



RECENZIIY

*by prof. Yana Ilieva Topalova, PhD, R4 Researcher,
Faculty of Biology of Sofia University "St. Kliment Ohridski" – member of the scientific jury with
Order I-65/29.04.2025.
of the Director of the Institute of Microbiology "Stefan Angelov" - BAS*

*of the DOCUMENTATION submitted for participation in the COMPETITION for the academic
position of Associate Professor in the field of Higher Education 4. Natural Sciences,
Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty
"Microbiology", announced in State Gazette No. 21 of 14.03.2025 for the needs of the Department
of Biotechnology, Laboratory "Bioremediation and Biofuels" - Institute of Microbiology "Stefan
Angelov" - BAS*

1. Overview of the competition documentation submitted by the candidate

In the announced in the State Gazette, **No. 21 of 14.03.2025**, and on the website of the Institute of Microbiology, competition for Associate Professor in 4.3. Biological Sciences (scientific specialty "Microbiology") for the needs of the Institute of Microbiology, the Department of Biotechnology, the Laboratory of Bioremediation and Biofuels, **the documents of one candidate – Chief Assistant Dr. Venelin Neychev Hubenov have been received on time.**

A quick look at the documents shows that the participant in the competition meets the conditions under Section IV of the LDASRB, respectively. – of Art. Art. 60 and 61 of the Rules of application LDASRB, as well as the requirements of the Regulations of the Bulgarian Academy of Sciences and the Institute of Microbiology "Stefan Angelov".

This review has been prepared in accordance with this regulatory framework, and is consistent with academic practice. The references for the minimum requirements under Article 26 of the Law on Biological Sciences for the scientific field 4.3. Biological Sciences, professional field "Natural Sciences, Mathematics and Informatics", the reference for the additional criteria for the growth of the academic staff at the Institute of Microbiology "Stefan Angelov" - BAS are filled in in accordance with the regulatory requirements. **The entire volume of documentation for the competition, in addition to being in accordance with the requirements for the given academic position of Associate Professor, is diligent, understandable, orderly designed with respect for details and accuracy of facts.**

2. Career development and thematic profile of the candidate

The only participant in the announced competition – Chief Assistant Dr. Venelin Neychev Hubenov – is a researcher at the Institute of Microbiology - Bulgarian Academy of Sciences on a permanent employment contract. He has 13 years, 2 months and 28 days of work experience, almost all of which was spent in the Institute of Microbiology. Partner and participant in teams for innovative and modern biotechnological research and has gained **valuable experience in the field of microbial anaerobic technologies**. I can responsibly state here that the entire professional path of Dr. Hubenov

is the path of a scientist with **a vocation, talent and deep motivation** to create scientific products – projects, articles, utility models, to introduce and modify methods for scientific research.

Dr. Hubenov was born in 1982 and graduated with a Bachelor's degree in Biotechnology from the Bulgarian State University and a Master's degree in Environmental Biotechnology from the Faculty of Biology of Sofia University "St. Kliment Ohridski". Kliment Ohridski" in 2007, since then I have known him and have been his teacher in basic microbiological and biotechnological disciplines. For a short time, he worked as a biotechnologist at the Asen Zlatarov Bulgarian State University, after which he started working at the Institute of Microbiology of the Bulgarian Academy of Sciences, where he developed his doctoral dissertation on **"Anaerobic degradation of organic waste in mesophilic and thermophilic cultivation mode"**, which he defended in 2015. Most of his professional experience was spent in the First Department of Microbiology. He grew from an assistant to a chief assistant, a position he has held since 2021. He is fluent in English, Russian and German at a basic level. There are two protected utility models. In his academic growth he has gone through two specializations abroad - at the University of Bologna in Italy and Narbonne - France. The topics of both specializations are in the field of his narrower scientific specialization – biochemistry and microbiology of methanogenesis. He is a member of the editorial board of the journal Biotechnology and Biotechnological Equipment. **Apart from the factual biographical data, I would like to point out that Dr. Hubenov is modest and tactful, diplomatic, loyal, but at the same time proactive and looking for innovations, stable and persistent in pursuing research goals. He perfectly integrates into a scientific team, manages to show understanding and support to his partners, which makes him a desirable member of successful, innovative teams.**

3. Research activity of the applicant

3.1. Review and analysis of publications and scientometric data

The general impression of the documentation presented under the competition is that several features can be brought to the fore before the detailed analysis: **focus on specific scientific topics, depth in the development of priority biotechnology topics, combination of technological aspects and functional-mechanism justification, strong innovative but pragmatic elements of the developments**, Excellent integration and correspondence of the subject matter of the laboratory and the department for which the competition for Associate Professor has been announced.

In the competition for Associate Professor, the candidate has submitted a total of 27 papers /full list of publications/, which were created after acquiring the scientific and educational degree of Doctor. Of these, 21 are referencing in Scopus and 27 in Google Scholar, 6 of them Dr. Hubenov is the first author. 94 citations on WoSc/Scopus are presented, 7 in other databases, achieved if for the entire scientific career 35.835, H-index 6, participation in 9 scientific projects /8 national and one international/. The author participates in two useful models, one of which is the first author. With these scientometric indicators, Dr. Hubenov meets the so-called additional criteria for the growth of the academic staff at the Institute of Microbiology – BAS for the academic position of Associate Professor. He has presented 16 participations in conferences, of which 8 are international. He has participated in scientific forums with posters and 2 reports.

The scientometric indicators of Assist. ace. Hubenov according to the point system, compared to the minimum requirements according to the Law on the Protection of Persons with Disabilities Act, have been filled in - exactly, I have reflected them below in the short table:

Indicator group	Evidence presented	Requirements for an Associate Professor	Number of points achieved by the candidate
A.	Dissertation on the topic of Doctoral Thesis	50	50
B.	Habilitation work / 5 pcs. Scientific publications with information about habilitation work Publications B4.1. – Q4.5.	100	107
C.	Scientific publications in ref. publications and indexed in world-famous databases Web of Science/Scopus – 15 articles – G7.1-D7.12, G9-1 - Utility Model	220	224
G.	Citations in scientific journals, according to the requirements	60	195
E.			
ALL OF IT			576

All the above numerical data are evidence of **two important intermediate conclusions**: 1/ The candidate has a scientific production that fully meets the requirements for the position of "Associate Professor"; 2/ He is a well-known researcher and creator nationally and internationally with his developments in the field of anaerobic biodegradation of waste products and the production of methane and hydrogen.

3.2. Main scientific and scientifically applied contributions to the habilitation reference and as overall contributions to the publication activity

I will evaluate the described scientific production from my position as a researcher and lecturer, with more than 42 years of work experience in the Biological Sciences and in the University **Research Work in Biology** and the various eco-biotechnological fields in various aspects and scientific educational degrees.

The scientific contributions of Assist. ace. Prof. Hubenov can be systematized in the following scientific directions, which are related to the topics of the announced competition for Associate Professor.

A. Methods for pre-treatment of lignocellulose materials for application as raw materials for anaerobic biodegradation. Publications of the G7-2.

Research in this direction is valuable because it leads to an increase in the amount of biogas and the content of methane in it by shortening the phases of hydrolysis, acidogenesis and acetogenesis. Pretreatment with ultrasound (400 W) and microwaves presents an important and promising result for the technology.

B. Optimization of two-stage processes for anaerobic biodegradation with the production of hydrogen and methane as energy carriers. Publications B4-1, B4-3, B4-4, G7-3, D7-4, G7-9

As important contributions, I accept the implemented and optimized processes for anaerobic biodegradation with the production of hydrogen and methane. In this direction, a two-stage anaerobic digestion system with an immobilized microbial consortium has been investigated, which is an innovative biotechnological approach. The approach provides increased production of methane and hydrogen and high efficiency of degradation of waste raw materials. It has been found that the temperature regime suitable for biodegradation of wheat straw is 55°C, which leads to a 2.5-fold increase in hydrogen production. The volatile fatty acids obtained in the bioreactor are a suitable substrate for the immobilized microbial consortium, which is formed in nearly twenty days of operation.

Another important contribution is the identification of the potential for co-degradation of agricultural and kitchen waste, as well as the positive modulatory role of cooking fat for the production of hydrogen.

Important technological parameters of the methanogenesis process aimed at obtaining high daily yields of hydrogen and methane have been established. The resulting energy is more than 40% compared to the traditional one-stage methane production process. These results are valuable for advising business lines in obtaining alternative valuable biofuels from waste biomass and solving important problems with biodegradable waste in the context of the circular economy. I highly appreciate these original and extremely valuable from a technological and business point of view.

C. Contributions in the field of deciphering the composition of microbial communities involved in the processes of anaerobic biodegradation with the production of hydrogen and methane. Publications: W4-1, W4-4, D7-6, D7-9

The composition and structure of microbial communities are key to anaerobic biodegradation and play an important role in the sequence of hydrolysis, acidogenesis, acetogenesis and methanogenesis processes. These are synergistically and syntrophically working communities that are analyzed with metagenomic and fluorescence techniques. Their composition and structure are a prerequisite for the efficient flow of energy generation processes and optimal implementation of anaerobic biodegradation in its different phases. **For this reason, I highly appreciate the results of the metagenomic study of the composition of communities.** The microbial composition of communities performing anaerobic biodegradation of lignocellulosic materials in the two-stage system has been established. It was found that hydrogen generation was most likely due to the presence of *Proteiniphilum saccharofermentans*, which made up 28.2% to 45.4% of the microbial community in the first and second bioreactors. In the methane generation reactor, archaeological representatives belonging to *Methanobacterium formicicum* (0.71% of the community), *Methanosarcina spelaei* (0.03%), *Methanothrix soehngenii* (0.012%) and *Methanobacterium beijingense* (0.01%).

In another system, consisting of two bioreactors with continuous stirring, the microbial species involved in the biodegradation of a by-product of the food industry – corn extract – have been identified. Metagenomic data confirm that the most abundant in both bacterial communities are representatives of *Firmicutes* – 58.61% and 36.49% in bioreactors 1 and 2, respectively. were found in significant quantities (22.91%) in the microbial community in Bioreactor 1, while in Bioreactor 2 they were 2.1%. *Bacteroidetes* are present in both bioreactors. *Euryarchaeota* makes up 0.4% of the content in the first bioreactor and 11.4% in the second. The dominant genera among the methanogenic archaea are *Methanothrix* (8.03%) and *Methanosarcina* (3.39%).

In a thermophilic process of anaerobic biodegradation of native lignocellulosic materials – corn stalks and wheat straw, it was found that more than a third of the bacteria belong to the class *Clostridia* (32.9%), followed by *Bacteroidia* (21.5%), *Betaproteobacteria* (11.2%), *Gammaproteobacteria* (6.1%) and *Alphaproteobacteria* (5%). The most well-known genera among them are *Proteiniphilum*, *Proteiniborus* and *Pseudomonas*. The share of archaea is 1.37% of the microflora in the thermophilic bioreactor, with the most common genera being *Methanocorpusculum*, *Methanobacterium*, *Methanomassiliicoccus*, *Methanoculleus* and *Methanosarcina*.

An important contribution to the practice is the deciphering of the co-decomposition of a mixture of pre-treated wheat straw and algae waste biomass in a ratio of 80:20 (w/w). This process has a potential for hydrogen and methane production with hydrogen concentrations of 42.5% and a maximum methane concentration of 56.1%. It was found that the bacteria *Thermocaproicibacter melissae* (44.9%) and *Clostridium cellulosi* (41.9%) participate in the consortium, carrying out substrate hydrolysis and acidogenesis in the first stage. Less common are *Thermoanaerobacterium butyriciformans*, *Calorimonas adulescens*, *Pseudomonas aeruginosa* and *Anaerocolumna chitinilytica*.

Methanogenesis is carried out by archaea, *Bathyarchaeota* (99.5%) and *Methanobacterium formicicum*. The most abundant bacterial strains in the methanogenic fermenter are *Abyssalbus ytuae* (30%), *Proteiniphilum acetatigenes* (26%) and *Ruficoccus amylovorans* (13%).

All these contributions to the microbial composition of communities and the associated biosynthesis processes of biofuels provide an excellent basis for the application of the microbial factor in the management and optimization of biogas and hydrogen production processes underlying clean technologies.

D. Contributions related to alternatives for the disposal of the waste liquid fraction (biosludge) obtained after anaerobic biodegradation processes. Publications B4-2, G7-1, G7-8, G9-1

This fraction can be used directly as a fertilizer (if it meets the relevant sanitary and hygienic standards) or the mineral-rich biosludge can be used as a medium for the development of microalgae. Cultivating microalgae in waste biosludge has been found to be a promising cost-effective and environmentally friendly strategy for accumulating algae biomass and producing valuable products.

E. Contributions on the possibilities of applying anaerobic biodegradation systems for the absorption of organic waste in long-term manned spaceflights. Publication B4-5

During long-term manned missions, solving organic waste problems can be solved through circular systems, with orchestrated anaerobic biodegradation. The experiments reached a high degree of anaerobic digestion - up to 72%. The composition of the bacterial community has been established, with the most common species: *Bacteroides oleiciplenus*, *Clostridium butyricum* u *Ruminiclostridium papyrosolvens*.

F. Contributions on the antimicrobial activity of nanocomposite films. Publications: G7-10 and G7-11

This group of contributions is aimed at elucidating the potential of nanocomposite films to exhibit antimicrobial properties. The antimicrobial properties of nanocomposite materials have been determined, which change their properties when inorganic substances are introduced into them. The antimicrobial properties of nanocomposite films based on biodegradable polylactic acid (PLA) and PLA/polyvinylpyrrolidone (PVP) with embedded stabilized hydrozincite powder, as well as electrospinnable poly(lactic acid)/polyvinylpyrrolidone fibers containing green-synthesized hydrozincite (GHZ) emulsion were determined. The antibacterial activity of the materials was tested against a control strain of *Escherichia coli*. Biocomposite films with high antibacterial activity with high potential for application as packaging, medical materials, etc. have been obtained.

Dr. Hubenov also plans his future research, which is planned to be focused on various aspects of anaerobic biodegradation with the production of mainly gaseous energy carriers – hydrogen and methane, but also some products of anaerobic transformation such as organic acids.

The contributions thus summarized are valuable. They contain an original, fundamental and applied element. It would be good if the author had put these accents in their formulation. Looking at his scientific output, I believe that the contributions are formulated a little modestly. The materials allow them to be more extensive. I accept that in the articles on the biotechnological and molecular-genetic aspects of anaerobic biodegradation with the

production of methane and hydrogen, a key role was played by the candidate for Associate Professor – Assist. ace. Venelin Hubenov.

A characteristic feature of the candidate's scientific contributions is a clear focus on the anaerobic biodegradation of various organic raw materials and waste. This also forms the clear profile of Dr. Hubenov. It is evident from the scientific facts that the candidate has established himself as one of the best trained specialists in the field of anaerobic methanogenic processes not only on a national scale, but also abroad. These conclusions of mine are based on the numerous citations presented by the candidate, as well as participation in international scientific forums and projects. Another very positive emphasis in the contributions for me is that most of the research and scientific products are made in teams with versatile cross-disciplinary skills. This only confirms the valuable research qualities of Dr. Hubenov – to integrate into a scientific team and to play an important soldering, and in specific cases, leading role.

An important contribution is the application of metagenomic analysis to decipher the composition of microbial communities and to clarify the relative share of individual microbial groups in the production of methane and hydrogen. As for hydrogen of microbial origin based on waste – this is a development in the field of the most desirable, most innovative clean technologies. I strongly hope that in this regard, research will be expanded and focused on biofuel without a carbon footprint.

I cannot fail to emphasize a clearly distinguishable tendency to expand the perimeter and application parameters of well-controlled anaerobic biodegradation to the potential for creating complete ecosystems for space conditions, for creating and searching for applications of nanocomposite materials with antimicrobial properties. All contributions and the entire research output of Dr. Hubenov are aimed at an innovative search for solving key problems of environmental and biotechnological science and practice.

4. Research projects

A significant part of the publications authored or co-authored by Chief Assist. Venelin Hubenov are the result of the implementation of research and applied projects funded by various lines. The list of projects lists 11 projects in which the applicant has participated. Of these, 6 are funded by the NSF (one is under bilateral cooperation with China), two projects are international. Dr. Hubenov himself has been the head of 4 successfully completed projects – one funded by the NSF, one from the Institute of Microbiology of the Bulgarian Academy of Sciences and two projects funded by companies. All projects (11 in total) have **provided the material and technical opportunities** for teamwork and training/good practices of researchers in the field of anaerobic biotechnology. I believe that in the future these teams will be **sustainable** and will continue to work in the above priority areas, as well as will cooperate with other similar teams in the field in Bulgaria and abroad.

4. Educational and pedagogical activity of the candidate

No data on the educational and pedagogical activities of the candidate for associate professor have been presented. Although I know that he participated in the training of masters and bachelors-graduates as a consultant. Here I would especially like to emphasize his responsiveness in seeking help from colleagues developing the PhD in the field of biogas technologies.

5. Expert activity

Dr. Hubenov has not indicated direct data on expert activity. From the presented projects, financed by the companies TESI Ltd. and Allterco Robotics AD, it can be concluded that he has also carried out

expert activities for the benefit of the business. I think that in the future this potential of the future associate professor can be developed and expanded.

6. Participation in the organization and administrative activities of scientific institutions and organizations

Dr. Hubenov is an active member of the microbiological and biotechnological community in Bulgaria. He is a member of the Union of Scientists in Bulgaria, Department of Microbiology since 2010, a member of the National Society of Environmental Engineering and Environmental Protection and since 2022 he has been a member of the Management Board of the society, he has been a member of the organizing committee of national conferences and conferences with international participation organized by the society, he is a member of the editorial board of the journal Biotechnology and Biotechnological Equipment.

7. Summary commentary, personal impressions, critical remarks and recommendations

The analysis made so far of the professional development and achievements of Chief Assistant Dr. Hubenov shows that during his 13 years of work experience he has formed himself as a researcher at the Institute of Microbiology of the Bulgarian Academy of Sciences with **a pronounced profile and high qualification**, fully corresponding to and coinciding with the topics of the announced competition for Associate Professor. content and quality **meets the requirements for occupying the position of Associate Professor at the Institute of Microbiology-BAS.**

His clearly outlined profile of a highly qualified researcher in the field of anaerobic biodegradation of waste raw materials with a focus on the production of methane and hydrogen fully coincides with the theme of the announced competition for Associate Professor.

I have personal impressions of Venelin Hubenov since the distant 2007. I was his teacher and his partner later in organizational and research activities. He is a creatively fruitful, constructive, dialogical, result-oriented, desirable partner in research projects, a good and ethical person who supports his colleagues, a realistic-thinking, but innovative researcher. He has built an image of an open, contemporary, complex personality, with his own style and class, the kind that a HABILITATED RESEARCHER should be.

I am convinced that Assist. ace. Prof. Dr. Venelin Hubenov, fully meets the requirements of the RASRB and the additional criteria of the Institute of Microbiology for Associate Professor.

I have some minor criticisms of the candidate. It would be good to expand the contributions and be richer with a clearer emphasis on fundamental, applied, original and confirmatory elements. I noticed some small errors in the calculation of the points that I reflected in the review, but they do not affect the final positive result. It would be good to attach references for educational and consulting activities and the diploma for a Master of Environmental Biotechnology.

I also have a wish for the candidate. To construct a textbook on anaerobic transformation of waste raw materials and to be more active in the future when working with graduates and students. The current habilitation will help him with this and it will contribute to the creation of a youth school around the future Associate Professor – a habilitated specialist in the field of microbiology of anaerobic biotransformations and biofuels.

8. Conclusion

Based on the above analysis of the submitted tender documentation, and from my long-term impressions of Assist. ace. Prof. Venelin Hubenov, I believe that he meets the requirements for an Associate Professor formulated in the Law on Associate Professorship and in the Regulations of the Institute of Microbiology of the Bulgarian Academy of Sciences. Undoubtedly, he is a well-established

specialist in the field of microbiology, anaerobic biodegradation processes and technologies with a proven place and role in the research practice of the Institute of Microbiology of the Bulgarian Academy of Sciences.

In my opinion, this is a sufficient argument to recommend to the esteemed Scientific Jury and the Scientific Council of the Institute of Microbiology – in accordance with Article 29 b, paragraph 1, in conjunction with Article 29 of the LDASRB, as well as with Articles 60 and 61 of the Regulations for its implementation – **to vote for the acquisition by Chief Assistant Dr. Venelin Neychev Hubenov of the academic position of Associate Professor.**

25.06.2025

Reviewer:

/Prof. DSc Yana Topalova/