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RESEARCH PAPER

Study of biomass accumulation using different nutrient media

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Abstract

G. Bissenova, Z. Tekebayeva, B. Mussabayeva, A. Temirkhanov, and Z. Sarmurzina. 2024. Study of biomass accumulation using different nutrient media. Int. J. Agric. Nat. Resour. 1-9. In recent years, the use of microorganisms to stimulate plant growth and resistance, including biocontrol and maintenance or improvement of microbial activity and soil functionality, has increased. The aim of this work was to select nutrient media and to optimize the deep cultivation regimes of a biopreparation consisting of the *Bacillus pumilus* Pol P3(1) 10 B-RKM 0528, *Bacillus thuringiensis* Pb 30 B-RKM 0341, *Bacillus licheniformis* 356 B-RKM 0074 and *Serratia marcescens* Sh-2 B-RKM 0952 strains, which exhibit high growth-stimulating activity. Three different media were use to cultivate the biopreparation: standard meat-peptone broth medium, King's medium and Mueller-Hinton's medium. The main criterion for determining the effectiveness of the nutrient medium was the accumulation of specific components to yield the largest biomass of biopreparation cells. A comparative analysis of growth on the nutrient media was used to assess the viability and biomass accumulation of the biopreparation strains tested. The optimum cultivation period, maximum optical density of biomass growth, viability and optimum medium acidity during bacterial cultivation on nutrient media were determined both for the strains individually and as part of the biopreparation.

Keywords: cultivation, fermenter, bacteria, optical density, microorganism.

Introduction

Creating microbial complexes from agriculturally useful microbial strains aimed at enriching the soil is of great scientific and practical interest (Totubaeva et al., 2023). The choice of nutrient media is important for ensuring microbial suspension quality. The adequate nutritional status of microorganisms (nitrogen compounds, carbohydrates, micro- and macroelements and other biologically active substances) during cultivation depends on careful selection of nutrient media based on their individual characteristics (Titova et al., 2001; Rakhmetov et al., 2023). The most suitable method for producing bacterial biomass for biopreparations is deep cultivation (Tihonovich & Kruglov, 2006; Kalenska et al., 2023). The choice of cultivation conditions for each microorganism depends on the cultivation regime, such as temperature, agitation and aeration rates,

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hydrogen ion concentration (pH), redox potential (pH), oxygen partial pressure (pO_2) and glucose concentration (Sr).

This issue, though partially addressed by the scientific community, has gained complexity due to advancements in technology and approaches to biological processes (Marchenko et al., 2023; Wrzecińska et al., 2023). Ackermann and Tardito (2019) analyzed nutrient media content and characteristics, revealing key factors that influence the process, while also examining conditions for formulating the medium. Chao et al. (2019) proposed strategies for increasing biopreparation biomass and outlined essential tools and methods. Their main focus was on cultivation, investigating distinct properties in various media and bacterial strains. Moreover, Lee et al. (2019) assessed different bacterial strains suitable for cultivation, identifying crucial elements and considerations for choosing microorganism species.

The current study stands out from previous research by providing valuable insights into microbial biomass production for agriculture, especially plant cultivation. This study contributes by outlining the specifics of biomass accumulation in different media and underscores the need for future research to explore inhibiting factors in conventional media. The aim of the present study was to analyze and evaluate the process of biomass accumulation based on a number of features resulting from the introduction of specific cultures, including nutrient media. The specific objectives were as follows:

- determine nutrient medium parameters;
- describe the biopreparation strains;
- evaluate the growth dynamics of biopreparation under the influence of specific factors;
- carry out the cultivation of the biopreparation in different media and identify and assess the most tangible medium conditions where biomass accumulation of the biopreparation would take place.

Materials and Methods

The aim of the study was to develop a growth-stimulating biopreparation for agricultural crops consisting of the *Bacillus pumilus*, *Bacillus thuringiensis*, *Bacillus licheniformis* and *Serratia marcescens* strains. The selected strains were chosen based on their isolation from the rhizosphere of soil under wheat cultivation in the Akmola region. The media were chosen based on their composition and suitability for promoting the growth of the selected strains. The growth of the biopreparation was monitored over time by measuring the optical density and pH at specific intervals.

The strains were stored in the Republican Collection of Microorganisms under specific entry numbers. The growth of the strains was assessed on three liquid media: standard MPB (meat-peptone broth) medium, King's medium and Mueller–Hinton's medium to select the optimal media for the cultivation of the strains included in the biopreparation (*Bacillus pumilus* Pol P3(1) 10 B-RKM 0528, *Bacillus thuringiensis* Pb 30 B-RKM 0341, *Bacillus licheniformis* 356 B-RKM 0074 and *Serratia marcescens* Sh-2 B-RKM 0952). The composition of the nutrient media was as follows:

1. Standard MPB medium (Hi-Media), g/l: 5 g peptone, 5 g NaCl, 5 g meat extract, 1.5 g yeast extract and distilled water.

2. King's medium, g/l: 20 g of peptone, 15 g glycerine, 1.5 g $MgSO_4.7H_2O$, 1.5 g anhydrous K_2HPO_4 and 1.5 g distilled water.

3. Mueller–Hinton's medium, g/l: 17.5 g acid casein hydrolysate, 3.0 g meat extract, 1.5 g starch and distilled water.

Optimal cultivation conditions were established to achieve a high concentration of active cells. Cultivation was conducted at 37 °C in 250-ml Erlenmeyer flasks utilizing 100 ml of nutrient medium and an initial inoculum comprising 5-10% of the nutrient medium. This optimization occurred over three days on a shaker (Innova 44R, USA; New Brunswick Scientific) at 150 rpm. Liquid culture (LC) samples were collected at 8, 16, 24, 36, 48, and 72 hours after the start of cultivation. Koch's method was used to determine the number of bacterial cells, involving dilution preparation, culture inoculation on nutrient media in Petri dishes, and colony counting. The number of cells per ml of the study LCs were calculated using the following formula:

$$T = \frac{a \times 10^{n}}{V} \quad (1)$$

where T is the number of colony-forming units (CFU) in 1 ml, a is the average number of colonies grown after inoculation from a given dilution, V is the volume of suspension taken for inoculation and 10^n is the dilution factor.

The optical density (OD) and pH of the test media were also measured at 8, 16, 24, 36, 48 and 72 hours after the start of cultivation. During the course of periodic growth, the biomass of the biopreparation suspension was determined by the OD at 600 nm using a spectrophotometer (Agilent Cary 60, USA). The experimental data were analyzed via standard computer programmes.

Results

To create an ideal medium for microbial growth and maximize biomass accumulation, it is essential to carefully select nutrient components and study cultivation parameters. In the quest for the best medium for biopreparation cultivation, three options were evaluated: standard MPB medium, King's medium, and Mueller–Hinton's medium. Table 1 presents the growth results of both the strains and biopreparations on these media, revealing consistent growth and biomass accumulation across all four strains, irrespective of the medium used.

The strains included in biopreparations should exhibit a high viability rate. For this purpose, a viability index assessment of the bacillary strains *Bacillus pumilus* Pol P3(1) 10 B-RKM 0528, *Bacillus thuringiensis* Pb 30 B-RKM 0341, *Bacillus licheniformis* 356 B-RKM 0074 and *Serratia marcescens* Sh-2 B-RKM 0952, which were included in the biopreparation, was conducted. During the study, MPB, King's, and Mueller–Hinton's media were used, as good culture growth was previously observed. The viability data are presented in Table 2.

Almost all the strains had high viability on each medium, but the highest values were observed for *B. licheniformis* 356 strain B-RKM 0074 on Mueller–Hinton's medium (1.5×10^9 CFU/ml) supplemented with salts (4.0×10^8 CFU/ml). The lowest viability was observed for the *Bacillus*

Table 1. Comparative assessment of strain and biopreparation growth on liquid media

Name	Medium		
	MPB	King's	Mueller-Hinton's
Bacillus pumilus Pol P3(1) 10 B-RKM 0528	++++	++++	++++
Bacillus thuringiensis Pb 30 B-RKM 0341	+++	+++	++++
Bacillus licheniformis 356 B-RKM 0074	++++	++++	++++
Serratia marcescens Sh-2 B-RKM 0952	++++	+++	++++
Biopreparation	++++	++++	++++

Note: ++++ – very good growth, +++ – good growth, ++ – weak growth, + – very weak growth.

Table 2. Strain and biopreparation viability on different media

Name	Viability indicator, CFU/ml		
	MPB	King's medium	Mueller-Hinton's medium
Bacillus pumilus Pol P3(1) 10 B-RKM 0528	4.0×10^7	3.5×10^7	3.0×10^8
Bacillus thuringiensis Pb 30 B-RKM 0341	4.0×10^5	6.5×10^5	9.0×10^5
Bacillus licheniformis 356 B-RKM 0074	2.0×10^8	7.5×10^6	1.5×10^9
Serratia marcescens Sh-2 B-RKM 0952	6.0×10^7	2.0×10^8	1.5×10^8
Biopreparation	1.0×10^8	3.5×10^7	6.5×10^7

thuringiensis Pb 30 B-RKM 0341 strain (4.0×10^5 CFU/ml to 9.0×10^5 CFU/ml).

The *Bacillus pumilus* Pol P3(1) 10 B-RKM 0528, *Bacillus thuringiensis* Pb 30 B-RKM 0341, *Bacillus licheniformis* 356 B-RKM 0074, and *Serratia marcescens* Sh-2 B-RKM 0952 strains were subjected to batch cultivation experiments to assess the growth-stimulating biopreparation rate on three different media. The cultivation conditions were optimized to achieve a high concentration of active cells by cultivating the cells at 37 °C in 250-ml Erlenmeyer flasks with 100 ml of nutrient medium and 5-10% inoculum. The cultivation was conducted on a shaker at 150 rpm for three days, with LC samples taken at various time points. Koch's method was used to determine the bacterial cell counts, and the results are presented in Table 3 and Figure 1.

Figure 1 shows that the cell growth of the biopreparation on MPB medium exhibited a notable pattern. Over the course of 3 days, the cell titers increased at 16, 24, and 36 hours, followed by a sharp decline at 48 hours. Similarly, on Mueller–Hinton's medium, high cell growth occurred

Table 3. Cell growth viability of the biopreparation cultivated on different media (Koch's method)

Medium	Viability indicator, CFU/ml					
	8 hr	16 hr	24 hr	36 hr	48 hr	72 hr
MPB	15.0×10^7	89.0×10^9	53.5×10^7	103×10^9	8.0×10^9	23.0×10^9
King's	18.0×10^7	16.0×10^7	25.0×10^7	26.5×10^7	12.0×10^7	24.5×10^7
Mueller-Hinton's	26.0×10^7	11.0×10^9	35.5×10^7	17.0×10^9	26.0×10^9	4.5×10^9

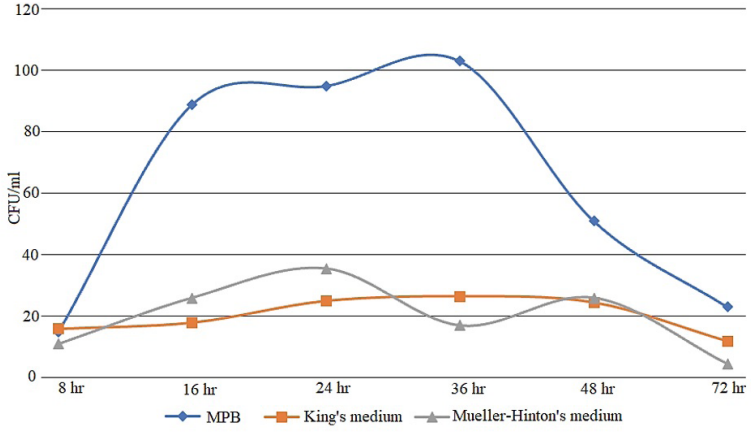

Figure 1. Dynamics of biopreparation cell growth viability when cultivated on different media.

at 16 and 24 hours, but there was a significant decrease by the 36th hour. On King's medium, cell growth was observed at the 24th, 36th, and 48th hours, with a rapid decrease at the 72nd hour. Additionally, OD of the biopreparation was monitored on three test media (MPB, King's, and Mueller–Hinton) at various pH values by measuring it at 8, 16, 24, 36, 48, and 72 hours after the start of cultivation. Biomass levels were assessed in terms of OD at 600 nm using an Agilent Cary 600 spectrophotometer, and the results are summarized in Table 4 and Figure 2.

The MPB medium was determined to be the most effective at increasing the OD of the biopreparation cells compared to other media. The OD of the biopreparation was 1.2631 to 2.3996 units (72 hours) on MPB medium, 1.5557 to 2.0540 units (48 hours) on King's medium, and 1.5613 to 2.4763 units (72 hours) on Mueller–Hinton's medium. One particular factor determining normal bacterial growth is the reaction to the medium. If the growth direction changes to an unfavorable direction, the microorganisms will stop growing, even if all the other medium conditions are adequate (Ackermann & Tardito, 2019).

The specific components that contributed to the greatest biomass accumulation in the studied nutrient media were found in the standard MPB medium. The composition of this medium was 5 g/l peptone, 5 g/l NaCl, 5 g/l meat extract, 1.5 g/l yeast extract and distilled water. These components were used in the cultivation of the strains included in the biopreparation, namely, *B. pumilus*, *B. thuringiensis*, *B. licheniformis*, and *S. marcescens.*

The pH values of the biopreparation in three different media were measured at intervals of 8, 16, 24, 36, 48, and 72 hours using an 827 pH lab meter (Metrohm, Switzerland). The pH levels

Table 4. Optical density of the biopreparation mixture cultivated on different media

Medium	Optical density over time, 600 nm					
	8 hr	16 hr	24 hr	36 hr	48 hr	72 hr
MPB	1.2631	2.3033	2.1486	2.2612	2.2109	2.3996
King's	1.5557	1.9357	2.0191	2.0427	2.0540	2.0486
Mueller-Hinton's	1.5613	2.2195	1.9105	2.0052	2.3848	2.4763

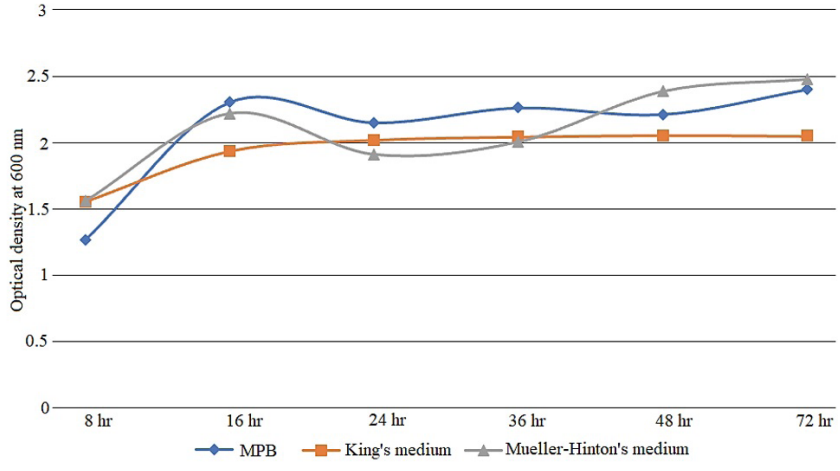

Figure 2. Dynamics of the optical density of the growth-stimulating biopreparation cultivated on three different media

varied across the three media, as shown in Table 5 and Figure 3. Over the three-day cultivation period, the pH of the biopreparation on MPB medium remained within the normal range of 6.5-7, while on King's medium, it was within the range of 5-6; on Mueller–Hinton's medium, it remained approximately 7. The acidity levels (pH) of the MPB and Mueller–Hinton's media, but not of King's medium, were consistent with the optimum medium reaction ranges for bacillary strains.

Thus, the main parameters of biomass accumulation of the biopreparation with a high growth-stimulating effect on agricultural crops were studied herein and optimized on different nutrient media. MPB and Mueller–Hinton's media were selected after cultivating the biopreparation on three different nutrient media. The selected media ensure maximum biomass yield and active cell titer via their compositions. All the experimental results demonstrated a maximum microbial mass content and high cell titer at 36–48 h after the start of cultivation.

Discussion

The topic addressed herein has been studied by a number of scholars, such as Bonnet et al. (2020), who explored the use of red blood cell mass from human blood as a nutrient base for microbial growth. Their study evaluated the growth characteristics of these nutrient media using an algorithm for cultivating test strains and compared them to standard media parameters. This research unveiled novel methods for determining biomass accumulation, offering promising prospects for future development and analysis of biomass accumulation features.

Table 5. pH of the biopreparation cultivated on different media

Medium	Optical density over time, 600 nm					
	8 hr	16 hr	24 hr	36 hr	48 hr	72 hr
MPB	6.5	7.5	7	7	7	7
King's	5	5	4.5	5	5	6
Mueller-Hinton's	7	7	6.8	7	7	7

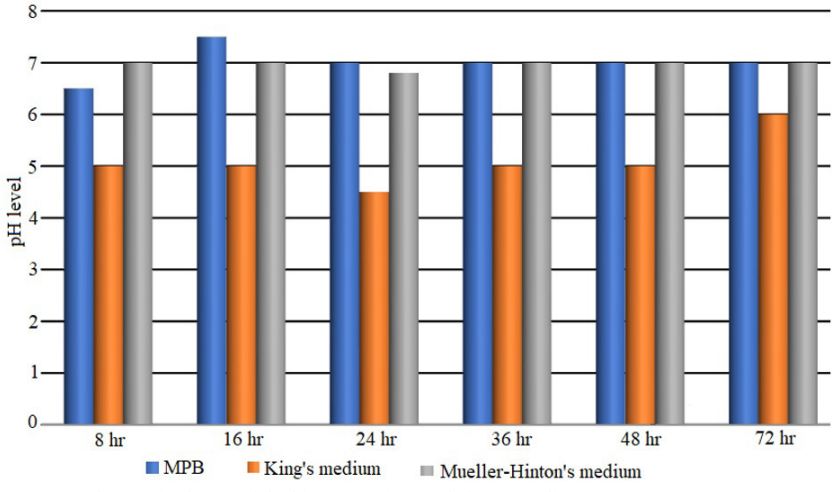

Figure 3. Changes in the pH of the biopreparation on different media

Prommer et al. (2020) also investigated biomass accumulation, emphasizing the importance of specific environmental conditions for optimal microorganism growth. They advocated for an in-depth analysis of morphological, physiological, and biochemical traits in microbial strains during cultivation, which they believe will facilitate the modeling of qualitative vectors for future work, especially in understanding the factors that attract diverse microorganisms for various media development processes.

Akcay and Avci (2020) identified cost-effective protein substrates with potential applications in biotechnology, especially in proteolytic component production. They obtained specific mathematical data related to the proteolytic structures' properties, enabling them to calculate the maximum proteolytic activity of *Bacillus bacteria*. The cultivation was conducted under specific conditions for 72 hours at 37 °C with varying bran protein and whole flour protein concentrations. Those researchers believe these findings can be applied to conduct a factor analysis for optimizing microorganism ratios in nutrient media. Perkovic et al. (2022) concentrated on creating optimal conditions for cocultivating probiotic cultures. They achieved this goal by analyzing and comparing various bacterial strains and examining the nitrogen composition of the nutrient medium, which affected the pH, cultivation duration, and generation quality.

Yeom et al. (2021) explored the use of soil microorganisms for enhancing soil fertility and promoting the growth of plants and fungi. They found that specific soil microorganisms can mineralize both organic and inorganic matter, improving the quality and efficiency of the minerals and organic compounds that enrich the soil with molecular nitrogen. These compounds also contribute to the synthesis of essential structures such as polysaccharides, proteins, and vitamins, ultimately leading to increased plant yields.

Conclusions

The study yielded valuable insights into microbial biomass production, resulting in the development of a growth-stimulating biopreparation designed for agricultural purposes, particularly plant cultivation. This biopreparation comprises several bacterial strains, namely, *Bacillus pumilus*, *Bacillus thuringiensis*, *Bacillus licheniformis*, and *Serratia marcescens.* Investigation of its growth dynamics and biomass accumulation across different media, namely, MPB, King's, and Mueller–Hinton media, revealed consistent and robust performance across all conditions and indicated high and active viability of the microorganisms involved.

The optical density of the biopreparation under various conditions was also investigated by cultivating the biopreparation simultaneously in MPB, King's, and Mueller–Hinton media. This analysis revealed key aspects of the biopreparation's biomass accumulation process, characterized by its potent growth-stimulating effect and suitability for cultivating agricultural crops, across a range of nutrient media. After cultivation, the most effective conditions for biopreparation development were determined, and MPB and Mueller–Hinton's media were selected due to their ability to yield maximum biomass and active microelement levels. Consequently, the unique features of biomass accumulation based on cultivation and activity within different nutrient media were elucidated.

This study has significant scientific and practical implications for agriculture, biotechnology, and related fields employing bacterial biomass for plant growth stimulation and microbial support. A comprehensive evaluation of the growth dynamics and biomass accumulation of multiple bacterial strains in diverse media offers valuable insights into microbial biomass production, particularly for plant cultivation. Emphasizing the consistent high viability and potent growth-stimulating effect of the biopreparation underscores the importance of understanding the specifics of biomass accumu-

lation in various media. This research not only highlights the essential microelements for plant growth in agriculture but also highlights the need for future investigations into inhibitory factors in conventional media to aid in optimizing microbial activity and biomass production for diverse agricultural and biotechnological applications.

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Resumen

G. Bissenova, Z. Tekebayeva, B. Mussabayeva, A. Temirkhanov, y Z. Sarmurzina. 2024. Estudio de la acumulación de biomasa utilizando diferentes medios nutritivos. Int. J. Agric. Nat. Resour. 1-9. En los últimos años, ha aumentado el papel de los microorganismos para estimular el crecimiento y la resistencia de las plantas, lo que incluye el biocontrol, así como el mantenimiento o la mejora de la actividad microbiana y la funcionalidad del suelo. El objetivo de este trabajo fue seleccionar medios nutritivos y optimizar los regímenes de cultivo en profundidad del biopreparado compuesto por cepas de Bacillus pumilis Pol P3(1) 10 B-RKM 0528, Bacillus thuringiensis Pb 30 B-RKM 0341, Bacillus licheniformis 356 B-RKM 0074 y Serratia marcescens Sh-2 B-RKM 0952 que proporcionan una elevada actividad estimuladora del crecimiento. Los investigadores utilizaron tres medios para cultivar el biopreparado, que son el medio estándar de caldo de carne y peptonas, el medio de King y el medio de Mueller-Hinton. Los principales criterios para determinar la eficacia del medio nutritivo son la acumulación de componentes específicos para producir la mayor biomasa de células del biopreparado. Los autores utilizaron un análisis comparativo sobre el crecimiento en medio nutritivo para evaluar la alta viabilidad y la acumulación de biomasa del biopreparado ensayado. Los investigadores determinaron el periodo óptimo de cultivo, la densidad óptica máxima de crecimiento de la biomasa, la viabilidad y la acidez óptima del medio durante el cultivo de bacterias en medios nutritivos, tanto individualmente como formando parte del biopreparado.

Palabras clave: Bacterias, cultivo, densidad óptica, fermentador, microorganismo.

References

Ackermann, T. & Tardito, S. (2019). Cell culture medium formulation and its implications in cancer metabolism. *Trends in Cancer, 5*(6), 329-332. https://doi.org/10.1016/j.trecan.2019.05.004

Akcay, F.A. & Avci, A. (2020). Screening and selection of media components for protease production by bacillus sp. EBTA6 using Plackett-Burman design. *Turkish Journal of Agriculture-Food Science and Technology*, *8*(6), 1250-1255. https://doi.org/10.24925/turjaf.v8i6.1250-1255.2986

Bonnet, M., Lagier, J.C., Raoult, D. & Khelaifia, S. (2020). Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes and New Infections, 34*, 1-11. https://doi.org/10.1016/j.nmni.2019.100622

Chao, Z., Liu, N., Zhang, P., Ying, T. & Song, K. (2019). Estimation methods developing with remote sensing information for energy crop biomass: A comparative review. *Biomass and Bioenergy, 122*, 414-425. https://doi.org/10.1016/j.biombioe.2019.02.002

Kalenska, S., Novytska, N., Kalenskyi, V., Osman, O.E.F. & Melnychenko, V. (2023). Efficiency

of the compensatory scheme of using Organic Acid Nano-Complex microfertilizers in spring wheat cultivation technology. *Plant and Soil Science*, *14*(4), 61-75. https://doi.org/10.31548/plant4.2023.61

Lee, S., Kang, M., Bae, J.H., Sohn, J.H. & Sung, B.H. (2019). Bacterial valorization of lignin: strains, enzymes, conversion pathways, biosensors, and perspectives. *Frontiers in Bioengineering and Biotechnology, 7*, 209. https://doi.org/10.3389/fbioe.2019.00209

Marchenko, T., Skakun, V., Lavrynenko, Yu., Zavalnyuk, O. & Skakun, Ye. (2023). Biometric parameters and yield of maize hybrids in dependence on agricultural technology elements. *Scientific Horizons*, *26*(11), 90-99. https://doi.org/10.48077/scihor11.2023.90

Perkovic, L., Djedović, E., Vujović, T., Baković, M., Paradžik, T. & Čož-Rakovac, R. (2022). Biotechnological enhancement of probiotics through co-cultivation with algae: Future or a trend? *Marine Drugs, 20*(2), 142. https://doi.org/10.3390/md20020142

Prommer, J., Walker, T.W., Wanek, W., Braun, J., Zezula, D., Hu, Y. & Richter, A. (2020). Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Global Change Biology, 26*(2), 669-681. https://doi.org/10.1111/gcb.14777

Rakhmetov, D., Bondarchuk, O., Rakhmetova, S., Rashydov, N. & Kutsokon, N. (2023). Biological, morphological and biochemical features of seeds of introduced genotypes of Cicer arietinum L. *Plant and Soil Science*, *14*(3), 97-100. https://doi.org/10.31548/plant3.2023.97

Tihonovich, I.A. & Kruglov, J.V. (2006). Microbiological aspects of soil fertility and problem so sustainable agriculture. *Plodorodiie*, *5*, 9-12. https://cyberleninka.ru/article/n/mikrobiologicheskie-aspekty-mnogoletnego-sistematicheskogo-primeneniya-gerbitsidov-v-zemledelii.pdf

Totubaeva, N., Batykova, A., Karches, T., Osmonaliev, A. & Sultanalieva, V. (2023). Ecological aspects in the use of soil enzymes as indicators of anthropogenic soil pollution. *Scientific Horizons, 26*(12), 88-98. https://doi.org/10.48077/scihor12.2023.88

Wrzecińska, M., Czerniawska-Piątkowska, E., Kowalewska, I., Kowalczyk, A., Mylostyvyi, R. & Stefaniak, W. (2023). Agriculture in the face of new digitization technologies. *Ukrainian Black Sea Region Agrarian Science*, *27*(3), 9-17. https://doi.org/10.56407/bs.agrarian/3.2023.09

Yeom, Y.H., Oh, J.H. & Yoon, M.H. (2021). Plant-growth promoting traits of bacterial strains isolated from button mushroom (*Agaricus bisporus*) media. *Journal of Mushroom*, *19*(3), 134-139. https://db.koreascholar.com/Article/Detail/409785