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Microbial and Enzymatic Biodegradation of Alginate and Chitosan-Based Biocomposite Films

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Extended Abstract

Global plastics production has been steadily increasing, driven by its versatile applications in industries such as packaging, agriculture and electronics. However, the combination of high production volumes and inadequate waste management has led to an accumulation of plastic and microplastics in the environment, causing significant damage to ecosystems[1], [2]. Tackling the impact of plastics on the environment requires a multi-faceted approach that includes the following: (i) the development of biodegradable plastic alternatives, (ii) a deeper understanding of the biodegradation process and (iii) the promotion of efficient recycling systems.

In our previous work, we developed biocomposite films using natural polysaccharides, alginate and chitosan, and examined their degradation in aqueous environment [3]. In this study, we investigated their biodegradation in soil and *in vitro* using four commercial microbial enzymes. The films included chitosan (Ch), chitosan with cellulose nanocrystals, acetylated chitosan-cellulose nanocrystals, alginate (A), alginate with cellulose nanocrystals, and acetylated alginate-cellulose nanocrystals. For soil degradation, film samples were placed either on top of the soil (TOP) or buried between layers (MID), with degradation monitored over time. MID films degraded significantly faster (around 30 days) due to greater microbial exposure and moisture retention. Ch-based films degraded faster than A-based films, despite the latter dissolving quickly in water. Ch-TOP films degraded in about 80 days, while A-TOP films took approximately 100 days. No significant differences were observed between different Ch- or A-based film types.

In vitro degradation was performed with lipase from *Rhizopus oryzae* (Lip), cellulase from *Aspergillus niger* (Cel), laccase from *Trametes versicolor* (Lac) and alcohol dehydrogenase from *Saccharomyces cerevisiae* (ADH) in phosphate buffer pH 6 and 7. During a 48h incubation at 37°C, we monitored the degree of depolymerization (degradation) by measuring the amount of released reducing sugars (3,5-Dinitrosalicylic acid (DNS) test). The remaining Ch-films (A-films dissolve in aqueous medium) were also analysed by FTIR. As reported in the literature [4], Lip cleaved glycosidic bonds in Ch, leading to some depolymerization. While ADH treatment caused visible changes in the films, the degradation mechanism remains unclear. Although Cel is known to act on chitosan and cellulose [5], our FTIR analysis showed only minor changes, likely because the degradation products (oligomers and monomers) exhibit similar spectral peaks to the intact polymer. The DNS test showed similar results with a high release of sugars with Cel and a moderate one with Lip. When a higher concentration of these enzymes was used, the amount of released sugars increased and, at pH 6, a complete degradation of the films was obtained with Cel. The microbial activity in the reaction mixtures (non-sterile conditions) affected the measurements of the DNS test, however, it could be countered by the addition of an inhibitor of microbial growth (sodium azide).

Keywords: alginate; chitosan; biocomposite films; biodegradation; soil; enzymes.

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