Abstracts of the published papers authored by Ivaylo Dimitrov Yotinov

presented for participation in a competition for the academic position of "associate professor" - 4.3. Biological Sciences (Hydrobiology - Water Management), announced in the State Gazette, no. 67, (04.08.2023)

Publications as per item B.4: Habilitation work – scientific papers in referenced and indexed journals by reputable scientific databases (Web of Science and Scopus):

B4.1 Todorova, Y., Lincheva, S., **Yotinov, I.,** & Topalova, Y. (2016). Contamination and ecological risk assessment of long-term polluted sediments with heavy metals in small hydropower cascade. *Water resources management*, 30, 4171-4184. https://doi.org/10.1007/s11269-016-1413-8; **(Web of science: IF=2.848; Q1) (SJR=1.422; Q1) 25 т.**

Abstract
The identification of contamination level and ecological risks, associated with heavy metal pollution of sediments in small hydropower cascade was done on the base of index analyses approach. The concentration of As, Cd, Cu, Hg, Pb, Zn, total organic carbon and percentage of fines were determined in sediments of two habitats in cascade sequence – river and dam. Correlation and multivariate analyses suggest that As, Cu, Pb and Zn are associated with similar anthropogenic source. Cadmium and mercury originate from different source and have specific moving. Based on the contamination and background indices the sediments in Middle Iskar cascade are moderate contaminated at least and are subject of intensive hydrological and technological mixing. The potential ecological risk index (PERI) classifies the sediments in dam site with the higher risk level. Suitable indicators for express assessment of metal pollution in “river” sediments are contamination/enrichment indices which are more sensitive for local concentration increase of less toxic metals. In “dam” sites the process of sedimentation affects strongly the degree of metals accumulation and differences in toxicity are clearly presented – PER/PERI in combination with content of fine sediment fractions and TOC have a potential for rapid identification of sediment-associated risks.

B4.2 Belouhova, M., **Yotinov, I.**, Schneider, I., Dinova, N., Todorova, Y., Lyubomirova, V., ... & Topalova, Y. (2022). Purposely Development of the Adaptive Potential of Activated Sludge from Municipal Wastewater Treatment Plant Focused on the Treatment of Landfill Leachate. *Processes*, *10*(3), 460. https://doi.org/10.3390/pr10030460; **(Web of science: IF=3.5 (2022), Q2 (2022); (SJR=0.529 (2022); Q2 (2022)) 20 т.**

Abstract
Biological treatment is a key technology in landfill leachate treatment However, often its efficiency is not high enough due to the pollutants in concentrations above the critical ones.

The present study aimed to investigate the adaptive responses that occur in activated sludge (AS) during landfill leachate purification. A model process with AS from a municipal wastewater treatment plant and landfill leachate in increasing concentrations was constructed. The data showed that when dilutions 25 and 50 times had been applied the structure of the AS was preserved, but the COD cannot be reduced below 209 mg O2 /L. The feed of undiluted leachate destroyed the AS structure as SVI was reduced to 1 mL/g, biotic index to 1, floc size was greatly reduced and COD remained high (2526 mg O2 /L). The dominant group of protozoa was changed from attached to free-swimming ciliates. An increase of the bacterial groups responsible for the xenobiotics elimination (aerobic heterotrophs, genera Pseudomonas, Acinetobacter, Azoarcus, Thauera, Alcaligenes) was registered. This was accompanied by a significant increase in free bacteria. The obtained data showed that for optimal treatment of this type of water it is necessary to include a combination of biological treatment with another non-biological method (membrane filtration, reverse osmosis, etc.).

B4.3 Yotinov, I., Belouhova, M., Foteva, A., Dinova, N., Todorova, Y., Schneider, I., ... & Topalova, Y. (2022). Application of Nanodiamonds in Modelled Bioremediation of Phenol Pollution in River Sediments. *Processes*, *10*(3), 602. https://doi.org/10.3390/pr10030602; **(Web of science: IF=3.5 (2022), Q2 (2022); (SJR=0.529 (2022); Q2 (2022)) 20 т.**

Abstract
The pollution of aquatic ecosystems is a big problem that has its impact on river sediments. In recent decades, an effective solution to this problem has been the application of bioremediation technologies. Nanoremediation is an innovative part of these technologies. We still know little about the efficiency of nanoparticles, especially nanodiamonds, in modelled conditions. The aim of the present study is to investigate the effect of nanodiamonds on the key parameters of modelled bioremediation of river sediments that are polluted with phenol, as well their effect on the structures and functions of microbial communities. An important indicative mechanism that was used is the application of fluorescent in situ hybridization for sediment microbial communities. The results of this study revealed the positive role of nanodiamonds that is associated with their intoxication with high concentrations of phenol. Readaptation was also found, in which the xenobiotic biodegra-dation potential evolved by increasing the relative proportions of non-culturable bacteria, namely Acinetobacter (at the 144th hour) and Pseudomonas (at the 214th hour). The results can help to find an effective solution to the question of how information from such precise molecular methods and the application of nanodiamonds can be translated into the accessible language of management and bioremediation technologies.

B4.4 Yotinov, I. D., Belouhova, M. V., Dinova, N. K., Todorova, Y. T., Schneider, I. D., & Topalova, Y. I. (2022). Adaptation of micro-and metafauna in activated sludge with microbial augmentation to shock loading with amaranth. *Biotechnology & Biotechnological Equipment*, *36*(1), 220-231. https://doi.org/10.1080/13102818.2022.2070437**; (Web of science: IF=1.4 (2022), Q4 (2022)) (SJR=0.317 (2022); Q3 (2022); 15 т.**

Abstract
Wastewaters from the industrial sector and, in particular, those from the textile industry have a diverse composition of organic dyes. These dyes are xenobiotics for living organisms and, falling into natural water, can lead to severe consequences for aquatic flora and fauna. This is the case with the azo-dyes, such as, for example, the amaranth. The adaptive responses of the micro- and metafauna communities in activated sludge towards shock loading of high concentrations of the azo-dyes in wastewater treatment plants (WWTPs) have been poorly studied. The aim of this research was to study the adaptation mechanisms of the communities of micro- and metafauna in activated sludge towards the shock loading of amaranth (200 mg/L)with and without the addition of Pseudomonas aureofaciens AP-9 as a bioaugmentation agent (3.03 × 107 cells/mL). To achieve this, the change in the quantities of key groups of micro- and metafauna was monitored in the course of a model biodegradation process involving real activated sludge from a WWTP and toxic pollutant amaranth at a concentration of 200 mg/L. The results showed that in the case of such shock loading with amaranth, the microfauna communities changed very quickly. In addition, a positive bioaugmentation effect of the added strain of Pseudomonas aureofaciens AP-9 was observed for the micro- and metafauna. The bacteria in a homogenous form provide easily accessible food for the macro-organisms and contribute to their adaptation in the conditions of a toxic shock. This is particularly pronounced in the representatives of free-swimming ciliates and small flagellates.

B4.5 Yotinov, I., Belouhova, M., Todorova, Y., Schneider I., Topalova Y. (2023) Influence of the azo-dye amaranth on the trophic structure of activated sludge in a model experiment. *Environ Sci Pollut Res* (2023). https://doi.org/10.1007/s11356-023-27406-2 ; (**Web of science: IF=5.8 (2022), Q1 (2022)) (SJR=0.944 (2022)); Q1 (2022)) 25 т.**

Abstract
The textile industry generates significant amounts of wastewater containing high concentrations of azo dyes. An important point in the process of purification of azo dyes is their influence on the activated sludge (AS) in wastewater treatment plants. Azo dyes, such as amaranth, play the role of xenobiotics. This article seeks to answer the question of how organisms manage to respond to xenobiotics remains very important and open, i.e., how they will react to toxic conditions. The aim of this research was to study how these changes are expressed in terms of the different trophic levels of AS. In our experiment, it was found that the dominant trophic units are significantly changed due to the xenobiotic entering the system. The data reveal the significant development of the bacterial segment (genus Pseudomonas and azo-degrading bacteria) at times of large amaranth removal. In the most active phase of amaranth biodetoxification (48 h), the culturable bacteria of the genus *Pseudomonas* change by about 40%, while the azo-degrading bacteria change by about 2%. Fauna organisms have a sharp change in the dominant groups—from attached and crawling ciliates and testate amoebas to the mass development of small and large flagellates. This is of great importance because micro- and metafauna play an important role in the detoxification process by ingesting some of the xenobiotics. This role is expressed in the

fact that after dying, macro-organisms release this xenobiotic in small portions so that it can then be effectively degraded by adapting to the amaranth biodegradation bacteria. In this study, it is clear that all these events lead to a decline in the quality of AS. But on the other hand, these allow AS to survive as a microbial community, and the fauna segment does not disappear completely.

Г.7. Scientific publications in publications that are referenced and indexed in world-famous databases with scientific information (WEB OF SCIENCE and SCOPUS), outside of the habilitation thesis.

Г7.1 **Yotinov, I.,** Lincheva, S., Kenderov, L., Schneider, I., & Topalova, Y. (2013). Evaluation of the self-purification in the waters of the micro-dams in the small hydroelectric power plants (hepps) Lakatnik and Svrazhen: Potential of the bioalgorithms. *Bulgarian Journal of Agricultural Science, 19*(2), 135-138. **(SJR=0.162; Q3) 15 т.**

Abstract
Environmental management requires the solution of problems through a comprehensive and thorough examination of all levels - from biochemical analysis to bio managerial approaches. The chemical and microbiological parameters of water in depth in micro-dams Lakatnik and Svrazhen have been investigated in June and September, 2011. Linear correlations between microbiological and hydrochemical indicators have been found. They give key information about the biochemical processes underlying the self-purification. Moreover, these correlations create a highly effective indicator system. The information from this indicator device will enable the preparation of forecast bioalgorithms for the express diagnostics of risk events in the waters of the studied micro-dams in the small HEPPs Lakatnik and Svrazhen. Our results show that these small dams provide excellent conditions for the implementation of self-purification processes in the water because of slow speed of the water in these reservoirs. The dams can be considered as natural treatment reactors where the microbial communities, operating in different layers of water, play a key role.

Г7.2 **Yotinov, I.,** Todorova, Y., Schneider, I., Daskalova, E., & Topalova, Y. (2017). Comparison of the Influence of Nanodiamonds and Single-Walled Nanotubes on Phenol Biodetoxification by Pseudomonas sp. *Journal of Nanoscience and Nanotechnology*, 17(2), 1031-1040. https://doi.org/10.1166/jnn.2017.12687 ; **(Web of science: IF=1.354; Q3) (SJR=0.326; Q2) 20 т.**

Abstract
Nanobiotechnologies are a rapidly growing field that offers new opportunities thus far unknown including regulation processes at a nano level. The biodetoxification and mechanisms of degradation of many xenobiotics have been studied and are well documented. There remains the important issue of the impact of nanomodulators on biodetoxification processes and their potential to optimize and regulate biodegradation of

recalcitrant xenobiotics. The purpose of the present study is to clarify in comparative terms the effect of carbon nanoparticles (single-walled nanotubes and nanodiamonds) on these processes. In order to achieve this objective analogous modeling of biodegradation processes was performed. The experiment was conducted in simplified conditions, using a microbial culture of Pseudomonas sp. We observed the influence of nanodiamonds (ND) and single-walled nanotubes (SWNT) on the basic kinetic parameters and key oxygenase enzymes of the bacteria from the genus Pseudomonas in the course of a model phenol biodegradation process. The results confirm the stimulating effect of ND on the initial stages of the biodetoxification processes. In comparison to the control variant there was an increase in the specific rate of phenol biodegradation (154%) and in the effectiveness of phenol elimination (151%). ND increase the activities of phenol-2-monooxygenase and catechol-2,3-dioxygenase respectively by 63,91% and 63,94% in comparison to the control variant. Under the same conditions SWNT have positive influence on the catechol-1,2-dioxygenase activity by 30,12% in comparison to the control. The data from this study are optimistic in relation to the future application of carbon nanoparticles, such as specific nanomodulators in bioremediation technologies for sediments, activated sludge, compost and other resources, polluted with xenobiotics.

Г7.3 Todorova, Y., Schneider, I., **Yotinov, I.,** Lincheva, S., & Topalova, Y. (2017). Potential of phosphatases for express assessment of self-purification at different types of pollution in running waters. *Water Practice & Technology*, *12*(4), 953-963. https://doi.org/10.2166/wpt.2017.103; **(Web of science: IF=0.17; Q4) (SJR=0.197; Q3) 15 т.**

Abstract
The potential of an express enzymological indicator - phosphatase activity index (PAI) - for assessment of different types of pollution and self-purification potential in running waters was evaluated for three river subcatchments, representative for different ecological situations and impacts. According to the values of correlation coefficients, a significant positive correlation existed among PAI and total microbial count, organic loading and phosphate concentrations. The enzyme activity is useful tool for early identification of risks from point discharge of different wastewaters (treated or non-treated). The role of PAI as an indicator is significant at case of disinfection after treatment of wastewaters in treatment plant - the aquatic microbial community at the discharge point is suppressed but not at a functional level. The results allow classical microbiological and chemical parameters (state variables) to be related directly to the dynamics of the transformation processes by functional variable - PAI.

Г7.4 Topalova, Y., Todorova, Y., Schneider, I., **Yotinov, I.**, & Stefanova, V. (2018). Detoxification potential and rehabilitation of activated sludge after shock loading of Sofia's wastewater treatment plant 'Kubratovo'with mazut. Water Science and Technology, 78(3), 588-601. https://doi.org/10.2166/wst.2018.329; **(Web of science: IF=1.624; Q3) (SJR=0.455; Q2) 20 т.**

Abstract
The shock loading of wastewater treatment plants (WWTPs) with toxic pollutants remains a critical problem with crucial significance for the technologies. On 5 November 2014, 30 tons of mazut were emitted in Sofia's WWTP 'Kubratovo', passing through equipment and damaging the functioning of the technological modules. The rehabilitation of activated sludge (AS) after shock loading as well as the development of detoxification activity were investigated. The hydrocarbon index of petroleum products, filamentous index (FI), sludge biotic index, sludge volume index (SVI), chemical oxygen demand (COD), biochemical oxygen demand (BOD5), oxygenases and succinate dehydrogenase activities were analyzed for a period of two weeks. The results show that independently from prolonged rehabilitation period, AS remained with filamentous bulking (SVI over 200 ml/g and FI over 1.107 μm/mg). At the same time, the detoxification potential of the AS was developed. Although the morphological and functional structure was still not fully recovered, the AS developed two adaptive mechanisms. First, activation of shorter, more effective ways for benzene ring cleavage, operated by catechol 2,3-dioxygenase; second, strong increase of succinate dehydrogenase activity, which is consistent with the activation of the degradation of trivial substrates for energy generating to overcome the intoxication and synthesis of oxygenases.

Г7.5 Marinova, P., Benova, E., Todorova, Y., Topalova, Y., **Yotinov, I.,** Atanasova, M., & Krcma, F. (2018). Surface-wave-sustained plasma torch for water treatment. In *Journal of Physics: Conference Series* (Vol. 982, No. 1, p. 012009). IOP Publishing. https://doi.org/10.1088/1742-6596/982/1/012009; **(SJR=0.221; без Q) 10 т.**

Abstract
In this study the effects of water treatment by surface-wave-sustained plasma torch at 2.45 GHz are studied. Changes in two directions are obtained: (i) changes of the plasma characteristics during the interaction with the water; (ii) water physical and chemical characteristics modification as a result of the plasma treatment. In addition, deactivation of Gram positive and Gram negative bacteria in suspension are registered. A number of charged and excited particles from the plasma interact with the water. As a result the water chemical and physical characteristics such as the water conductivity, pH, H2O2 concentration are modified. It is observed that the effect depends on the treatment time, wave power, and volume of the treated liquid. At specific discharge conditions determined by the wave power, gas flow, discharge tube radius, thickness and permittivity, the surface-wave-sustained discharge (SWD) operating at atmospheric pressure in argon is strongly non-equilibrium with electron temperature T e much higher than the temperature of the heavy particles (gas temperature T g). It has been observed that SWD argon plasma with T g close to the room temperature is able to produce H2O2 in the water with high efficiency at short exposure times (less than 60 sec). The H2O2 decomposition is strongly dependant on the temperature thus the low operating gas temperature is crucial for the H2O2 production efficiency. After scaling up the device, the observed effects can be applied for the waste water treatment in different facilities. The innovation will be useful especially for the treatment of waters and materials for medical application.

Г7.6 Todorova, Y., **Yotinov, I.,** Topalova, Y., Benova, E., Marinova, P., Tsonev, I., & Bogdanov, T. (2019). Evaluation of the effect of cold atmospheric plasma on oxygenases' activities for application in water treatment technologies. *Environmental technology*, *40*(28), 3783-3792. https://doi.org/10.1080/09593330.2018.1491631; **(Web of science: IF=2.213, Q3) (SJR=0.485; Q2) 20 т.**

Abstract
Plasma-based technologies take an increasing place in the new conceptions of wastewater management as a promising tool for the treatment of persistent organic pollutants with low biodegradability. Plasma major advantage is the synergy of diverse active components with high oxidative action and additional benefits as disinfection of treated water. But the bactericidal effect of plasma can influence the treatment effectiveness when this technology is used in combination with biological methods for the removal of pollutants. The aim of this paper is to study the effect of non-thermal atmospheric plasma torch on key enzymes from phenol biodegradation pathways in Pseudomonas aureofaciens (chlororaphis) AP-9. The strain was isolated from contaminated soils and had a high potential for biodegradation of aromatic compounds. The used plasma source is surface-wave-sustained discharge operating at 2.45 GHz in argon produced by an electromagnetic wave launcher surfatron type. The enzyme activities of phenol 2-monooxygenase (P2MO), catechol 1,2-dioxygenase (C12DO), catechol 2,3-dioxygenase (C23DO), protocatechuate 3,4-dioxygenase (P34DO) and succinate dehydrogenase (SDH) were measured in control and after plasma treatment of 10, 30 and 60 s. At short-time treatment, the activities of intradiol dioxygenases increased with 26% and 59% for C12DO and P34DO, respectively. Other oxygenases and SDH were inhibited with 35% even at 10 s treatment. Longer treatment times had a clear negative effect but SDH kept the higher activity at 60 s treatment compared to the oxygenases. Our data suggest that plasma-based technologies are a useful approach for post-treatment of aryl-containing wastewater in order to increase the effectiveness of biological removal.

Г7.7 Belouhova, M., Dinova, N., **Yotinov, I.**, Lincheva, S., Schneider, I., & Topalova, Y. (2021). FISH investigation of the bacterial groups anammox and Azoarcus-Thauera at treatment of landfill leachate. *Bulg. Chem. Commun*, *53*, 27. DOI: 10.34049/bcc.53.A.0007; **(SJR=0.168; Q4) 12 т.**

Abstract
The landfill leachate is heavily polluted wastewater produced in the landfills. The management of the purification of the leachate is especially challenging and that is why new approaches and indicators are needed. The quantity, localization, interaction, clustering of the key microbial groups, responsible for the critical transformation processes can be used as indication leading to better performance of the technology. This study is focused on two bacterial groups (Anammox and Azoarcus-Thauera cluster) which have potential to serve as indicators for the landfill leachate treatment. Their quantity and activity were studied by FISH during lab-scale treatment of leachate from the Municipal

Enterprise for Waste Treatment (MEWT), Sofia, Bulgaria. Two activated sludges (AS) were used – one from the MEWT and another form the WWTP (wastewater treatment plant) of Sofia. The obtained results showed that 74% of the COD was eliminated when leachate was diluted 50 and 25 times and 31% - when undiluted leachate was used. At the end of the process (21 day) the Azoarcus-Thauera group formed large aggregations in the AS from MEWT. They were 17.50% of the bacteria there while in the AS from the WWTP of Sofia they represented only 2.61%. The quantity of the anammox bacteria remained almost unchanged during the process and was 10.75% of the community from MEWT which eliminated 98 mg/L more ammonium ions at the end of the process and 6% from the community from the WWTP of Sofia. The two studied groups gave more complex information about the processes in the AS related to the elimination of the nitrogen and carbon containing pollutants. They could be used for better management of the biological processes during landfill leachate treatment.

Г7.8 Chobanova, A., Belouhova, M., **Yotinov, I.**, Dinova, N., Daskalova, E., Todorova, Y., ... & Topalova, Y. (2021). Adaptation of activated sludge to treatment of landfill leachate during model process. *BULGARIAN CHEMICAL COMMUNICATIONS*, 57. DOI: 10.34049/bcc.53.A.0007; **(SJR=0.168; Q4) 12 т.**

Abstract

Landfill leachate is generated from the waste degradation in landfill sites and rainwater infiltrates. Its treatment includes more often biological methods combined with physical and chemical methods. The availability of polycyclic aromatic carbohydrates, phenols, polychlorinated phenols, pesticides, heavy metals, and refractory organics in landfill leachate remains a critical technological problem during biological treatment. The effect of these toxic pollutants on activated sludge (AS) processes is related to deformations of AS structure /bulking or pin-point flocs/ and inhibition of biodegradation activity. One of the most economic and effective possibilities for problem solving is application of adaptation as a biological approach. The aim of the study is to assess the activated sludge from wastewater treatment plant to Municipal enterprise for waste treatment of Sofia City during model adaptation process with landfill leachate. The duration of adaptation process was 21 days. The results confirm that the leachate diluted 25X contains toxic xenobiotics, which concentration is close to the critical one for the development of adaptive potential of AS in the concrete experimental conditions. This dilution of the leachate and the ratio COD:BOD5 are appropriate for accomplishment of wastewater treatment process.

Г7.9 Belouhova, M., Daskalova, E., **Yotinov, I.**, Topalova, Y., Velkova, L., Dolashki, A., & Dolashka, P. (2022). Microbial diversity of garden snail mucus. *MicrobiologyOpen*, *11*(1), e1263. https://doi.org/10.1002/mbo3.1263; (Web of science: **IF=3.4 (2022); Q2 (2022)) (SJR=0.729 (2022); Q2 (2022); 20 т.**

Abstract

The search for new natural compounds for application in medicine and cosmetics is a trend in biotechnology. One of the sources of such active compounds is the snail mucus. Snail

physiology and the biological activity of their fluids (especially the mucus) are still poorly studied. Only a few previous studies explored the relationship between snails and their microbiome. The present study was focused on the biodiversity of the snail mucus used in the creation of cosmetic products, therapeutics, and nutraceuticals. The commonly used cultivation techniques were applied for the determination of the number of major bacterial groups. Fluorescence in situ hybridization for key taxa was performed. The obtained images were subjected to digital image analysis. Sequencing of the 16S rRNA gene was also done. The results showed that the mucus harbors a rich bacterial community (10.78 × 1010 CFU/ml). Among the dominant bacteria, some are known for their ability to metabolize complex polysaccharides or are usually found in soil and plants (Rhizobiaceae, Shewanella, Pedobacter, Acinetobacter, Alcaligenes). The obtained data demonstrated that the snail mucus creates a unique environment for the development of the microbial community that differs from other parts of the animal and which resulted from the combined contribution of the microbiomes derived from the soil, plants, and the snails.

Г7.10 Todorova, Y., Benova, E., Marinova, P., **Yotinov, I.**, Bogdanov, T., & Topalova, Y. (2022). Non-Thermal Atmospheric Plasma for Microbial Decontamination and Removal of Hazardous Chemicals: An Overview in the Circular Economy Context with Data for Test Applications of Microwave Plasma Torch. *Processes*, *10*(3), 554. https://doi.org/10.3390/pr10030554; **(Web of science: IF=3.5 (2022), Q2 (2022); (SJR=0.529 (2022); Q2 (2022)) 20 т.**

Abstract

The transformation of our linear “take-make-waste” system to a cyclic flow of materials and energy is a priority task for society, but the circular use of waste streams from one industry/sector as a material input for another must be completely safe. The need for new advanced technologies and methods ensuring both microbiological safety and the removal of potential chemical residues in used materials and products is urgent. Non-thermal atmospheric plasma (cold atmospheric plasma—CAP) has recently attracted great research interest as an alternative for operative solutions of problems related to safety and quality control. CAP is a powerful tool for the inactivation of different hazardous microorganisms and viruses, and the effective decontamination of surfaces and liquids has been demonstrated. Additionally, the plasma’s active components are strong oxidizers and their synergetic effect can lead to the degradation of toxic chemical compounds such as phenols and azo-dyes.

Г7.11 Belouhova, M. V., **Yotinov, I. D.,** & Topalova, Y. I. (2023). Nanodiamonds improve amaranth biodegradation in a lab-scale biofilter. *Biotechnology & Biotechnological Equipment*, *37*(1), 317-328. https://doi.org/10.1080/13102818.2023.2191744; **(Web of science: IF=1.4 (2022), Q4 (2022)) (SJR=0.317 (2022); Q3 (2022)) 15 т.**

Abstract

Nanodiamonds (ND) are nano-particles with a size of 4–5 nm which are intensively investigated for a number of properties that make them suitable for bio-applications. They

could be chemically modified, they have a high adsorption capacity, high mechanical and chemical stability. Because ND have all these properties, they are also suitable for application in specific wastewater treatment and especially when the treated water is polluted with toxic compounds. The effect of ND applied in laboratory sand biofilter was investigated in this research. Synthetic wastewater with a model toxicant, the azo-dye amaranth, was used. The results showed that, in the presence of ND, the efficiency of amaranth removal remained high (more than 90%) in concentrations close to the critical one. The level of carbon-containing pollutants (assessed as chemical oxygen demand (COD) and total organic carbon (TOC)) decreased. The number of bacteria from the key detoxification groups increased 98 times for Pseudomonas sp. and 105 times for azo-degrading bacteria, while aerobic heterotrophs increased only 6 times after ND were applied. The abundance of Pseudomonas spp. after ND addition was confirmed with fluorescence in-situ hybridization. The activities of the key enzymes increased with 26–57%. Also, the activities of cathechol-1,2-dioxygenase and catechol-2,3-dioxygenase were found after ND addition but not before that. The obtained data showed that after ND were introduced in the wastewater treatment system, the number of biodegrading bacteria increased. They synthesized large amounts of azoreductases and oxygenases that led to the improved parameters of azo-detoxification process.

Г7.12 Dinova N, Peng W., Belouhova M., Lia C., Schneider I., Niea E., **Yotinov I.**, Duana H., Todorova Y., Lü F., Zhang H., Topalova Y., He P., (2023) Functional and molecular approaches for studying and controlling microbial communities in anaerobic digestion of organic waste: A review, *Reviews in Environmental Science and BioTechnology*, *22*, 563–590 (**Web of science: IF=14.4 (2022), Q1 (2022)) (SJR=2.410 (2022)); Q1 (2022)) 25 т.**

Abstract

Anaerobic digestion (AD) has been studied for centuries, but its operation still mainly relies on physicochemical indicators. Recent advanced molecular biological tools can unveil the nature of the AD process since microbial activity is directly related to digester performance. The paper summarized up-to-date microbiological and molecular biological analysis techniques applied in AD, including PCR-based techniques, electrophoresis, next-generation sequencing, MS-based techniques, and visualization-based techniques. In addition, the paper also reviewed the techniques that link microbial identity and activity to ecological function. Molecular biological techniques can identify microbial activity and AD process disturbance, but research on on-site analysis of microbial communities for a full-scale system is lacking. One of the most suitable methods for studying microbial communities in anaerobic digesters is fluorescence in situ hybridization, which does not require preparative isolation and cultivation. Another very important method is the use of physiological fluorescent probes to reveal the functional characteristics of methanogenic communities by CTC (5-cyano-2,3-ditolyl tetrazolium chloride)/DAPI (4'-6 diamino-2 phenylindole), which is a very rapid, sensitive and informative assay. These methods, together with the application of confocal laser microscopy, and the study of polyphosphate granules and Co-factor 420 of the microbial communities allow us a very effective and targeted functional-molecular control of the processes in anaerobic digesters.

Indicator 9. Invention, patent or utility model, for which a protective document has been issued in due order.

Г9.1 Utility model № 3227 U1/19.09.2019, Yana Topalova, Pavlina Dolashka, Nellie Zheleva, Irina Schneider, Yovana Todorova, Mihaela Belouhova, **Ivaylo Yotinov**, Elmira Daskalova, Lyudmila Velkova, Mariya Todorova, Nora Dinova

Abstract

The cosmetic composition includes a first active ingredient comprising peptides and proteins from a water-soluble extract of land snail slime Helix aspersa in the form of a serum with the conventional cosmetic additives, and a second active ingredient comprising an aqueous suspension of nanodiamonds. The weight ratio of the serum: nanodiamonds in the composition is 1:1, and the water-soluble extract of the land snail slime Helix aspersa is further enriched with fractions with molecular weight in the ranges above 10 kDa, 30 kDa and 50 kDa. The content of the first active ingredient in the serum is from 15% to 30%, and the content of the second active ingredient - nanodiamonds, in the finished composition, is 50 ppm. The cosmetic composition of the utility model has a proven cellular action, as it permeates deep into the cells, provides a soft peeling of the surface of the epidermis, and has antibacterial action, and also increases the detox and cell protection of and causes accelerated rates and increase of protein synthesis in skin cells. It will find application in cosmetics for eliminating various skin problems such as acne, rosacea, age spots and freckles, skin irritations, wrinkles, facial lines, including crow's feet, nasolabial folds, scars, stretch marks, dead skin cells that need exfoliation. Furthermore, the composition will find application in the recovery of the skin after dermatological interventions - injection mesotherapy, lifting procedures, peeling procedures, laser skin rejuvenation, and after fillers and Botox interventions for and so forth.