Rheological Properties of Clay-Polymer Composite Hydrogels

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Abstract. Contamination by heavy metals poses many threats, affecting food chain safety, food quality, and the agricultural production. However, highly contaminated environments are reservoirs of heavy metal tolerant microbes, such as *Stenotrophomonas bentonitica* that is found in natural bentonite. In this work, we describe the synthesis and rheological characterization of alginate-cellulose hydrogels at different polymeric proportions, with best results for 30/70 W/W alginate/cellulose proportion and final concentration of 1%wt. For this concentration, we added bentonite at 1%wt to encapsulate *S. bentonitica* cells. A hydrogel precursor was created by adding $CaCO_3$ in combination with D-glucono-δ-lactone, which was additionally cross-linked with a $CaCl_2$ solution. Alginate beads were used to assess their metal bioremediation potential. The rheological properties under shear of the hydrogels were analysed for the different conditions and the results were related with microstructural changes analysed by HRSEM.

Keywords: Polymer · Clay · Hydrogel · Composite · Rheology · Bacteria · Bioremediation

1 Introduction

Many anthropogenic human activities such as ore mining and metallurgy contribute to discharge of high concentrations of heavy metals in the environment [1]. These impacted sites harbour many heavy metal tolerant microbes which play a crucial role in decreasing their toxicity and bioavailability. As conventional remediation techniques entail high costs and low contaminant specificity, remediation using microorganisms, known as bioremediation, is gaining importance for the clean-up of environmental pollutants

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[2]. The immobilization of microorganisms is a procedure which provides several advantages, as the matrices can improve the preservation of cell integrity under limiting conditions, provide specific properties to microbes and ease the separation/recuperation of cells and elements [3].

Among the different strategies for immobilization, polymers of natural origin to fabricate highly porous hydrogels are mostly preferred due to their inherent biocompatibility, availability and low cost [4]. In addition, nanoclays such as bentonite offer new opportunities for composite hydrogel design due to their relative low-cost, easy fixation of bacteria, durability, and high mechanical strength [5, 6]. It is also well known that bentonite clays are characterized by the presence of diverse groups of microbes including bacteria with high heavy metals bioremediation potential (e.g., *Stenotrophomonas bentonitica* BII-R7) [2].

We propose here the use of novel clay-polymer hydrogels for the encapsulation of *S. bentonitica* BII-R7 and the bioremediation of metal ions. In particular, we searched a compromise between the optimization of the mechanical properties of the composite hydrogels and the amount of biopolymer and clay. For this, we prepared alginate-cellulose hydrogels at different polymer proportions and characterized the rheological properties. Then, we added bentonite 1% wt. to the most stable hydrogels and encapsulated *S. bentonitica* cells.

2 Experimental Methods

2.1 Bentonite Treatment

Raw bentonite was purchased from Sigma Aldrich (USA) and the exchangeable cations were substituted by Ca^{2+} ions following the protocol reported in [7].

2.2 Hydrogel Preparation

Hydrogels were prepared following a previously reported two-step protocol [8]. First, 1 wt% water solutions were respectively prepared with sodium alginate (ALG, PanReac AppliChem ITW Reagents, Spain) and 2-hydroxyethyl cellulose (CEL, Mv$\sim$1300000, Sigma Aldrich, USA). Then, 1 wt% ALG/CEL solutions were prepared by mixing the previous ones at different proportions from 100/0 W/W (alginate hydrogel as a control sample) to 20/80 W/W. To initiate the first step of the gelation process, we added 4 mg of calcium carbonate ($CaCO_3$, Sigma Aldrich, USA) and 14.24 mg of D-glucono-δ-lactone (GDL, Sigma Aldrich, USA) per mL of ALG/CEL solution and homogenized by magnetic stirring. The mixture was then rapidly placed in the mold and was additionally cured in a second step by the addition of a 0.3M calcium chloride solution ($CaCl_2$, Sigma Aldrich, USA). In some cases, bentonite (BENT, 1 wt.%) and *S. bentonitica* BII-R7 cells (1.4 Optical Density, OD) were introduced into the hydrogels by mixing with the ALG/CEL 30/70 W/W solution, before the $CaCO_3$ addition.

2.3 Hydrogels Characterization

The rheological properties of the hydrogels were determined at 25.0 ± 0.1 ºC using a rheometer Discovery HR-1 (TA Instruments) equipped with a plate-plate geometry of 40 mm of diameter. The linear viscoelastic region (LVR) of the samples was determined by strain amplitude sweep tests at a constant frequency of 1 Hz and stepwise increasing shear strain amplitude (γ). High Resolution Scanning Electron Microscopy (HRSEM) images were taken using a AURIGA (FIB-FESEM) from Carl Zeiss SMT equipment. Bioremediation assays were carried out incubating alginate (100/0 hydrogels) beads in Luria Bertani medium (LB) with the addition of 2mM Se(IV).

3 Results and Discussion

Alginate hydrogels (100/0 W/W) were taken as a control group and alginate/cellulose hydrogels at different proportions were prepared with the aim of reducing the charge load and production cost without affecting the mechanical properties of the hydrogels (see Fig. 1). As observed, the greater the amount of alginate, the greater the values of the viscoelastic moduli (G' and G"), since this is the charged polymer that interacts with the Ca^{2+} ions, while the cellulose is just interpenetrated in the network, but not cross-linked. Among all the proportions, 30/70 W/W was chosen as the optimal one, since G' and G" have similar values before and after the addition of $CaCl_2$, which could be taken as an indication of the saturation of crosslinking, while considerably minimizing the amount of alginate but maintaining good rheological properties.

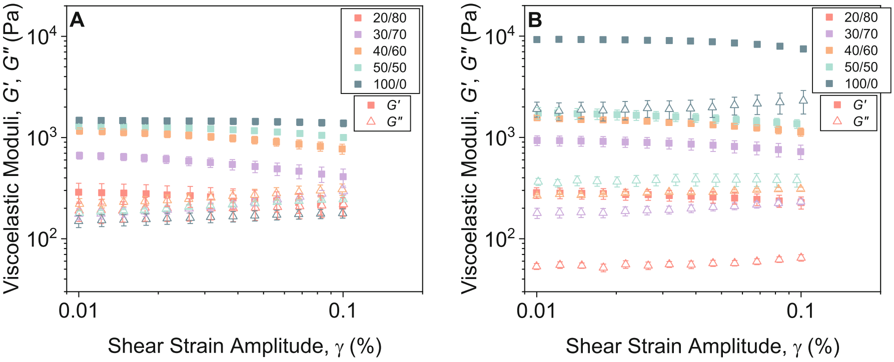

Fig. 1. Storage (G') and loss (G") moduli corresponding to the LVR for polymeric hydrogels at different proportions of alginate/cellulose **(a)** before and **(b)** after $CaCl_2$ 0.3M addition.

Then, we added 1wt% of bentonite and *S. bentonitica* BII-R7 at 1.4 optical density to the 30/70 hydrogels (Fig. 2a). A clear increase in viscoelastic moduli was observed after the addition of bentonite, which is perfectly integrated into the network thanks to the previous ion exchange. Although the encapsulation of BII-R7 entails a decrease in G' and G" with respect to clay-polymer hydrogels, they maintain good rheological properties, of the same order of magnitude as hydrogels without bentonite. Finally, the control alginate beads (100/0 W/W) showed a Se(IV) removal up to 70% thanks to the bacteria encapsulation (see HRSEM images in Figs. 2b and 2c; quantitative results not shown here).

4 Conclusions

It was possible to prepare low-cost polymeric hydrogels, with an adequate internal structure and rheological properties by combining alginate and cellulose. An optimal ratio of both polymers was found to be 30/70 W/W. Bentonite was added to improve mechanics and cell growth and *S. bentonitica* BII-R7 was effectively encapsulated in the clay-polymer hydrogels. Finally, premiliminar selenium reduction measurements were carried out for alginate hydrogels obtaining up to 70% of removal.

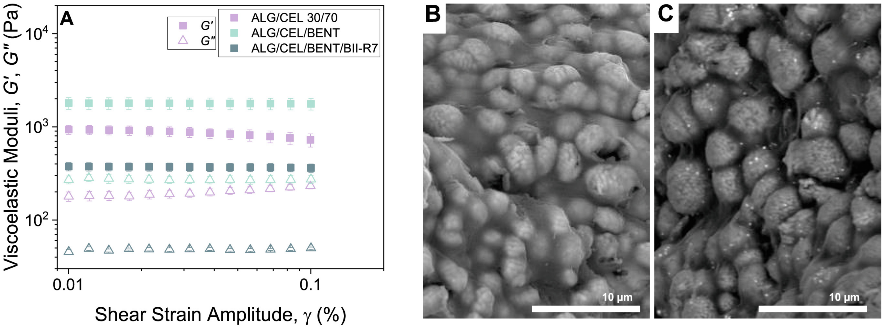

Fig. 2. **(a)** Vicoelastic moduli at the LVR for 30/70 W/W hydrogels contining bentonite at 1 wt% and BII-R7 at 1.4 OD. HRSEM images of alginate beads with BII-R7 cells without selenium **(b)** and with selenium nanoparticles **(c)**.

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References

1. Li, C., Zhou, K., Qin, W., Tian, C., Qi, M., Yan, X., Han, W.: A review on heavy metals contamination in soil: effects, sources, and remediation techniques. Soil. Sediment. Contam. **28**(4), 380–394 (2019)
2. 2.Lopez-Fernandez, M., Jroundi, F., Ruiz-Fresneda, M.A., Merroun, M.L.: Microbial interaction with and tolerance of radionuclides: underlying mechanisms and biotechnological applications. Microbiol. Biotechnol. **14**(3), 810-828 (2021)
3. Bouabidi, Z., El-Naas, M., Zhang, Z.: Immobilization of microbial cells for the biotreatment of wastewater: a review. Chem. Lett., **17**, 241-257 (2019)
4. Mehrotra, T., Dev, S., Banerjee, A., Chatterjee, A., Singh, R., Aggarwal, S.: Use of Immobilized Bacteria for Environmental Bioremediation: A Review. J. Environ. Chem. Eng. **9**(5), 105920 (2021)

5. Kim, Y.H., Yang, X., Shi, L., Lanham, S.A., Hilborn, J., Oreffo, R.O.C., Ossipov, D., Dawson, J.I.: Bisphosphonate nanoclay edge-site interactions facilitate hydrogel self-assembly and sustained growth factor localization. Nat. Commun. **11**(1), 1365 (2020)
6. Chen, J.P., Lin, Y.S.: Decolorization of azo dye by immobilized *Pseudomonas luteola* entrapped in alginate-silicate solgel beads. Process. Biochem. **42**(6), 934–942 (2007)
7. 7.Abrougui, M.M., Lopez-Lopez, M.T., Duran, J.D.G.: Mechanical properties of magnetic gels containing rod-like composite particles. Philos. Trans. A Math. Phys. Eng. Sci. **377**(2143), 201802182 (2019)
8. Gila-Vilchez, C., Bonhome-Espinosa, A.B., Kuzhir, P., Zubarev, A., Duran, J.D.G., Lopez-Lopez, M.T.: Rheology of magnetic alginate hydrogels. J. Rheol. **62**, 1083 (2018)