#### **SCIENTIFIC OPINION**

by Assoc. Prof. Dr. Radoslav Ignatov Abrashev, Institute of Microbiology "Stephan Angeloff", BAS

**regarding:** competition for the academic position of "Associate Professor" professional field 4.3. Biological Sciences, scientific specialty Microbiology, for the needs of the "Stephan Angeloff" Institute of Microbiology, BAS, presented to a scientific jury, formed by order No. I-65/29.05.2025 of the Director of the "Stephan Angeloff" Institute of Microbiology, BAS.

In the competition for "ASSOCIATE PROFESSOR", announced in the State Gazette, issue 21 of 14.03.2025, documents have been received from Dr. Venelin Neychev Hubenov, Chief Assistant Professor in the "Bioremediation and Biofuels" Laboratory at the Department of Biotechnology at the "Stephan Angelovff Institute of Microbiology, Bulgarian Academy of Sciences (SAIM, BAS).

### 1. GENERAL PRESENTATION OF THE PROCEDURE AND THE CANDIDATE

To participate in the competition, Dr. Venelin Neychev Hubenov has submitted the necessary documents and materials proving the fulfillment of the requirements for occupying the academic position of "Associate Professor" on an electronic medium. All of them are in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, as well as the Regulations of the Institute of Microbiology "Stephan Angeloff", BAS. The documentation for the competition is well compiled and comprehensively reflects the research and project activities of the candidate.

Dr. Venelin Hubenov has a Bachelor's degree in Biotechnology from the University "Prof. Dr. Asen Zlatarov", Burgas (2005) and a Master's degree in Biotechnology in Environmental Biotechnology from Sofia University "Kliment Ohridski" (2007). In 2015, he received the educational and scientific degree "Doctor" at the SAIM-BAS. His scientific career began in 2009, where he successively worked as a specialist, assistant (2012) and chief assistant (2016). The candidate's work experience is more than 13 years.

Dr. Hubenov is actively involved as a member of the Union of Scientists in Bulgaria, the Microbiology Section, the John Atanasov Union of Automation and Informatics, and the National Society for Ecological Engineering and Environmental Protection, of which he has been a member of the board of directors since 2022. He also participates in the editorial board of the journal Biotechnology & Biotechnological Equipment.

Dr. Hubenov's growth as a specialist was undoubtedly contributed by his specializations at INRA, Narbonne, France, and the University of Bologna, Italy.

The candidate's scientific and research activities are entirely related to the topic of the competition and reflect current and promising directions in the field.

### 2. CHARACTERISTICS OF THE CANDIDATE'S ACTIVITY

Dr. Venelin Hubenov is a co-author of 28 scientific papers, of which 26 scientific articles and 2 utility models. For the competition, he presents 21 scientific articles and 1 utility model. Fifteen articles have been published in journals with IF, the total IF is 35.835 with an h index of 6 (Scopus). *Compliance with the requirements of the LDASRB* 

### **Reference for the minimum requirements:**

- Indicator A abstract for the ONS "doctor" 50 points;
- Indicator C 5 scientific articles (3 in journals Q1; 1 Q2; 1 Q4) 107 points;
- Indicator D 12 scientific articles (6 in journals Q2; 3 Q3; 2 Q4; 1 without quartile) and 1 utility model - 224 points;
- ➤ Indicator E 94 citations (WoS/SCOPUS) 188 points;

### **Compliance with additional requirements of the SAIM, BAS**

The candidate is a co-author of 27 articles (required 20) and is the first author of 6 (required 5). The total number of citations is 126/Scopus (instead of the required 100). His IF for the entire scientific career is 35.835 at requirement 20, with an h index of 6/Scopus at requirement 5. Dr. Hubenov is a participant in 11 projects (at requirement 3), 4 of which he is a leader. Of the 8 research projects presented, 6 are funded by the Bulgarian National Science Foundation and 2 are internationally funded by European programs. His activities also include 3 scientific and applied projects, 2 of them with Bulgarian companies, and 1 concerns the development and implementation of standards in the laboratory practice of the Institute of SAIM, BAS.

The report on the fulfillment of the minimum requirements for the academic position "associate professor" shows that the candidate scored 576 points, which covers and exceeds the required 430 points. In addition, Dr. Hubenov also exceeds the additional requirements of the SAIM-BAS.

### 3. EVALUATION OF THE CANDIDATE'S RESEARCH ACTIVITY

The research activity of Dr. Venelin Hubenov fully corresponds to the topic of the competition. It is focused on the field of one of the most promising areas of modern microbiology - the development of processes for bioconversion of waste materials and their use as a sustainable raw material for obtaining valuable biotechnological products. In this context, the use of microbial agents for the degradation of organic waste is a widely applied solution, which arouses growing interest among scientists and business circles. In addition, biotechnological solutions can be both economically effective and environmentally friendly.

The candidate's works submitted for the competition outline six scientific areas in which important scientific and applied contributions have been formulated.

# **3.1.** Research on methods for pretreatment of lignocellulosic materials for their application as raw materials for anaerobic biodegradation

The results corresponding to this direction are reflected in publication D7-2. Along with the increased demand for energy and growing public concern for the environment, in recent years much attention has been paid to the possibility of using organic or biodegradable waste, including lignocellulosic waste, as sources of renewable energy. It should be emphasized that anaerobic degradation of such materials can mitigate environmental damage and produce biogas and

bioenergy with high economic efficiency. The main raw materials for biogas production are plant residues (wheat, rice and corn straw, corn, and others) and livestock waste. Studies have been conducted on the pretreatment of lignocellulosic waste in the production of biomethane by physical and chemical methods (temperature, pH, ultrasound, polyethylene glycol, NaOH, NH<sub>4</sub>OH).

# **3.2.** Research on two-stage anaerobic biodegradation processes with hydrogen and methane production as energy carriers (publications C4-1, C4-3, C4-4, D7-3, D7-4, D7-9)

Anaerobic digestion with biogas production is a multifunctional concept for renewable energy production. This includes the effect of waste treatment as a tool for reducing greenhouse gases, as a means of protecting water quality in streams and aquifers, converting low-value materials into high-value ones, environmental protection, and many others. The candidate presents scientific articles that develop in detail the efficient use of microbial consortia for the degradation of wheat straw or corn extract (a waste product from the processing of corn grain for starch extraction) in a two-stage process in a bioreactor for the production of biohydrogen and biomethane. The same direction includes studies on the joint degradation of agricultural and kitchen waste for hydrogen production.

# **3.3.** Establishing the composition of microbial communities involved in the processes of anaerobic biodegradation with hydrogen production and those involved in the processes for methane production (C4-1, C4-4, D7-6, D7-9)

The use of appropriate microorganisms in anaerobic biodegradation is one of the main approaches for a successful biotechnological process. The articles included in this direction present studies on the identification of microbes participating in the consortium depending on the type and conditions of the operating mode, the waste materials used, and their pre-treatment. The role of microbial communities of bacteria and archaea, changes in their composition, and their synergistic interactions in order to achieve successful application in energy production have been studied.

# 3.4. Alternatives for the use of the waste liquid fraction (bio sludge) obtained after the processes of anaerobic biodegradation (C4-2, D7-1, D7-8, D9-1)

Waste sludge can be utilized in various environmentally beneficial processes that are included in the circular economy strategy. For this purpose, the alternative for the use of the liquid fraction from anaerobic degradation in the cultivation of microalgae has been studied. The possibilities of obtaining valuable biologically active compounds and biofertilizers from algae on this basis have been discussed. For this development, the author's team has received a Certificate of Registration of a Utility Model.

### **3.5.** Study of the possibilities for the application of anaerobic biodegradation for the use of organic waste in long-term manned space flights (C4-5)

Waste management is a critical issue for long-term space missions with a long duration and distance from Earth. Bringing waste back to Earth or simply dumping it into space is neither practical nor sustainable. In recent years, efforts have been made to develop methods for managing waste during space missions through anaerobic digestion. This direction is reflected in the data on microbial degradation of cellulose, which is contained in substrates similar in composition to the hygiene materials used by space crews.

### 3.6. Determination of antimicrobial activity of nanocomposite films (D7-10, D7-11)

The candidate is a co-author of scientific articles that join the growing activity of scientists to propose a solution to the problem of drug resistance. Nanocomposites based on biodegradable polylactic acid (PLA) and PLA/polyvinylpyrrolidone (PVP) with embedded stabilized hydrozincite powder, as well as electrospun PLA/PVP fibers containing green synthesized hydrozincite (GHZ), with pronounced antibacterial activity, have been developed.

# In my opinion, the most important contributions of the candidate can be formulated as follows:

1. A method for pretreatment of lignocellulosic waste with polyethylene glycol, NH<sub>4</sub>OH, and ultrasound under mesophilic conditions has been developed and its higher efficiency in producing biogas has been proven.

2. An innovative biotechnological approach for producing hydrogen and methane has been presented.

3. A two-stage anaerobic digestion system with an immobilized microbial consortium with high efficiency for producing energy from waste materials has been developed. New data have been obtained on increasing the yield of hydrogen production from wheat straw when using a temperature regime of 55°C. The obtained data are a contribution to the concept of the circular economy.

4. It has been proven that the use of waste cooking oil (5%) as a co-substrate acts as a positive modulator of anaerobic digestion for hydrogen production without inhibiting the hydrolysis phase.

5. New information has been obtained on the role of the microbial consortium in the effective utilization of complex waste substrates.

- For the first time, the composition of archaeal and bacterial consortia in a two-stage system for biodegradation of lignocellulosic waste and by-products from the food industry has been identified.
- Metagenomic analysis provides detailed information on the structure of microbial communities and changes in the ratio between species for the correct course of the process in the direction of methane or hydrogen.

6. Successful cultivation of microalgae on a decolorized liquid fraction after anaerobic degradation with methane production has been achieved. It has been established that the obtained algal biomass can be used as a biofertilizer in free or immobilized form to stimulate the growth of some valuable plants.

7. A process for anaerobic degradation of cellulose in materials similar to those for the personal hygiene of astronauts has been developed based on a selected and genetically identified community of non-pathogenic bacteria. With the experiments conducted in terrestrial conditions in a mesophilic regime, a high degree of cellulose degradation has been achieved.

8. Nanocomposites with high antibacterial activity (PLA and PLA/PVP/hydrozincite and PLA/PVP/GHZ) have been developed. They are promising candidates as alternative materials in various food packaging applications.

I positively assessed the scientific research activities of Dr. Venelin Hubenov. The studies are complex with the participation of specialists with different qualifications. The contribution of the

candidate, however, is well outlined. I assess the contributions of Senior Asst. Venelin Hubenov as the significant, both original scientific and those with applied significance.

### **4. CONCLUSION**

The documents and materials presented by Senior Asst. Prof. Dr. Venelin Neychev Hubenov meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the LDASRB, and the relevant Regulations of SAIM, BAS. The candidate has a sufficient number of scientific papers for the competition, published after the materials used in the defense of the Doctoral Dissertation. They define Senior Asst. Prof. Dr. Venelin Hubenov as a professionally competent specialist in the field of microbiology and, more specifically, anaerobic microbial degradation of waste materials.

Based on the analysis of the scientific articles submitted in the competition and their significance, I strongly recommend that the Scientific Jury prepare a report-proposal to the Scientific Council of the Institute of Microbiology "Stephan Angeloff" at the Bulgarian Academy of Sciences for the election of Chief Assistant Professor Dr. VENELIN NEICHEV HUBENOV to the academic position "ASSOCIATE PROFESSOR" at the SAIM-BAS in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological Sciences, Scientific specialty Microbiology.

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/Assoc. Prof. Radoslav Abrashev, PhD/