydrogen content.

A metagenomic analysis of bacterial communities carrying out degradation processes of lignocellulose's and cellulose's substrates in a fermenter was carried out.

A collection of microorganisms was created, assimilating plant waste only as a source of carbon and nitrogen and suitable for addition to compost.

Significant and original scientific results have been achieved in the study of the antitumor potential of newly isolated polysaccharides from red microalgae, which, apart from being scientific, will also provide a perspective for the application of the obtained results.

In vitro, the antitumor activity and the absence of adverse effects of trehalosolipid biosurfactants naturally extracted from *Nocardia farcinica* and *Rhodococcus wratislaviensis* have been demonstrated, which shows potential for their biomedical application, reduced cancer cell viability but did not affect isometric contraction.

CONCLUSION

The documents and materials presented by Assoc. Dr. Lyudmila Kabaivanova meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the relevant Regulations of the BAS and the Institute of Microbiology - BAS.

The presented scientific research is of high level and actual subjects. T is aimed at establishing original theoretical knowledge with a perspective for biotechnological application. The obtained results are original, topical, of public importance and reveal opportunities and prospects for new research on current problems, aimed at the needs of real biotechnological productions. Evaluating the scientific activity of Assoc. Dr. Lyudmila Kabaivanova in a complex way, it can be claimed that her work resulted in significant fundamental scientific and scientific-applied contributions.

The results achieved by Assoc. Prof. Kabaivanova in research and teaching activities fully correspond to and exceed the minimum national requirements, as well as the increased criteria of Regulations for its Implementation of the Bulgarian Academy of Sciences and the Institute of Microbiology - BAS for occupying the academic position "Professor".

The detailed analysis of the materials and scientific works presented in the competition and the scientific, scientific-applied and applied contributions contained in them are grounds for giving my convinced positive assessment and recommending to the Scientific Jury to propose to the members of the Supervisory Board of the Stefan Angelov Institute of Microbiology " to vote positively for the occupation of the scientific position "Professor" by Assoc. Dr. Lyudmila Vladimirova Kabaivanova.

17.10.2022 Reviewer:

(Assoc. Prof. Zlatka Alexieva)