TESTING AND ASSESSING FUNCTIONALITY OF KERATIN HYDROLYSATE WITH AGRICULTURAL APPLICATION ON WHEAT SEEDS

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ABSTRACT. This work aimed to obtain, characterize and evaluate keratin hydrolyzate with specific properties for applications in agriculture. The KerKAgr product, made by hydrolysing sheep wool with potassium hydroxide, was applied to wheat seeds. Physico-chemical analyses show that the keratin hydrolyzate obtained is rich in minerals, nitrogen and protein substances necessary in the plant growth process. Keratin hydrolyzate, KerKAgr, was used in treatments on Tamino and Mirastar wheat seeds, and the observations made showed an improvement in the germination rate and a stimulation of plant growth. KerKAgr 3% and 5% was used to treat wheat seeds and then germination and plant growth were monitored. The effect of keratin hydrolyzate was evaluated for 10 days on the two types of wheat (Tamino and Mirastar) treated with keratin hydrolyzate. The results of the treatments indicated an increased stimulation of seed germination by 10-20% compared to the control and a growth of wheat plants by 7.1-21.7% compared to the control. The biostimulating effect of keratin hydrolyzate has been highlighted. The good results obtained in the applications of keratin hydrolyzate in agriculture show that keratin hydrolyzate can be the basis for obtaining new biomaterials with various applications.
KEY WORDS: keratin hydrolyzate, biostimulator, seed germination

TESTAREA ȘI EVALUAREA FUNCȚIONALITĂȚII HIDROLIZATULUI DE CHERATINĂ CU APLICAȚIE ÎN AGRICULTURĂ PE SEMINȚE DE GRÂU

REZUMAT. Această lucrare a avut ca scop obținerea, caracterizarea și evaluarea hidrolizatului de cheratină cu proprietăți specifice pentru aplicații în agricultură. Produsul KerKAgr, obținut din lână de oaie prin hidroliză cu hidroxid de potasiu, a fost aplicat pe semințe de grâu. Analizele fizico-chimice arată că hidrolizatul de cheratină obținut este bogat în minerale, azot și substanțe proteice necesare în procesul de creștere a plantelor. Hidrolizatul de cheratină, KerKAgr, a fost utilizat în tratamente pe semințe de grâu, tip Tamino și Mirastar, iar observațiile făcute au arătat o îmbunătățire a ratei de germinare și o stimulare a creșterii plantelor. KerKAgr 3% și 5% a fost folosit pentru tratarea semințelor de grâu și apoi s-au monitorizat germinarea și creșterea plantelor. Efectul hidrolizatului de cheratină asupra celor două tipuri de grâu tratate cu hidrolizat de cheratină (Tamino și Mirastar) a fost evaluat timp de 10 zile. Rezultatele tratamentelor au indicat o stimulare crescută a germinării semințelor cu 10-20% față de martor și o creștere a plantelor de grâu cu 7,1-21,7% față de martor. Efectul biostimulator al hidrolizatului de cheratină a fost evidențiat. Rezultatele bune obținute în aplicațiile hidrolizatului de cheratină în agricultură arată că hidrolizatul de cheratină poate sta la baza obținerii de noi biomateriale cu aplicații diverse.
CUVINTE CHEIE: hidrolizat de cheratină, biostimulator, germinarea semințelor

TEST ET ÉVALUATION DE LA FONCTIONNALITÉ DE L'HYDROLYSAT DE KÉRATINE AVEC APPLICATION AGRICOLE SUR LES GRAINES DE BLÉ

RÉSUMÉ. Ce travail visait à obtenir, caractériser et évaluer un hydrolysat de kératine aux propriétés spécifiques pour des applications en agriculture. Le produit KerKAgr, obtenu à partir de laine de mouton par hydrolyse avec de l'hydroxyde de potassium, a été appliqué sur des graines de blé. Les analyses physico-chimiques montrent que l'hydrolysat de kératine obtenu est riche en minéraux, substances azotées et protéiques nécessaires au processus de croissance des plantes. L'hydrolysat de kératine, KerKAgr, a été utilisé dans les traitements des graines de blé Tamino et Mirastar, et les observations réalisées ont montré une amélioration du taux de germination et une stimulation de la croissance des plantes. KerKAgr 3% et 5% ont été utilisés pour traiter les graines de blé puis la germination et la croissance des plantes ont été suivies. L'effet de l'hydrolysat de kératine sur les deux types de blé traités avec l'hydrolysat de kératine (Tamino et Mirastar) a été évalué pendant 10 jours. Les résultats des traitements ont indiqué une stimulation accrue de la germination des graines de 10 à 20 % par rapport au témoin et une augmentation des plants de blé de 7,1 à 21,7 % par rapport au témoin. L'effet biostimulant de l'hydrolysat de kératine a été mis en évidence. Les bons résultats obtenus dans les applications de l'hydrolysat de kératine en agriculture montrent que l'hydrolysat de kératine peut être la base pour l'obtention de nouveaux biomatériaux avec diverses applications.
MOTS CLÉS : hydrolysat de kératine, effet biostimulant, germination des graines

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INTRODUCTION

The increase in population and the decrease in the area of arable land represented a challenge for the development of agriculture [1-3]. Ensuring the supply of sufficient and quality food is an important project of all countries in the world. The need for fertilizer application has played a critical role in agricultural production. It is estimated that 2.50×106 tons of fertilizers are used in agriculture every year [1, 2]. Application of these fertilizers can increase crop yield by 56%-58% and grain yield by 31%-32% [1-3]. The excessive application of chemical fertilizers directly results in the pollution of the agricultural environment, through soil acidification, soil hardening, nutrient imbalance and surface water pollution, etc. [4]. Some fertilizers also contain heavy metals; such as Zn, Cu, Co and Cr. Long-term use will lead to soil environmental damage and metal pollution [5, 6]. In order to mitigate environmental pollution, increase nutrient use efficiency, and develop sustainable ecological agriculture, eco-fertilizers have been widely produced [5-7]. Renewable materials are brought to attention because they could provide sustainable solutions for the production of ecological fertilizers, such as cellulose [8], chitosan [9, 10], alginate [11], starch [12, 13], and even branch waste of mulberry [14] and flax thread waste [15]. Besides the renewable natural materials mentioned, keratin derived from industrial by-products has started to be studied for use in agriculture [5-7]. Keratin is a biomass resource [16], which contains functional groups, it is renewable, degradable, non-toxic and has a low price. The protein substances that make up keratin can be a precursor of substances that restore humus and organic carbon in the soil [17]. It can also stabilize organic nitrogen in the soil [18].

A large part of sheep wool waste is produced in the processing of skins [19]. Sheep wool waste is one of the main sources of keratin, which presents some properties such as availability, non-toxicity, bio-degradation and bio-compatibility, etc. Keratin extracted by chemical methods is easily degraded by microorganisms into amino acids, which can increase microbial activity, improve soil structure and physical and chemical properties, and improve plant growth [1, 2, 19]. The use of waste for the production of fertilizers promotes industrial sustainability and the transition to a green economy [1, 2]. This research aims to provide an innovative strategy for resource utilization of sheep wool waste through its recycling and sustainable agricultural development. This study reported an environmentally friendly multifunctional fertilizer based on keratin from sheep wool waste. Keratin as a natural polymeric material is composed of amino acids that can increase the activity of microorganisms and improve the soil environment [1, 2, 19]. Keratin has recently appeared in various applications based on natural biomaterials. In addition to the potential to assemble into organized structures, keratin also consists of bioactive elements suitable for initiating biocompatible interactions with various substrates [20]. Keratin-rich wastes pollute the environment and are released in increasing quantities as by-products of agro-industrial or leather tanning processes [21-23]. Keratin from renewable sources in the leather industry is abundant, available and easy to harvest. Extracted keratin hydrolysates can be processed into different products using specific techniques established for a wide range of fields [24, 25]. Keratinous materials have a high protein content consisting of at least 17 amino acids that can be used in animal feed or agricultural fertilizers [19]. Degradation of keratin waste may therefore provide a cheap source of protein and amino acids [26]. The development of an ecological waste-based fertilizer is important in modern agriculture, due to the costly, incomplete degradation and supply restrictions of traditional fertilizers [4, 5, 7]. This could be an ecological multifunctional fertilizer rich in nutrients and with soil restoration action [4, 5, 7].

EXPERIMENTAL

Materials and Methods

In the experimental part, the obtaining, characterization and evaluation of keratin hydrolyzate with specific properties for applications in agriculture was pursued. Wool waste was purchased from sheep breeders in Constanța county, sodium carbonate and ammonia were supplied by SC Cristal RChim SRL, potassium hydroxide was supplied by Lachner. Keratin hydrolyzate was obtained from wool waste by alkaline hydrolysis with potassium hydroxide. All reagents used were for laboratory grade.

Obtaining Keratin Hydrolyzate

The wool recovered from the waste resulting from the processing of natural furs was degreased with 1% Na_2CO_2, 2% NH_3 (sol. 25%), 1% detergent, 1:10 water, 45°C, stirring, 3 hours, dried, shredded and hydrolyzed by the alkaline method, with 8% potassium hydroxide, at 97°C, stirring, 4 hours to obtain keratin hydrolyzate with protein macromolecular chains with a high degree of cleavage. The alkaline hydrolysis process consisted of the following stages: degreasing the raw wool, drying, shredding, alkaline hydrolysis, decantation, filtration and separation of the keratin hydrolyzate obtained. Keratin hydrolyzate, obtained by alkaline hydrolysis with potassium hydroxide, can be used in agriculture as a fertilizer to supply minerals and organic nitrogen in the development of plants grown on poor soils. The process of alkaline hydrolysis with potassium hydroxide by optimizing the technological parameters of temperature, reaction times, homogenization of the reaction mass and going through the sequence of work stages leads to obtaining keratin hydrolyzate rich in growth biostimulants and nutrients, important for ecological agriculture and as a rich source of organic nitrogen and mesonutrient sulfur for plants.

Characterization of Keratin Hydrolyzate

The keratin hydrolyzate obtained by alkaline hydrolysis was characterized by physico-chemical analyses regarding the content in: dry matter (SR EN ISO 4684:2006), ash (SR EN ISO 4047:2002), total nitrogen (SR ISO 5397:1996), protein substance (SR ISO 5397:1996), pH (STAS 8619/3:1990).

Physico-Chemical Analysis of Keratin Hydrolyzate for Applications in Agriculture

The physico-chemical characteristics highlight the functionality of the keratin hydrolyzate obtained with potassium hydroxide as follows: the content in ash (11.61%) shows that the keratin hydrolyzate is rich in mineral substances, total nitrogen (14.12%) and protein substance (85.49%) as an important source of organic nitrogen, the slow-release nutrient source for the development of plant crops (Table 1).

Table 1: Physico-chemical characteristics of the keratin hydrolyzate obtained by hydrolysis with potassium hydroxide for agriculture, KerKAgr

Characteristics	Dry substance, %	Ash, %	Total nitrogen, %	Protein substance, %	pH, pH units
No	1	2	3	4	5
KerKAgr	5.17	11.61	14.12	85.49	10.05

*The values for items 3 and 4 are related to the dry substance.

Analysis of Keratin Hydrolyzate by Electrophoresis

The keratin hydrolyzate obtained was characterized by determining the molecular weight distribution using SDS-PAGE electrophoresis. One of the most important factors in obtaining bioactive peptides with functional properties is their molecular weight. In the experimental study to determine the molecular masses of the proteins, the high polydispersity was taken into account, due to the presence of polypeptides, oligopeptides and free amino acids and generally having molecular masses between 3 and 198 KDa. KerKAgr was analyzed by acrylamide gel electrophoresis in the kuroGEL Verti 10x10 cm system, Vertical Dual-Gel Units, VWR Austria. Proteins were visualized by blue staining with Coomasie Blue Stain R250.

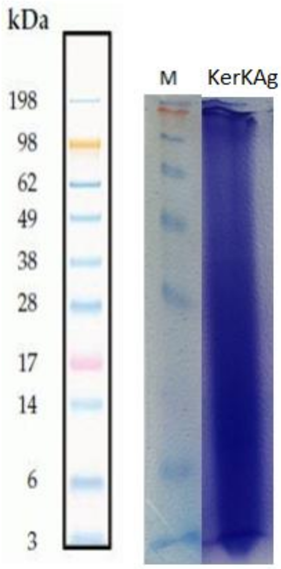

Figure 1. The range of molecular masses corresponding to the marker, M, and molecular mass distribution in keratin hydrolyzate, KerKAgr

The marker has a known molecular mass range of 3-198 KDa. Keratin hydrolyzate contains proteins with varying molecular masses, in the range of 3-198 KDa, throughout the marker domain. The electrophoretic pattern of wool keratin (Figure 1) shows the main two groups of proteins associated with keratin, intermediate filamentous proteins and matrix proteins. High molecular weight bands (45-60 kDa) attributed to filamentous proteins with a low sulfur content and low molecular weight bands attributed to proteins with a high sulfur content (20-10 kDa) and high glycine /tyrosine (6-9 kDa) content can be observed [27]. These considerations highlight the presence of proteins with a high sulfur content but also rich in glycine/tyrosine with low molecular masses in the keratin hydrolyzate extracted with potassium hydroxide, but also proteins with a low sulfur content and high molecular mass attributed to the proteins filamentous.

Determination of particle size by DLS

KerKAgr was analyzed with Zetasizer Nano ZS equipment, Malvern. Three measurements were made to determine the size of the particles and the Zeta potential. Two major populations were measured (Figure 2) at 164 nm and at 1106 nm, with an average of 527 nm and polydispersity of 0.739. These data confirm the analyzes obtained by the electrophoresis method of obtaining compounds with molecular masses lower than 20KDa, but also some compounds with high molecular masses.

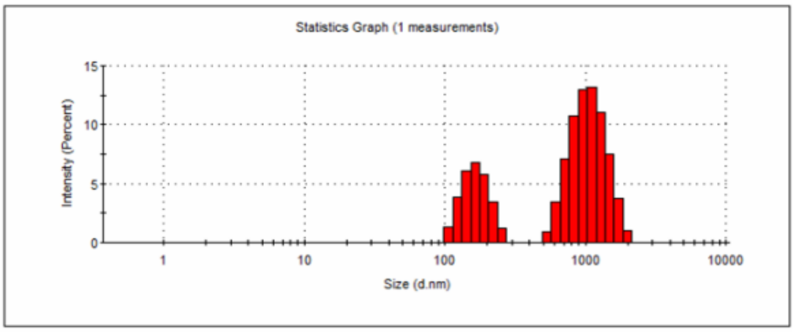

Figure 2. Histogram of particle size distribution in keratin hydrolyzate, KerKAgr

Zeta potential of -33 mV is an indicator of sample stability. A sample is considered stable if the potential value is outside the range (-30; +30 mV). The values included in this range suggest the tendency of agglomeration or deposition of particles. Also, the sign represents the type of charges on the surface of the particles, in the present case being negative charges (Figure 3).

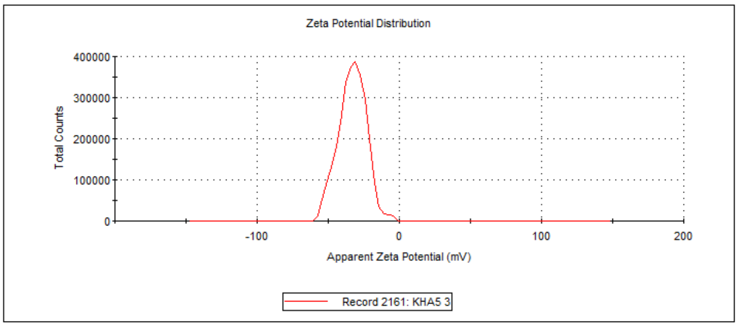

Figure 3. Zeta potential of keratin hydrolyzate obtained with potassium hydroxide, KerKAgr

RESULTS AND DISCUSSION

Testing and Evaluating the Functionality of Keratin Hydrolyzate on Wheat Seeds

The functionality of keratin hydrolysates has been tested and evaluated by using them in treatments applied to various types of wheat seeds. The keratin hydrolyzate obtained by alkaline hydrolysis in the presence of potassium hydroxide was used in different concentrations to treat the wheat seeds and then their growth was monitored.

Two types of wheat were treated with keratin hydrolyzate: Tamino and Mirastar. Wheat grains were treated with KerKAgr keratin hydrolyzate using the dilutions of 3% and 5% in water compared to the control sample germinated in water.

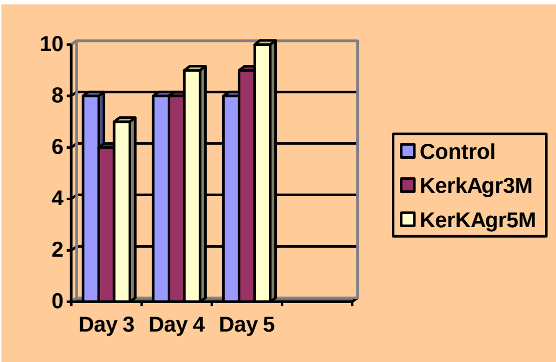

Figure 4. Germination rate for Mirastar wheat type

Mirastar and Tamino seeds treated with 3% and 5% KerKAgr had a higher germination rate on days 4 and 5 of the experiment compared to the control by up to 20% (Figures 4, 5). For the Tamino wheat type, treated with 5% KerkAgr, a higher germination rate is observed in all days of the experiment compared to the control (Figure 5).

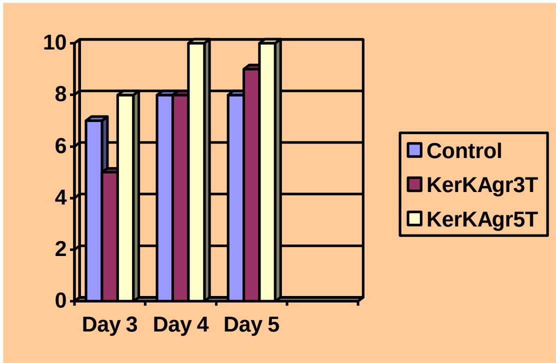

Figure 5. Germination rate for the Tamino wheat type

When measuring the length of the plants in the case of the Tamino type of wheat, an accelerated growth effect of the treatment with KerKAgr is seen compared to the control plants, in all days of the experiment, noticing a higher increase for seeds treated with 5% KerKAgr (Figure 6).

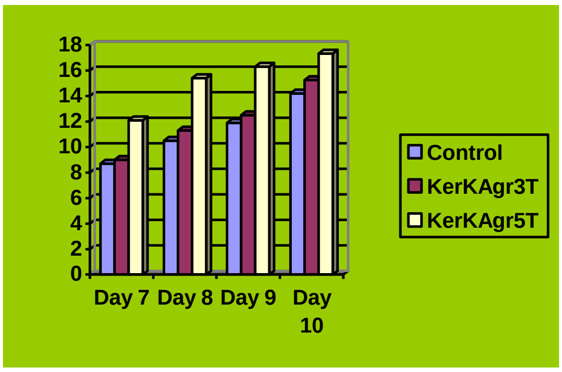

Figure 6. Average plant length (cm) for the Tamino wheat type

In the case of the growth of Mirastar wheat, an acceleration of the growth of plants treated with KerKAgr compared to the control is observed and plants continued to grow throughout the experiment (Figure 7).

The results of the treatments indicated a stimulation of the growth of wheat plants until 7.1% for Mirastar and 7.4% for Tamino wheat in the case of using the 3% dilution and 14.3% for Mirastar and 21.7% for Tamino wheat in the case of using the 5% dilution compared to the control (Figures 6, 7).

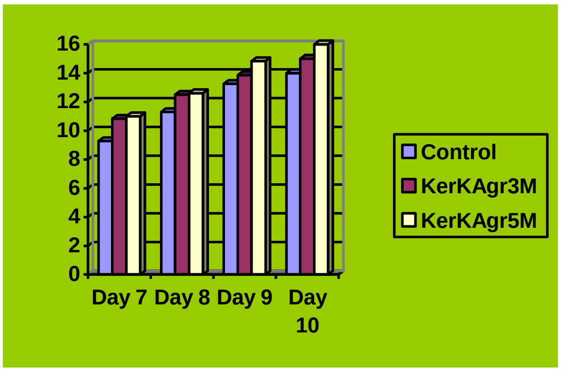

Figure 7. Average plant length (cm) for Mirastar wheat type

Keratin hydrolyzate has 10%-20% higher biostimulating effects for the germination of wheat seeds compared to the control sample and the growth of plant length was 7.1-21.7% higher than the control. The KerKAgr product made by hydrolysis with potassium hydroxide has specific properties for applications in agriculture. Physico-chemical analyses show that the hydrolyzate is rich in minerals, nitrogen and protein substances necessary in the plant growth process. KerKAgr keratin hydrolyzate was used in treatments on wheat seeds (Tamino and Mirastar) and the observations made showed an improvement in the germination rate and a stimulation of plant growth.

CONCLUSIONS

An eco-friendly multifunctional fertilizer based on keratin from sheep wool was prepared. Keratin hydrolyzate, KerKAgr, was obtained by alkaline hydrolysis with potassium hydroxide, and characterized physico-chemically. KerKAgr keratin hydrolyzate was tested and evaluated in treatments on wheat seeds and a 10-20% higher stimulation of the seed germination rate and a 7.1-21.7% higher plant length was observed compared to the control sample. The good results obtained in the applications of keratin hydrolyzate in agriculture show that keratin hydrolyzate can be the basis for obtaining new biomaterials with various applications. The utilization of wool by-products from the leather processing industry leads to a decrease in the amount of waste and the prevention of environmental pollution.

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REFERENCES

1. Chen, Y., Li, W., Zhang, S., A Multifunctional Eco-friendly Fertilizer Used Keratin-based Superabsorbent as Coatings for Slow-release Urea and Remediation of Contaminated Soil, *Prog Org Coat*, **2021**, 154, 106158, https://doi.org/10.1016/j.porgcoat.2021.106158.
2. Ren, X., Zeng, G., Tang, L., Wang, J., Wan, J., Liu, Y., Yu, J., Yi, H., Ye, S., Deng, R., Sorption, Transport and Biodegradation – An Insight into Bioavailability of Persistent Organic Pollutants in Soil, *Sci Total Environ*, **2018**, 610–611, 1154–1163, https://doi.org/10.1016/j.scitotenv.2017.08.089.
3. Lu, Y., Song, S., Wang, R., Liu, Z., Meng, J., Sweetman, A.J., Jenkins , A., Ferrier, R.C., Li, H., Luo, W., Wang, T., Impacts of Soil and Water Pollution on Food Safety and Health Risks in China, *Environ Int*, **2015**, 77, 5-15, https://doi.org/10.1016/j.envint.2014.12.010.
4. Li, S.X., Wang, Z., Malhi, S., Tian, X.H., Chapter 7: Nutrient and Water Management Effects on Crop Production, and Nutrient and Water Use Efficiency in Dryland Areas of China, *Adv Agron,* **2009**, 102, 223-265, https://doi.org/10.1016/S0065-2113(09)01007-4.
5. Doran, J., Soil Health And Global Sustainability: Translating Science Into Practice, *Agr Ecosyst Environ*, **2002**, 88, 2, 119-127, https://doi.org/10.1016/S0167-8809(01)00246-8.
6. He, Y., Wu, Z., Tu, L., Li, C., Encapsulation and Characterization of Slow-release Microbial Fertilizer from the Composites of Bentonite and

Alginate, *Appl Clay Sci*, **2015**, 109, https://doi.org/10.1016/j.clay.2015.02.001.

7. Xiao, X., Yu, L., Xie, F., Bao, X., Liu, H., Ji, Z., Chen, L., One-step Method to Prepare Starch-based Superabsorbent Polymer for Slow Release of Fertilizer, *Chem Eng J*, **2017**, 309, 607-616, https://doi.org/10.1016/j.cej.2016.10.101.
8. Lateef, A., Oloke, J., Gueguim Kana, E.B., Bello, B.Y., Keratinolytic Activities of a New Feather-degrading Isolate of *Bacillus cereus* LAU 08 Isolated from Nigerian Soil, *Int Biodeterior Biodegradation*, **2010**, 64, 2, 162-165, https://doi.org/10.1016/j.ibiod.2009.12.007.
9. Li, T., Lü, S., Zhang, S., Liu, M., Lignin-based Multifunctional Fertilizer for Immobilization of Pb (II) in Contaminated Soil, *J Taiwan Inst Chem Eng*, **2018**, 91, https://doi.org/10.1016/j.jtice.2018.06.025.
10. Murray, H.H., Traditional and New Applications for Kaolin, Smectite, and Palygorskite: A General Overview, *Appl Clay Sci*, **2000**, 17, 5–6, 207-221, https://doi.org/10.1016/S0169-1317(00)00016-8.
11. Xie, L., Liu, M., Ni, B., Zhang, X., Wang, Y., Slow-release Nitrogen and Boron Fertilizer from a Functional Superabsorbent Formulation Based on Wheat Straw and Attapulgite, *Chem Eng J*, **2011**, 167, 1, 342-348, https://doi.org/10.1016/j.cej.2010.12.082.
12. Dang, X., Yuan, H., Shan, Z., An Eco-friendly Material Based on Graft Copolymer of Gelatin Extracted from Leather Solid Waste for Potential Application in Chemical Sand-fixation, *J Clean Prod*, **2018**, 188, https://doi.org/10.1016/j.jclepro.2018.04.007.
13. Wang, W., Wang, A., Synthesis and Swelling Properties of pH-sensitive Semi-IPN Superabsorbent Hydrogels Based on Sodium Alginate-g-poly(sodium acrylate) and Polyvinylpyrrolidone, *Carbohydr Polym*, **2010**, 80, 4, 1028-1036, https://doi.org/10.1016/j.carbpol.2010.01.020.
14. Aciego Pietri, J.C., Brookes, P.C., Relationships between Soil pH and Microbial Properties in a UK Arable Soil, *Soil Biol Biochem*, **2008**, 40, 7, 1856-1861, https://doi.org/10.1016/j.soilbio.2008.03.020.
15. Sanchez, C., Lignocellulosic Residues: Biodegradation and Bioconversion by Fungi, *Biotechnol Adv*, **2009**, 27, 2, 185-194, https://doi.org/10.1016/j.biotechadv.2008.11.001.
16. Ni, B., Liu, M., Lü, S., Multifunctional Slow-release Urea Fertilizer from Ethylcellulose and Superabsorbent Coated Formulations, *Chem Eng J*, **2009**, 155, 3, 892-898, https://doi.org/10.1016/j.cej.2009.08.025.
17. Liu, W., Qiu, R., Water Eutrophication in China and the Combating Strategies, *J Chem Technol Biotechnol*, **2007**, 82, 9, 781-786, https://doi.org/10.1002/jctb.1755.
18. Conley, D.J., Paerl, H., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., Controlling Eutrophication: Nitrogen and Phosphorus, *Science*, **2009**, 323, 5917, 1014-1015, https://doi.org/10.1126/science.1167755.
19. Berechet, M.D., Gaidau, C., Niculescu, M., Recovery of Renewable Resources from Leather and Fur Industry through Development of Advanced Ecological Materials, *Agriculture & Food*, **2018**, 6, 138-148, ISSN 1314-8591.
20. Singh, V., Wang, S., Ng, K.W., 2.25 Keratin as a Biomaterial, in: P. Ducheyne (ed.), *Comprehensive Biomaterials II, Volume 2: Biologically Inspired and Biomolecular Materials*, **2017**, 542–557, https://doi.org/10.1016/B978-0-12-803581-8.09317-6.
21. Călin, M., Constantinescu-Aruxandei, D., Alexandrescu, E., Răut, I., Badea Doni, M., Arsene, M.-L., Oancea, F., Jecua, L., Lazăr, V., Degradation of Keratin Substrates by Keratinolytic Fungi, *Electron J Biotechnol*, **2017**, 28, 101–112, https://doi.org/10.1016/j.ejbt.2017.05.007.
22. Lange, L., Huang, Y., Busk, P.K., Microbial Decomposition of Keratin in Nature – A New Hypothesis of Industrial Relevance, *Appl Microbiol Biotechnol*, **2016**, 100, 2083-96, https://doi.org/10.1007/s00253-015-7262-1.
23. Jin, H-S., Park, S.Y., Kim, K., Lee, Y-J., Nam, G-W., Kang, N.J., Lee, D.W., Development of Akeratinase Activity Assay Using Recombinant Chicken Feather Keratin Substrates, *PLoS One*, **2017**, 12, e0172712, https://doi.org/10.1371/journal.pone.0172712.
24. Kreplak, L., Doucet, J., Dumas, P., Briki, F., New Aspects of the α-helix to β-sheet Transition in Stretched Hard α-keratin Fibers, *Biophys J*, **2004**, 87, 640-7, https://doi.org/10.1529/biophysj.103.036749.
25. Bragulla, H.H., Homberger, D.G., Structure and Functions of Keratin Proteins in Simple, Stratified, Keratinized and Cornified Epithelia, *J Anat*, **2009**, 214, 516–59, https://doi.org/10.1111/j.1469-7580.2009.01066.x.
26. Chandrakant, R.H., Saransh, S.J., Ananda, J.J., Dipak, V.P., Valorization of Keratin Based Waste, *Process Saf Environ Prot*, **2017**, 115, 85-98, https://doi.org/10.1016/j.psep.2017.08.045.
27. Vasconcelos, A., Freddi, G., Cavaco-Paulo, A., Biodegradable Materials Based on Silk Fibroin and Keratin, *Biomacromolecules*, **2008**, 9, 1299–1305, https://doi.org/10.1021/bm7012789.