REVIEW

BY

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REGARDING: Competition for the academic position "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (specialty Microbiology) under the Law for the Development of the Academic Staff in the Republic of Bulgaria, announced in State Gazette Issue No. 21 of March 14, 2025 for the needs of the "Biotechnology" Department, "Bioremediation and Fuels" Laboratory at the Institute of Microbiology - Bulgarian Academy of Sciences.

This review has been prepared in accordance with Order No. I-65/29.04.2025 of the Director of the "Stephan Angeloff" Institute of Microbiology - Bulgarian Academy of Sciences (IMikB) by decision of the Scientific Council of IMikB, Protocol No. 6 of 29.04.2025, according to which I have been included in the scientific jury for a competition announced in State Gazette issue No. 21 of March 14, 2025.

CANDIDATE: Chief Assistant Dr. Venelin Neychev Hubenov

The review of the documents shows that the procedure for opening and announcing the competition has been followed. The submitted set of materials is in accordance with the requirements of Chapter IV, Section III. of the Terms and Conditions for occupying the academic position "Associate Professor" from the Regulations for the terms and conditions for acquiring scientific degrees and occupying academic positions at the Institute of Microbiology at the Bulgarian Academy of Sciences and includes all necessary documents.

I know the candidate, I do not have common publications with him, and I am not in a conflict of interest, as per the law.

BRIEF BIOGRAPHICAL DATA OF THE CANDIDATE

Venelin Neychev Hubenov was born on June 6, 1982, in Burgas. In 2005, he obtained a Bachelor's degree in Biotechnology from "Prof. Dr. Asen Zlatarov" University - Burgas, and in 2007, a Master's degree in Ecological Biotechnology from the Faculty of Biology at "St. Kliment Ohridski" Sofia University. In the period 2007-2008, he worked as a biotechnologist at "Prof. Dr. Asen Zlatarov" University - Burgas. In the period 2012-2017, he was an assistant at the "Stephan Angeloff" Institute of Microbiology - BAS, where in 2015 he defended his dissertation on the topic: "Anaerobic Degradation of Organic Waste in Mesophilic and Thermophilic Growth Regimes" and acquired a PhD degree.

Since 2021, he has been a Chief Assistant in the "Biotechnology" Department, "Bioremediation and Fuels" Laboratory. He is fluent in English, Russian, and German.

The candidate has two specializations in well-known European laboratories - in 2018 in Ravenna, Italy, and in 2011 in Narbonne, France, in the field of biochemistry and microbiology of methanogenesis. He is a member of the editorial board of the journal Biotechnology and Biotechnological Equipment.

ASSESSMENT OF THE SUBMITTED MATERIALS IN THE COMPETITION

In this competition, Dr. Hubenov presents a list of 17 scientific papers, and the total number of publications is 27 after acquiring his PhD. The presented publications are distributed by groups of indicators, according to the requirements of the ZRASRB in the respective professional field, as follows: 16 publications and reports published in scientific journals, peer-reviewed and indexed in world-renowned scientific information databases, of which 5 are under indicator B and 11 under indicator G. The table for indicator G also includes 1 publication in a non-refereed journal with scientific peer review, as well as one utility model of which he is the first author. The total impact factor for his entire scientific career is 35.835, H-index 6.

The scientific area that the candidate develops is in the field of anaerobic degradation of organic waste for biofuel production and related biotechnological applications. His contributions focus on developing and optimizing biotechnological processes for the production of renewable energy and valuable products from organic waste, in the context of the circular economy. Key achievements include: highly efficient anaerobic degradation, innovative waste utilization, integrated systems for circular economy, identification of key bacterial and archaeal consortia involved in different degradation stages, synthesis of biodegradable fibers and films (PLA/PVP) with embedded hydrozincite obtained through "green synthesis," demonstrating antibacterial and photocatalytic properties for applications such as food packaging and water purification.

MAIN SCIENTIFIC CONTRIBUTIONS

The main contributions from the research activities that Chief Assistant Dr. Venelin Neychev Hubenov has summarized and presented in this competition are in six main areas:

- Investigation of methods for pre-treatment of lignocellulosic materials for their application as raw materials for anaerobic biodegradation.
- Investigation of two-stage processes for anaerobic biodegradation to obtain hydrogen and methane as energy carriers.
- Determination of the composition of microbial communities involved in hydrogen production processes from anaerobic biodegradation and those involved in methane production processes.
- Alternatives for utilizing the liquid waste fraction (biosludge) obtained after anaerobic biodegradation processes.
- Investigation of the possibilities for applying anaerobic biodegradation for the utilization of organic waste in long-term piloted space flights.
- Determination of the antimicrobial activity of nanocomposite films.

I believe that the contributions from the research that Dr. Hubenov has presented in the competition documents are formulated quite concisely. Among the most significant, I highlight those related to the production of biomethane and biohydrogen from lignocellulosic waste and process optimization through pre-treatment.

- An approach for pre-treatment with ammonium hydroxide (NH_4OH) and polyethylene glycol (PEG) has been adapted, which led to increased methane yield (percentage content of methane in biogas).
- The application of ultrasound pre-treatment of raw materials ensures a high biogas yield with minimal energy consumption. This process improves substrate accessibility. Lignocellulosic materials have a strong and complex structure that is difficult for microorganisms to degrade. Ultrasound destroys this structure, breaks down cell walls, and increases the surface area of the material, making it more accessible to enzymes and bacteria. This allows microorganisms to process organic matter more easily and quickly, leading to higher yields of biogas (and thus biomethane) in a shorter time. Ultrasound treatment has also been shown to be highly efficient per unit of energy input.

Application of two-phase anaerobic degradation increases energy efficiency, based on monitoring the influence of various parameters:

- Temperature regimes;
- Selection of substrate ratio and optimization of organic loading levels for thermophilic conditions and hydraulic retention time;
- Influence of inoculum on the biodegradation of lignocellulosic waste and hydrogen production, addition of spent vegetable oils;
- Metagenomic analysis was conducted to identify bacterial, archaeal, and fungal communities involved in anaerobic degradation processes;
- Identification of key genera and species of bacteria and archaea responsible for hydrolysis, acidogenesis, acetogenesis, and methanogenesis was carried out;
- Various agricultural wastes such as cattle manure, wheat straw, corn stalks, and corn extract were investigated as renewable and inexpensive raw materials;
- A concept for an integrated system is presented, where the liquid phase from the anaerobic digestate is used as a nutrient medium for cultivating microalgae. The accumulated algal biomass can be a source of high-value bioproducts, animal feed, fertilizers, or returned to the bioreactor as a co-substrate to increase biogas yield.

The most valuable contribution for the discovery of high-value products is the cultivation of microalgae in the liquid phase of anaerobic digestate (biosludge) after the anaerobic digestion process. The digestate, which is otherwise a waste product from biogas production, is transformed into a valuable nutrient medium for algae. This reduces costs and environmental impact. The resulting microalgal biomass is a source of various biologically active substances.

Returning the biomass to the bioreactor allows for recycling, which helps increase biogas/biomethane production.

A photosynthetic CO_2 fixation approach was used: Microalgae effectively fix carbon dioxide, contributing to the reduction of greenhouse gases and providing an additional environmental benefit. The short growth cycle and easy accumulation of biomass make microalgae cultivation economically viable and with great potential for subsequent beneficial applications. The inclusion of microalgae turns the waste stream into a source of multiple valuable products, contributing to a circular economy and sustainability.

In addition, Dr. Hubenov developed a computerized system for control and monitoring of operational parameters (temperature, pH, biogas composition, etc.) in pilot bioreactors. A new mathematical model of thermophilic anaerobic degradation of wheat straw for methane production,

verified with experimental data, was created. Microorganisms were isolated from the digestate, some of which have known pathogenic potential, and their antimicrobial resistance to various antibiotics was investigated. This factor is extremely important for the safety of digestate utilization.

The main achievements in the application of two-phase anaerobic degradation can be classified as:

- Separation of the anaerobic process into two stages – biohydrogen production (hydrolysis/acidogenesis) and biomethane production (methanogenesis) in separate reactors or phases, often under thermophilic conditions (55°C).
- Increased energy efficiency - up to over 40% higher total energy yield compared to single-phase systems, due to optimized conditions for different microbial groups.
- Simultaneous production of two types of biogas: biohydrogen and biomethane, which are valuable energy carriers.
- Utilization of specific waste streams as co-substrates, e.g., spent vegetable oils, algal biomass, corn extract, horse manure, waste from space missions in anaerobic digestion. The addition of spent vegetable oils under thermophilic conditions eliminates the need for expensive pre-treatment. In this aspect, the contribution regarding the possibilities of applying anaerobic biodegradation systems for organic waste utilization in long-term piloted space flights is extremely important. This achieves effective waste management and their transformation from a problem into valuable resources.
- Integrated effective circular economy systems are based on the recycling of algal biomass and its return to the digester. This actually increases biogas yield and closes the loop by transforming waste streams into valuable nutrient media and resources. This is a step towards eliminating the need for expensive synthetic nutrient media for algae and reducing pollution and dependence on fossil fuels.
- The development and implementation of computer systems for automatic monitoring and control of bioreactors allows for more precise control and adjustment of operational parameters to achieve maximum efficiency. In this aspect, modeling supports a deeper understanding of biochemical and microbial processes, as well as predicting results. Last but not least, the reduction in the need for manual intervention should also be noted.

Identification of key microorganisms and their roles is essential for process optimization. Through targeted inoculation, the selection or adaptation of microbial consortia for specific substrates and conditions is optimized. Dr. Hubenov's research shows potential for new applications and opens up opportunities for the production of sustainable food packaging, water purification, and more.

A significant contribution can also be noted regarding the development of new materials such as the synthesis of biodegradable polymer films and fibers (PLA/PVP) with embedded nanoparticles (hydrozincite), obtained through "green synthesis" (using plant extracts). Environmentally friendly synthesis undoubtedly reduces the use of toxic chemicals and energy.

Overall, the work of Dr. Hubenov and his team demonstrates a strong commitment to innovative biotechnological solutions for sustainable resource management and energy production, applying both experimental and theoretical approaches for a comprehensive understanding and optimization of complex biological processes.

The fact that Dr. Venelin Hubenov is a follower of the school created by Prof. Dr. Lyudmila Kabaivanova deserves admiration, as he follows and further develops the trend regarding the application of innovative and complex methods in his research, primarily aimed at optimizing bioenergy production and waste utilization within the framework of the circular economy.

The publications submitted for participation in the competition for associate professor are in the field of the announced competition, namely the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (specialty Microbiology). The directions for his future work are also duly described. They are a natural continuation of the research work carried out so far, and the prospects are for their deepening.

GENERAL ASSESSMENT OF THE CANDIDATE'S COMPLIANCE WITH THE MANDATORY REQUIREMENTS OF THE LAW ON THE DEVELOPMENT OF THE ACADEMIC SECTOR OF THE REPUBLIC OF BULGARIA

Dr. Hubenov's publication activity fully meets the requirements for the advancement of academic staff. The fact that he has publications in international journals positioned in Q1 with high impact, such as Molecules, Fermentation, Life, etc., is also noteworthy. 126 citations in Scopus have been observed.

He has presented 16 participations in international scientific forums, 9 participations in national, and 2 international projects, leading 4 of them.

According to the presented statement for compliance with the minimum requirements of the ZRASRB and those of IMikB - BAS, Chief Assistant Dr. Venelin Neychev Hubenov meets the national and specific requirements for occupying the academic position "Associate Professor", as follows:

For indicator A - 50 points with a defended doctoral dissertation.
For indicator B - from the requirements of the ZRASRB, 3 articles in quartile Q1 and one each in quartiles Q2 and Q4 are presented, and the sum of points is 107, with 100 required.
For indicator G - Dr. Hubenov presents 6 articles published in journals with quartile Q2; 3 in quartile Q3; and 2 in quartile Q4, as well as one patent. The sum of points is 224, with 200 required.
For indicator D - 195 points from 60 required.
Total points: **576, with 400 required.**

CRITICAL REMARKS - some technical inaccuracies are observed, which I do not find significant.

CONCLUSION

The analysis of the scientific and research activity of Chief Assistant Dr. Venelin Neychev Hubenov shows that his expertise is entirely within the field of the announced competition. The obtained results are original, current, and of public importance. The contributions have a significant fundamental character, as well as a clear practical orientation - they reveal opportunities and prospects for developing new technologies for an extremely pressing global problem - the production of biofuels by utilizing waste materials and creating effective circular economy systems.

The candidate's scientific and research activity covers and exceeds the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Application of the ZRASRB, the Regulations of the Bulgarian Academy of Sciences, as well as the Institute of Microbiology "Stephan Angeloff" - BAS. In view of all the above, I can conclude that Chief Assistant Dr. Hubenov is a well-established researcher with a strong scientific profile.

As a member of the scientific jury for the announced competition**, I give a positive evaluation of the scientific contributions of Chief Assistant Dr. Venelin Neychev Hubenov and confidently recommend to the esteemed jury members to vote for his election to the academic position "Associate Professor" in professional field 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (specialty Microbiology) under the ZRASRB, announced for the needs of the "Biotechnology" Department, "Bioremediation and Fuels" Laboratory at the Institute of Microbiology "Stephan Angeloff" - Bulgarian Academy of Sciences.**

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