Study of Collagen Hydrogel Biodegradability Over Time

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Hydrogels have drawn the interest of researchers over the past decade, due to their intrinsic properties for applications in medicine, agriculture [1,2], food industry, pharmaceuticals, environmental protection and biomaterials, etc. Obtaining hydrogels with collagen structure by hydrolyzing pelt waste and using them for applications in agriculture is a novelty, considering that the collagen structure is currently used only in medicine. The paper presents an experimental model for obtaining hydrogels with collagenous structure from pelt waste resulting from the liming process. As a biological material, the hydrogel with collagen structure is a complex medium, optimal for the development of a variety of biodeteriogenic organisms due to a considerable amount of protein as well as the presence of lipids and carbohydrates that provide an excellent nutrient substrate for many biological species. During the phase of incorporation into the soil of the collagen hydrogel, proteolytic bacteria develop particularly, stimulated by humidity and possibly by temperature, thus producing biodegradation.The paper presents the methodology for studying the biodegradability of collagen hydrogel with encapsulated nutrients.

Keywords: biodegradability, hydrogels, pelt waste, tannery, soil

Biodegradation is a process of decomposition of organic substances by enzymes produced by living organisms, especially bacteria and microscopic fungi, by which the cycle of elements in nature is ensured.

The total carbon content (C) of the product subjected to biodegradability is found in the form of three final products:

- CO_2 is the respiration product of microorganisms;
- Residue from the polymer or any product that is formed, and
- Biomass produced by microorganisms through reproduction and growth.

Total biodegradation occurs when the carbon (C) is completely removed from the medium. The residue consists of fragments of plastic material or metabolites produced in biodegradation processes [3-7].

Experimental part

An innovative process was developed with the purpose of obtaining a collagen structure hydrogel with encapsulated nutrients through *direct* hydrolysis of pelt waste in acidic medium in combination with other polymers (polyacrylamide, starch, urea, cellulose, etc.).

The result was a gelatinous, elastic and semi-transparent collagen hydrogel with encapsulated nutrients (HZ), with a *p*H of 6.2-6.9 [8-10].

Table 1 presents the physico-chemical analyses of the pelt waste hydrolysate and HZ hydrogel, performed in the ICPI Testing and Quality Control Research Department.

By means of this process a technology can be established for the conversion of pelt waste into collagen hydrogels with encapsulated nutrients that can be used as fertilizers in agriculture (especially in horticulture) [11-14].

Results and discussions

The biodegradation study of the HZ collagen hydrogel with encapsulated nutrients was performed by means of 2 test variants:

- studying biodegradation of hydrogel in the soil - by measuring weight loss;
- studying biodegradation of hydrogel in the laboratory, according to the SR EN ISO 14852: 2005 standard.

Studying biodegradation of hydrogel in the soil by measuring weight loss

The biodegradability of HZ hydrogel was studied by incorporating the hydrogel in the soil. HZ hydrogel is semi-transparent, smooth and flexible but is not fragile. This hydrogel with encapsulated nutrients was deposited on biodegradable textile supports with cellulose and polyamide fibers.

Table 1
PHYSICO-CHEMICAL CHARACTERISTICS OF HYDROLYSATE AND HZ HYDROGEL

No.	Characteristics	UM	Sample code /Determined values*	
			Hydrolysate	HZ Hydrogel
1	Dry substance	%	16.17	**30.14**
2	Ash	%	22.26	**17.95**
3	Total nitrogen	%	11.94	**11.28**
4	Protein substance	%	67.10	**63.39**
5.	P and K oxides	%	2.29	**0.80**

** Values for ash, total nitrogen, protein substance and metal oxides are reported free from volatile matter.*

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To study biodegradability, two plastic boxes of 25 x 33 x 17 cm were filled with a composting medium of 3:1 alluvial mollisol Aldeni - Buzau and Zaharadnicky Compost.

This is a plant compost made in the Czech Republic. Compost is produced by mixing vegetable raw materials and adding limestone and trace elements. The compost contains a large amount of humus required for plant growth. Biodegradability of hydrogel films was investigated by soil burial test.

In this case of the natural variant, the experimental environment showed oscillating temperature variations (from -1°C to 21°C) as well as precipitation (snow, sleet and rain) given the experimental period (February - April 2017).

In the case of the vessel introduced into the oven, the temperature of 25°C was maintained, the composting medium having a *p*H value ranging from 6.8 to 7.2 and a moisture content of about 30%, favorable for the growth of microorganisms that play an active role in biodegradation.

The degree of biodegradation was determined by weight loss over time, with assessments performed every week of the 5-week experiment.

The parameters of the soil/compost combination (temperature, humidity) have an important influence on the biodegradability of the HZ collagen hydrogel. Under these experimental conditions, the biodegradation process takes place in two stages at different rates, namely in the first week with a slower rate until the population of the film with microflora and the second stage with an almost double hydrogel degradation rate under the influence of bacterial species. The rate of the second stage of collagen hydrogel biodegradation approaches the rate of the entire process determined for the 5 weeks of experimentation.

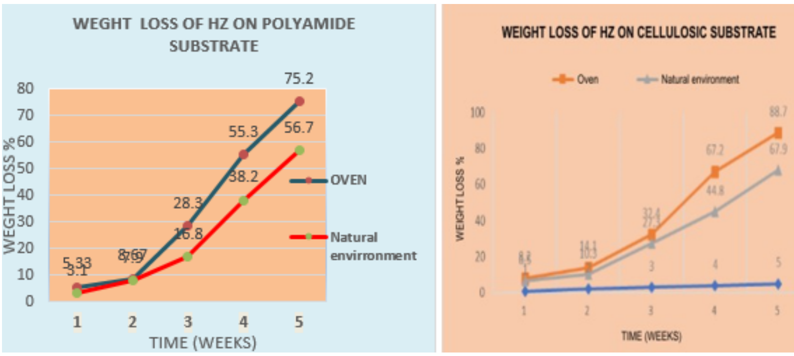
Analyzing the evolution of weight loss (%) based on the incubation time shown in figure 1, it was noticed that after 5 weeks, the hydrogel sample with polyamide fiber substrate placed in the oven had lost 75.2% of weight, compared to the sample deposited in natural environment, with 56.7% weight loss. The degradation process rate is more intense in the case of hydrogel on cellulosic substrate, which degraded after 5 weeks - broken down. These values suggest that the temperature of the medium influences the rate of material degradation, as the amount of material to be consumed by the microorganisms varies.

The evolution of the biodegradation process for the HZ collagen hydrogel in the soil-compost environment is shown in figures 2-3.

Determination of final aerobic hydrogel biodegradability

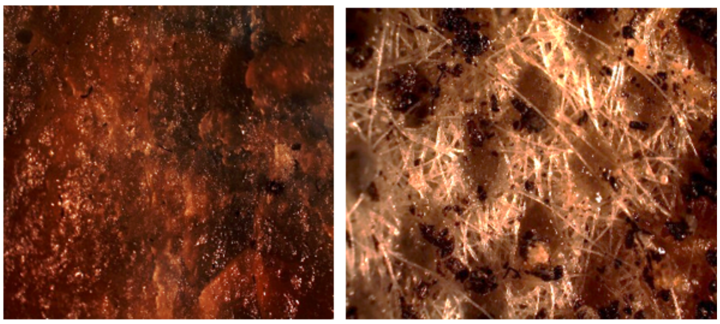
SR EN ISO 14852: 2005 establishes the method for determining the degree of aerobic biodegradability of plastics, including those containing additives, by measuring the amount of released carbon dioxide. The test material, in a synthetic medium, is exposed to an inoculum from compost or soil under laboratory conditions.

The conditions used in this international standard do not meet the optimal conditions to achieve maximum biodegradability, but the standard is intended to determine the potential biodegradability.



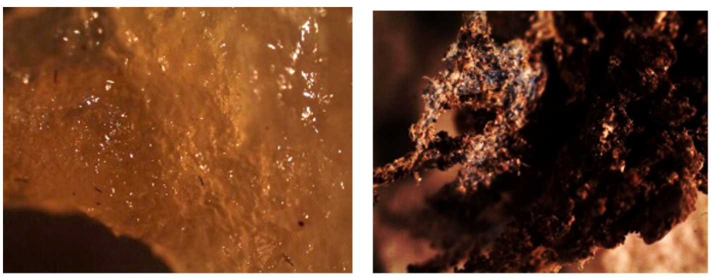
a b

Fig.1. Kinetics of weight loss (%) based on incubation time (weeks) of HZ hydrogel on substrate of a) polyamide fibers and b) cellulose fibres



a) HZ on polyamide substrate 20x b) degraded HZ on polyamide substrate 20x

Fig. 2. Optical microphotographs for HZ hydrogel on polyamide fiber substrate (a) before and b) after degradation



a) HZon cellulose fiber substrate 20x b) HZ degraded on cellulose fiber substrate 20x

Fig. 3. Optical microphotographs for HZ hydrogel on cellulose fiber substrate (a) before and b) after degradation

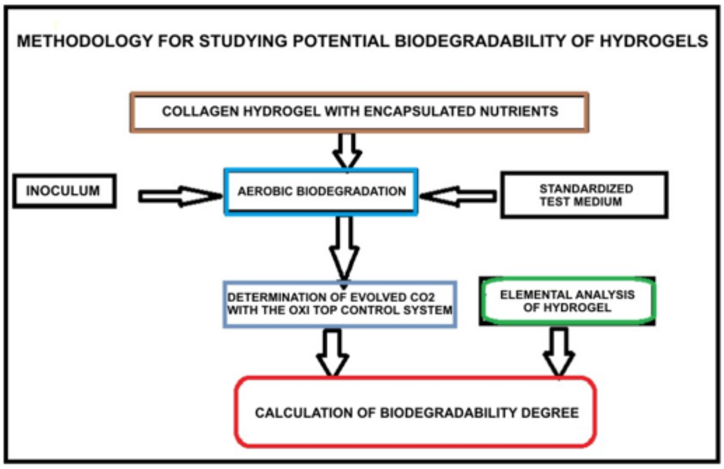


Fig. 4. Diagram of methodology for studying the biodegradability of collagen hydrogels

The method allows for improved biodegradability assessment by calculating a carbon balance. The method applies to the following materials:

- Natural and / or synthetic polymers, copolymers and mixtures thereof;
- Plastic materials with additives such as plasticizers, dyes or other compositions;
- Water-soluble polymers.

A standardized test medium and inoculum from soil and/or compost (Zaharadnicky Compost) were used according to the standard in force.

The Oxi Top-Control System (Germany) was used to determine carbon dioxide. This device is based on the development of mercury-free technology, the measured values are stored in the controller - Oxi BOD system. The principle of measurement is based on the fact that microorganisms absorb oxygen from the amount of air in the bottles and the samples (hydrogel) are organically degraded. The carbon dioxide formed in this process is the respiration product of the microorganisms.Due to the reduction in oxygen, the bottle pressure decreases and this is detected and stored in the measuring heads. After transferring data from the controller, the information is used to determine the BOD value.

During the experiments there were also cases when the BOD carbon dioxide curves were undervalued, i.e. undefined, due to the small amount of hydrogel introduced into the Oxi Top system. When introducing an amount of 30 mg, 56.3 mg/L CO_2 are obtained for HZ hydrogel, and 4.2 mg/L CO_2 for the control sample. The theoretical amount of carbon dioxide ($ThCO_2$) evolved from the hydrogel is calculated using the formula:

$$ThCO_2 = m \times X_0 \times \frac{44}{12} \quad \text{where:}$$

M = mass of the test material, in milligrams; X_o = the carbon content of the test material determined from the chemical formula or calculated from an elemental analysis and expressed as a mass fraction; 44 and 12 molecular weight of BOD and atomic mass of carbon, respectively.

Biodegradation, D_i, is calculated as follows:

$$D_i\ \% = \frac{\sum(CO_2)_T - \sum(CO_2)_B}{ThCO_2} \times 100 \quad \text{where:}$$

$\Sigma(CO_2)_T$ = the amount of evolved CO_2 from the test material

$\Sigma(CO_2)_B$ = the amount of evolved CO_2 from the control vial

Elemental analysis for HZ hydrogel was performed at Petru Poni Institute in Iasi.

Experimental data show:

$$D_i\ \% = 67.6\ \%$$

The degree of biodegradability of the collagen hydrogel determined with the Oxi Top System is therefore 67.6%.

Methodology for studying the potential biodegradability of hydrogels

As a result of experiments carried out, it was found that the method of studying hydrogel biodegradation according to standard SR EN ISO 14852: 2005 *Method of determining evolved carbon dioxide using the Oxi Top-Control System* is more efficient and more precise than the method of studying biodegradation of hydrogel in the soil. This method may be the suitable methodology for studying the biodegradability of HZ collagen hydrogel with encapsulated nutrients. Figure 4 presents the methodology for studying the biodegradability of collagen hydrogel with encapsulated nutrients.

Conclusions

Considering the main objective of the paper, which is to obtain hydrogels with a collagen structure for use in agriculture, at this stage, a study of biodegradability of the collagen hydrogel with encapsulated nutrients was carried out, also selecting the biodegradability calculation methodology.

1. HZ collagen hydrogel was obtained from pelt waste subjected to biodegradation;
2. The biodegradation of HZ collagen hydrogel with encapsulated nutrients was achieved by means of two test variants:

a) studying biodegradation of hydrogel in soil by measuring weight loss;

b) studying biodegradation of hydrogel by applying the SR-EN ISO 14852 standard, *Determination of the ultimate aerobic biodegradability in an aqueous medium—Method by analysis of evolved carbon dioxide*.

3. As a final conclusion it can be said that as a result of experiments carried out, the method of studying hydrogel biodegradation by determining evolved carbon dioxide using the Oxi Top-Control System is more efficient and more precise than the method of studying biodegradation of hydrogel in the soil.

This method may be the suitable methodology for studying the biodegradability of collagen hydrogels.

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References

1. BOGZA, G.E., CHELARU, L., BITERE, E., POROCH, V., SULEA, D., COSTULEANU, M., Rev. Chim.(Bucharest), **67**, no. 11, 2016, p. 2295

2. NITIPIR, GC., MARIN, S., MARIN, M.M., KAYA, M.A., GHICA, M.V., MEDERLE, N., Rev. Chim. (Bucharest), 68, no. 11, 2017, p. 2605
3.V. OROS, AL. OZUNU, 2002, Testele de biodegradabilitate. ed. Univ. Transilvania Brasov.
4.OZUNU AL., CARMEN TEODOSIU, 2002. Prevenirea poluarii mediului. Ed. Univ. Transilvania Brasov.
5.LARS PASSAUER, UTE HAMER, ACS Sustainable Chemestry & Engineering 2015"www.researchgate.net/publictions// 280946776.
6.NILADRI ROY, NABANITA SAHA, PETR SAHA Recent Researches in Geography, Geology, Energy, Environment and Biomedicine 2015
7. FRANCESCO F. MONTESANO et al. / Agriculture and Agricultural Science Procedia 4 (2015) 451 – 458
8..ZAINESCU, G., CONSTANTINESCU, R. R.,SIRBU, C., Rev. Chim. (Bucharest), **68**, no. 2, 2017, p. 393
9.ZAINESCU, G., ALBU, L., DESELNICU, D., CONSTANTINESCU, R. R., VASILESCU, A. M., NICHITA, P., SIRBU, C., Mat. Plast., **51**, no. 1, 2014, p. 90
10. ZAINESCU, G., CONSTANTINESCU, R. R.,.PETRE, V., DESELNICU, C.D., Rev. Chim. (Bucharest), **67**, no.1, 2016, p. 48
11. CONSTANTINESCU, R. R, CRUDU, M., ZAINESCU, G., COARA, G. Rev. Chim. (Bucharest) **66**, no. 8, 2015, p. 1244
12. CONSTATINESCU R. R., CRUDU M., ZAINESCU G. A., CRUDU A., VOICU P., , 15th SGEM, Albena Bulgaria 19-21, 2015 pp 747.
13. SAHA, P, SAHA, N., ROY, N., Hydrogel Wound Covering Patent no: 302405, 2011. Czech Republic
14. GAIDAU, C., NICULESCU, M.D., EPURE, D.G., TUDORACHE, M., RADU, E., STOICA, R., IGNAT, M., GIDEA, M., Rev. Chim. (Bucharest), 68, no. 12, 2017, p. 2783

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