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Short communication

Tensile properties of yeast cell-loaded Ca-alginate gel layers

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ABSTRACT

The tensile properties of Ca-alginate gel strips (2.0% (w/v) alginate) containing varying amounts of yeast cells (0.1–5.0%, w/v) were investigated. The stress, strain and energy at failure of yeast-filled alginate samples showed very similar evolutions as a function of the initial cell content, i.e., they peaked at a cell content of 0.5–0.8% (w/v). By contrast, no clear effect of cell loading on Young's modulus could be highlighted at moderate yeast concentration (≤1.0%, w/v) but the elastic modulus significantly decreased at higher cell loading. These results are discussed in light of literature data on particulate–polymer composites.

1. Introduction

Alginate hydrogels are widely used for a broad range of applications, including mainly cell and drug encapsulation, but also tissue and food engineering. The successful implementation of alginate-based structures is closely dependent on their mechanical properties which are commonly assessed by compression tests and, to a lesser extent, extensometry [1]. Within the area of cell encapsulation, the two methods, i.e., compressive [2] and tensile [3,4] measurements, have been applied to biofilms to test the mechanical stability of these natural immobilized-cell systems, in which microbial cells are englued in their own extracellular polymeric substances whose alginate is a major constituent [5]. The tensile properties of biofilms reflect their cohesive strength [4] that is likely to control natural biomass detachment or sloughing caused by antifouling treatments. As concerns artificial cell entrapment, however, only a few studies of the compressive behavior of cell-loaded alginate hydrogel constructs have been published so far [6] and tensile tests have been even more rarely reported, whatever the nature of entrapped cells, i.e., microbial [7] or eucaryotic [8].

In a recent paper [6], we investigated the compressive properties of alginate gel layers containing varying amounts of yeast cells using static uniaxial compression tests. The presence of yeasts led to the weakening of the gel structures, this effect increasing with the immobilized-cell content. The influence of yeast cell content on the tensile mechanical behavior of alginate samples is reported here.

2. Materials and methods

2.1. Alginate and yeasts

A commercial sodium alginate obtained from SKW Biosystems (Baupte, France) was used. The macromolecular characteristics of this alginate, extracted from *Lessonia trabeculata*, are the following: weight average molecular weight ($\overline{M_w}$), 652,000 g mol^{-1}; number average molecular weight ($\overline{M_n}$), 350,000 g mol^{-1}; polydispersity index ($\overline{M_w}/\overline{M_n}$), 1.86; M/G ratio (the ratio of mannuronic to guluronic units in alginate molecule), 0.5.

Commercial freeze-dried baker's yeast cells (*Saccharomyces cerevisiae*; Vahiné, Monteux, France) were weighted and suspended in known volumes of sterile deionized water. These calibrated cell suspensions were thoroughly homogenized by magnetic stirring before their introduction into alginate solution as described below.

2.2. Preparation of calcium alginate gel samples

Strip-shaped samples of ionically crosslinked alginate hydrogel were prepared according to the diffusion-controlled procedure already described [6]. Sodium alginate powder was dissolved in deionized water and supplemented with yeast cells from calibrated suspensions to yield a 2.0% (w/v) concentration of alginate and varying concentrations of yeasts (0–5.0%, dry w/v). The cell-loaded polysaccharide solution was degassed under vacuum for 2 h to

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eliminate most air bubbles and poured into the rectangular opening (80 mm × 30 mm) of a Plexiglas plate (2.5 mm thickness) fastened to another plastic plate. The cavity was covered with a microporous membrane sheet (Protan BA 83 nitrocellulose membrane from Whatman, Dassel, Germany; pore size, 0.2 μm) that was held in position by a third perforated plastic plate. The whole structure was immersed for 2.5 h in 100 mM calcium chloride solution under gentle agitation to allow gel formation by diffusion of Ca^{2+} ions through the microporous membrane. The rectangular gel sheet was then removed from its housing and stocked for 15 h in 100 mM $CaCl_2$ solution at 4 °C. Finally, the gel piece was carefully cut to thin strips (40 mm × 3 mm × 2.5 mm) using a scalpel.

A minimum of 20 identical alginate strips was constructed and submitted to tensile testing for each yeast cell concentration. All specimens were kept for 1 h at ambient room conditions of 20 °C and 50% relative humidity before testing.

2.3. Tensile tests

The tensile properties of cell-loaded alginate strips were evaluated at room temperature (20 °C) and RH (50%) using a Universal Testing Machine from Instron (Model 5543; Canton, MA, USA) equipped with mini wedge grips (Model 2716-016, 1kN, Instron) and a 5 N load cell. The alginate specimen was fastened with adhesive tapes to a rectangular pasteboard piece having a central square (10 mm × 10 mm) opening. The whole structure was clipped on both sides of the opening and the pasteboard piece was cut to release the gel strip for tensile test. In this way, the gel sample endured no damage during mounting and end effects during elongation were minimized so that sample fracture most often occurred between the two grips (tensile data were discarded when this was not the case). A constant elongation speed of 2.54 mm min^{-1} was applied in all experiments. After data treatment with specific software developed by the manufacturer (Merlin V22082), tensile (stress–strain) curves were constructed, from which the mechanical characteristics of alginate gel samples were obtained (see Fig. 1).

2.4. Data processing and statistical analyses

Microsoft Office Excel (Microsoft Corporation, Santa Clara, CA, USA) and Sigmaplot 10.0 (Jandel Scientific, Corte Madeira, CA, USA) were used for tension data processing and descriptive statistics. Detection of outliers (Grubbs' test) was performed using the free GraphPad Software available at http://graphpad.com/qickcalcs/grubbs1.cfm.

3. Results and discussion

Typical tensile stress–strain curves were obtained for all tested alginate specimens, whatever their yeast contents (Fig. 1). The following parameters were determined from these plots: strain (ε_f), stress (σ_f) and energy (W_f) at fracture, tensile (Young's) modulus (E). Energy at fracture W_f was given by the area below the curve between origin and the breaking point (coordinates: ε_f and σ_f). Young's modulus E corresponded to the slope of the stress–strain curve during the linear region, limited to the first 10% of strain. Slopes of regression lines over this elongation range displayed statistically the best correlation coefficients compared to higher strains, with r^2 values always above 0.98. Significant outliers ($p < 0.01$) among experimental ε_f, σ_f, W_f and E values were removed before calculation of means and standard errors on the means. Very briefly, σ_f (i.e., tensile strength) and W_f values are representative of the gel strength and toughness, respectively, Young's modulus characterizes the stiffness and elastic behavior of the gel and its tensile deformability is reflected by the strain at fracture (i.e., ultimate elongation).

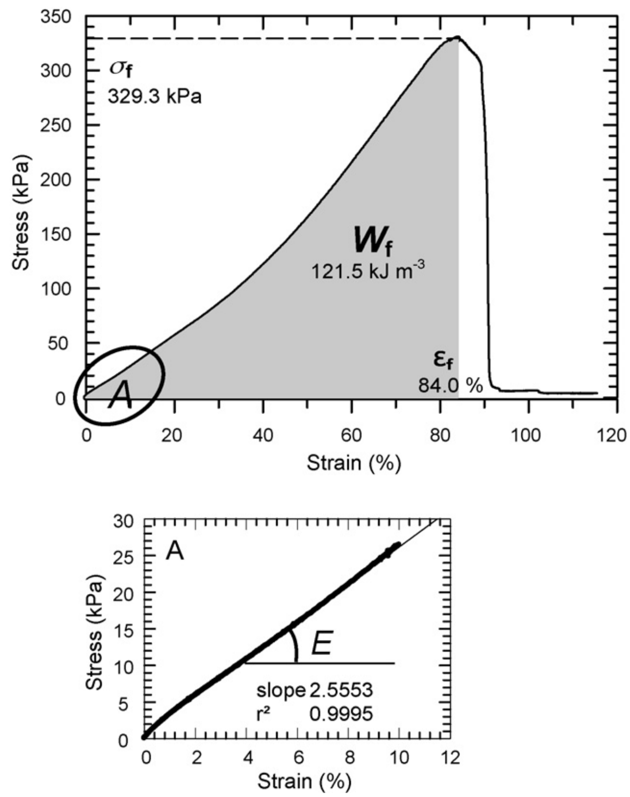

Fig. 1. Typical stress vs. strain tensile curve of an alginate gel strip. Illustrative values of the parameters (ε_f, strain at failure; σ_f, stress at failure; W_f, energy at failure; E, Young's modulus) were obtained for a yeast loading of 1.0% (w/v). The determination of Young's modulus is detailed in inset (A).

Fig. 2 illustrates variations in the values of gel tensile parameters resulting from entrapment of increasing amounts of yeast cells. The stress, strain and energy at failure of yeast-loaded alginate strips showed very similar evolutions as a function of the initial cell content, i.e., ε_f, σ_f and W_f values rose to a maximum that was reached at a cell content of 0.5–0.8% (w/v) and progressively decreased for higher cell concentrations. By contrast, no clear evolution of Young's modulus with cell content could be highlighted at moderate yeast concentrations (≤1.0% w/v) but E values endured a significant decrease at higher cell contents. Literature data comparable with these results are reduced to their simplest expression. In an early work [7], Krouwel et al. reported a decrease in the tensile strength of a 2% (w/v), 2.5-mm thick alginate slab loaded with 5% (w/v) baker yeast cells compared to the cell-free sample, i.e., an average σ_f value of *c.* 170 kPa compared to *c.* 250 kPa. They noted no significant influence of cell presence on the strain at failure, ε_f values ranging between 65 and 80%. These numerical values of σ_f and ε_f for the neat hydrogel are consistent with the present results, but the effects of yeast loading on σ_f and ε_f values are not.

The cell-loaded alginate hydrogel may be considered as a particulate-filled composite material. The deformation processes and overall mechanical behavior of such materials are determined by the intrinsic properties of both the neat polymer matrix (alginate) and the filler particles (yeasts), by particle size and loading, and also particle/matrix interactions [9]. *S. cerevisiae* cells are relatively large spherical microparticles, with an average diameter of *c.* 5 μm [6]. They behave like an elastic material with a Young's modulus in the range of 1–2 MPa [10], i.e., they are noticeably more rigid that the crude alginate gel matrix. On the other hand, the cell surface of *S. cerevisiae* is known to be negatively charged over a wide range of pH and ionic strengths [11]: owing to electrostatic repulsive forces, the occurrence of strong interactions between yeast cells and

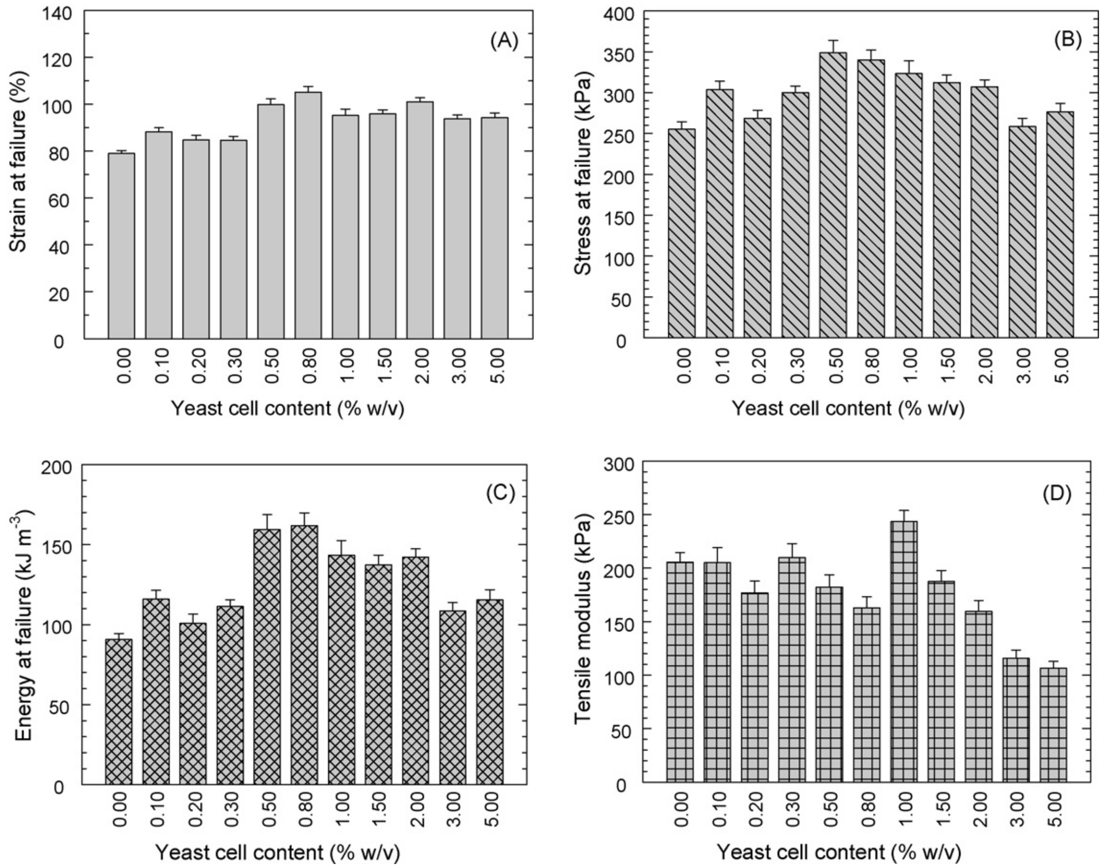

Fig. 2. . Influence of yeast cell load on the tensile properties of alginate gel strips (A, strain at failure ε_f; B, stress at failure σ_f; C, energy at failure W_f; D, Young's modulus *E*). Bars indicate errors on the means (*n* values ranging between 20 and 25).

(negatively charged) alginate chains is therefore improbable. Taking into account these features of yeast cells, the present results can be discussed in light of the general rules expressed by Fu et al. in a recent paper [9] reviewing the effects of particle size, particle/matrix interface adhesion and particle loading on the stiffness, strength and toughness of particulate–polymer composites:

- Stress at fracture (tensile strength). The composite strength is favoured by both the small size of particles and a strong interfacial bonding between particles and polymer matrix. Various trends of the effect of particle loading on composite strength have been reported, among which an initial increase in strength up to a maximum value, followed by a decrease at higher particle loading.
- Energy at fracture (toughness). Composite fracture toughness is affected by particle size and interfacial adhesion between particles and matrix. Varying effects (i.e., increase in toughness or brittleness) have been reported as a consequence of varying toughening and failure mechanisms. Toughness is also affected by particle loading. Frequently, toughness peaks at a medium filler loading and decreases with more filler added.
- Young's modulus (stiffness). As a general rule, the elastic modulus of the composite is enhanced by adding particles whose modulus is higher than that of the matrix. This improvement of stiffness is dependent on filler particle size—more pronounced when particles are small (e.g., nanoparticles). Since Young's modulus is measured at relatively low deformation, the matrix–particle adhesion strength does not noticeably affect the elastic modulus.

Here, the variations of σ_f and W_f as a function of yeast cell content were consistent with those observed for other particulate–polymer composites, i.e., an optimal value did exist for particle loading. However, the size and electric charge of *S. cerevisiae* cells are not favourable to a large improvement of tensile stress of yeast-loaded alginate gel. This improvement was moderate but significant (Fig. 2B) as was that of toughness (Fig. 2C). The creation of ionic crosslinks between alginate chains and yeast cells via Ca^{2+} ions may explain the reinforcement of the yeast-loaded gel at low cell content, this effect being balanced by partial screening of interchain crosslinking sites during the gel formation process at higher particle loading. The same explanation holds for the decrease in Young's modulus at high yeast content while the quasi independence of *E* value on yeast content at low cell loading probably originates from the size of filling particles.

The effects of yeast cells on ε_f, σ_f and W_f tensile values are noticeably different from those observed previously [6] on compressive parameters. In particular, the stress, strain and energy at failure of yeast-loaded alginate gel disks submitted to uniaxial (unconfined) compression tests were little influenced by moderate particle loading ($\leq$1%, w/v) and endured a huge decrease at the highest tested cell contents (2–5%, w/v). A better agreement stands between the effects of cell content on the compressive and tensile moduli, however, as both moduli decreased in the presence of yeasts. Comparative measurements of quasi-static compression and tension data for alginate gels have been seldom reported. Alginate is usually considered showing similar behaviors in tension and compression [12] but, at large deformations, it has been shown to be more rigid in tension than in compression [13], like other calcium-crosslinked gels such as gellan [14]. The compressive strength of a brittle material (such as alginate [7]) is generally higher than its tensile strength [15]. These data, i.e., higher σ_f and lower *E* values in compressive than in tensile tests, agree with our previous, compressive [6] and present, tensile results on neat and yeast-loaded alginate. The reasons for different yeast effects on compressive and tensile parameters of yeast-loaded alginate remain to be elucidated, however.

References

[1] J.L. Drury, R.G. Dennis, D.J. Mooney, The tensile properties of alginate hydrogels, Biomaterials 25 (2004) 3187–3199.

[2] V. Körstgens, H.-C. Flemming, J. Wingendern, W. Borchard, Uniaxial compression measurement device for investigation of the mechanical stability of biofilms, J. Microbiol. Meth. 46 (2001) 9–17.

[3] A. Ohashi, T. Koyama, K. Syutsubo, H. Harada, A novel method for evaluation of biofilm tensile strength resisting erosion, Water Sci. Technol. 39 (1999) 261–268.

[4] E.H. Poppele, R.M. Hozalski, Micro-cantilever method for measuring the tensile strength of biofilms and microbial flocs, J. Microbiol. Meth. 55 (2003) 607–615.

[5] M. Wloka, H. Rehage, H.-C. Flemming, J. Wingender, Rheological properties of viscoelastic biofilm extracellular polymeric substances and comparison to the behavior of calcium alginate gels, Colloid Polym. Sci. 282 (2004) 1067–1076.

[6] G.-A. Junter, F. Vinet, Compressive properties of yeast cell-loaded Ca-alginate hydrogel layers: comparison with alginate–$CaCO_3$ microparticle composite gel structures, Chem. Eng. J. 145 (2009) 514–521.

[7] P.G. Krouwel, A. Harder, N.W.F. Kossen, Tensile stress–strain measurements of materials used for immobilization, Biotechnol. Lett. 4 (1982) 103–108.

[8] G.M. Williams, T.J. Klein, R.L. Sah, Cell density alters matrix accumulation in two distinct fractions and the mechanical integrity of alginate–chondrocyte constructs, Acta Biomater. 1 (2005) 625–633.

[9] S.-Y. Fu, X.-Q. Feng, B. Lauke, Y.-W. Mai, Effects of particle size, particle/matrix interface adhesion and particle loading on mechanical properties of particulate–polymer composites, Compos. Part B: Eng. 39 (2008) 933–961.

[10] T. Svaldo-Lanero, O. Cavalleri, S. Krol, R. Rolandi, A. Gliozzi, Mechanical properties of single living cells encapsulated in polyelectrolyte matrixes, J. Biotechnol. 124 (2006) 723–731.

[11] Y.-I. Chang, P.-K. Chang, The role of hydration force on the stability of the suspension of *Saccharomyces cerevisiae*—application of the extended DLVO theory, Colloid Surf. A 211 (2002) 67–77.

[12] G.M. Williams, K.R. Gratz, R.L. Sah, Asymmetrical strain distributions and neutral axis location of cartilage in flexure, J. Biomech. 42 (2009) 325–330.

[13] T. Boontheekul, H.-J. Kong, D.J. Mooney, Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution, Biomaterials 26 (2005) 2455–2465.

[14] J. Tang, M.A. Tung, J. Lelievre, Y. Zeng, Stress–strain relationship for gellan gels in tension, compression and torsion, J. Food Eng. 31 (1997) (1997) 511–529.

[15] L.E. Nielsen, R.F. Landel, Mechanical Properties of Polymers and Composites, 2nd edition, Marcel Dekker, New York, Basel, 1994, p. 250.