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Study of biodegradability of chitosan-based antibacterial films

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Abstract. In one of our previous works, we obtained films based on chitosan, glycerol, and iron (III) cations. These films possess antibacterial properties, thus they can find applications in the food industry and medicine. In this study, we evaluated the biodegradability of previously described films by keeping them in soil for 4 weeks. For the control experiment, we utilized soil that underwent thermal treatment several times during the experiment. 4 out of 5 samples demonstrated mass loss slightly different from the mass loss in the control experiment (the difference in mass loss compared to the control for these samples did not exceed 3%). This might indicate that their mass loss was solely related to the dissolution process. The last sample, containing chitosan, iron chloride, glycerol, and a chitosan-derived cation, exhibited a 40% decrease in mass, which is 26% more compared to the control experiment.

1 Introduction

Currently, one of the main environmental problems is environmental pollution with polymer waste[1, 2]. Polypropylene films are widely used because of their strength and hydrophobic properties, but they are difficult to recycle and degrade[3]. The main approach to solving this problem is the development of films from biodegradable polymers. Films made from natural materials such as cellulose, alginates, starch and proteins are excellent environmentally friendly alternatives to traditional polymers due to their biodegradability and renewability. However, they have a few problems, such as limited mechanical strength and moisture resistance compared to traditional synthetic films. This may limit their use in some packaging conditions. Also, although they are biodegradable, the rate of decomposition may vary depending on environmental conditions[4-6].

Over the past few decades, a large amount of research has been carried out on the development of biodegradable antibacterial films for use in both medicine and food industry[7, 8]. Such films can be used to heal wounds and burns, as well as to create food packaging for vegetables and fruits, including edible ones.

One of the promising polymers for creating these films is chitosan (**Figure 1**). Its biological properties include biocompatibility, biodegradability, non-toxicity, and antibacterial and antioxidant properties [9-11]. This natural polymer does not have a negative

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impact on the environment [12]. In one of our previous works [13], we obtained films based on chitosan, iron(III) chloride and a chitin derivative [13].

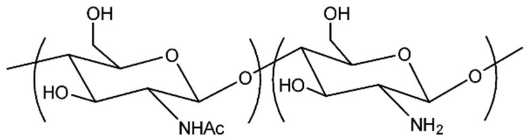

Fig. 1. Chemical structure of chitosan.

The resulting films demonstrated antibacterial activity and were characterized using a complex of physicochemical methods of analysis. However, the ultimate goal of the research cycle was to obtain not only highly active antibacterial films, but also those with a high tendency to biodegradation. Numerous studies have been devoted to the study of the biodegradation of chitosan-based films. For example, films based on chitosan and zinc oxide demonstrated complete decomposition after 8 weeks [14]. In the same study, films based on chitosan and iron(III) oxide completely biodegraded after only 6 weeks. In another study focusing on the biodegradation of chitosan films with the addition of Quercus polyphenol extract, complete decomposition was achieved within 3-14 days depending on the soil [15]. Even adding a small amount of chitosan (10%) to polyethylene films enables their complete biodegradation within six months [16].

Thus, the aim of this study was to assess the biodegradation potential of previously obtained films using the immersion method in soil.

2 Materials and methods

2.1 Preparation of the films

In the previous study, films denoted as **A2**, **B5**, **C10**, and **D15** were obtained. These films differed from each other in the content of chitosan-iron(III) nanoparticles (Fe(III)-CS-NPs). The obtained films had different mechanical properties and antibacterial activity. Additionally, a film **Q** was synthesized, which also contained a cationic derivative of chitosan (**CD**). This film demonstrated the most pronounced antibacterial properties, as well as more optimal mechanical characteristics. The composition of the films, as well as their mechanical and antibacterial properties, are presented in **Table 1**. A detailed methodology for obtaining the films is provided in one of our papers [13].

Table 1. The content characteristics of the elaborated films.

Sample	Mass ratio				Tensile strength (N/mm^2)	Elongation at break (%)	*E. Coli* inhibition zone (mm)
	Chitosan	Glycerol	Fe(III)-CS-NPs	CD			
A2	50	30	1	0	3.6	30.0	9.4 ± 0.1
B5	20	12	1	0	4.0	31.3	9.9 ± 0.1
C10	10	6	1	0	8.9	41.6	11.2 ± 0.2
D15	6,7	4	1	0	4.3	27.0	11.6 ± 0.2
Q	8	6	1	2	11.7	75.0	14.2 ± 0.1

2.2 Biodegradation determination method

To determine the biodegradability of the obtained films, a method involving measuring the change in mass of the film when placed in soil was employed. The soil was obtained from a forested area (Moscow region, Valuyevo village). The soil was taken from a depth of about 5-10 cm. The obtained soil had a pH of 5.1. We did not assess the elemental and bacterial composition of the soil, however, soil samples from this forest were previously described by other researchers [17]. Based on their data, the soil density from this forest is 0.82 ± 0.14 g/cm3, the amount of sand (0.05–2.00 mm) is 21.8 ± 6.6%, and the amount of silt (0.002–0.05 mm) is 70.5 ± 5.7%. Microbiological parameters such as microbial biomass carbon, determined using the substrate-induced respiration method, and microbial nitrogen, determined using the fumigation–extraction method, were 990 ± 266 μg/g and 135 ± 84 μg/g, respectively. A portion of the soil was placed in a glass container and heated for 24 hours at 120°C. Subsequently, the dried and sterilized soil was weighed (with a 24% loss in mass due to water evaporation). The lost moisture was replenished by adding distilled water. This soil was used for control experiments, aiming to distinguish between mass loss due to dissolution and degradation.

Film samples were placed in a desiccator for a day, then weighed and buried in a certain amount of soil (70g) to a depth of about 4cm. Every week, the samples were cleaned of soil, placed in the desiccator for a day, weighed, and returned to the soil. Meanwhile, the soil of control samples underwent another day of thermal treatment at 120°C, followed by replenishment of lost water mass. To maintain moisture and a favorable environment for bacterial growth and development, approximately 10ml of distilled water was added to the samples weekly.

3 Results

For most samples, the final difference in mass compared to control samples was no more than 3%, indicating the absence of biodegradation in this soil (Table 2). However, for sample **Q**, the difference was 26%, and mold was also observed on this sample, the vigorous growth of which could explain the initial increase in mass. It is also conceivable that sample **Q**, despite its strong antibacterial properties demonstrated in our previous work [13], may serve as a substrate for various fungal species.

Table 2. The change in film mass as a percentage of the initial mass of the film dried in the desiccator. The samples with index «**t**» were placed into soil that had undergone the thermal treatment described earlier.

Sample	Week 1	Week 2	Week 3	Week 4	The difference in mass compared to the control
A2	80	79	76	75	-1%
A2t (control)	77	77	76	74	
B5	79	83	81	78	-2%
B5t (control)	75	76	74	76	
C10	83	85	77	79	-2%
C10t (control)	75	76	78	77	
D15	64	62	61	58	3%
D15t (control)	77	80	66	61	
Q	104	90	76	60	26%
Qt (control)	87	90	88	86	

4 Conclusion

In our previous study, we obtained films with antibacterial properties based on chitosan. These films differed in their iron(III) content. One of the films also contained a cationic derivative of chitosan. The process of obtaining the films is described in our previous work.

In this experiment, these films were placed in soil for 4 weeks to assess the rate of their biodegradation. Additionally, a control experiment was conducted using soil that had been thermally treated for significantly reduce the number of microorganisms. Thus, the mass loss in the control experiment was primarily associated with the dissolution of the films.

For 4 out of 5 films, the difference in mass loss compared to the control was not higher than 3%. This may indicate the absence of biodegradation of these films under these conditions. The film containing the cationic derivative of chitosan demonstrated a mass loss of 40% in 4 weeks, which is 26% higher compared to the control experiment. Thus, this film has a pronounced potential for biodegradation. It is also worth noting the vigorous growth of fungal colonies. This growth explains the increase in mass during the first week of the experiment. It is possible that this film may serve as a substrate for fungi.

Comparing our results with those of other studies, one can observe how significantly the composition of the films as well as the soil composition affect the rate of their biodegradation. In previous studies, the complete degradation time of the films could range from 3 days to over half a year. Therefore, it can be concluded that longer experiments using various types of soil and their characterization are necessary.

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References

1. Lin, Z., et al., Environmental Pollution, **304,** 119159 (2022)
2. Ouyang, Z., et al., Journal of hazardous materials, **424**, 127461 (2022)
3. Wu, F., M. Misra, and A.K. Mohanty, Progress in Polymer Science, **117**, 101395 (2021)
4. Andrady, A.L., J.E. Pegram, and S. Nakatsuka, Journal of environmental polymer degradation, **1**, 31-43. 1993
5. Bano, K., et al., Current pharmaceutical biotechnology, **18(5)**, 429-440 (2017)
6. Dharma, H.N.C., et al., Membranes, **12(3)**, 345 (2022)
7. Cui, C., et al., Trends in Food Science and Technology, **116**, 854-869 (2021)
8. Kanikireddy, V., et al., Int J Biol Macromol, **164**, 963-975 (2020)
9. Abd El-Hack, M.E., et al.,. International Journal of Biological Macromolecules, **164**, 2726-2744 (2020)
10. Chandrasekaran, M., K.D. Kim, and S.C. Chun, Processes, **8(9)**, 1173 (2020)
11. Verlee, A., S. Mincke, and C.V. Stevens, Carbohydr Polym., **164,** 268-283 (2017)
12. Crini, N., et al., Environmental Chemistry Letters, **17**. 2019
13. Khubiev, O.M., et al., BioTech, **12(3)** (2023)
14. Wrońska, N., et al., Foods, **12(18)**, 3519 (2023)
15. Oberlintner, A., et al., **21**, 101318 (2021)
16. Makarios-Laham, I. and T.-C. Lee, Journal of environmental polymer degradation, **3(1)**, 31-36 (1995)
17. Ananyeva, N.D., et al., Eurasian Soil Science, **56(1)** 87-100 (2023)