Mechanical properties of sugar beet Ca-pectate gel usable for cell immobilisation and heavy metal accumulation

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Abstract

After alkaline hydrolysis, pectin extracted from sugar beet pulp is able to form gels that can be used for whole cell entrapment and heavy metal accumulation. The purpose of the present work was to appraise the ability of sugar beet pectin (SBP) gel particles to accommodate the stresses encountered in bioreactors. We investigated the rheological properties (mechanical behavior) of SBP gels shaped as flat membranes and compared them to those of citrus pectin and algal alginate gel membranes. We evaluated the fracture properties of the different gel membranes by constructing stress–strain curves (obtained by uniaxial compression at constant speed). Stress and strain at fracture, Young’s modulus, fracture and flow energies were determined. SBP and citrus pectin gel membranes were less resistant to stress than algal alginate membranes, with stresses at fracture of $1.7 \times 10^5$ N m$^{-2}$ (SBP) and $4.83 \times 10^5$ N m$^{-2}$ (citrus pectin) as compared to $2.44 \times 10^6$ N m$^{-2}$ for alginate. A chain flow phenomenon prior to fracture was observed in the case of pectin gel membranes (flow energies: $3500$ J m$^{-3}$ for SBP; $4400$ J m$^{-3}$ for citrus pectin, negligible for alginate). These results show that pectin gels are more heterogeneous than those of algal alginate and behave as a viscoelastic material, in opposition with alginate gels that display an elastic behaviour. The relatively low mechanical resistance of SBP gels may impede their long-term utilization in the biological treatment of wastes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Alginate; Ca-pectate gels; Immobilized cells; Pectins; Polysaccharide gels; Rheological properties; Sugar beet pulp

1. Introduction

Polysaccharide gel immobilized microorganisms can be used to remove heavy metal ions from dilute aqueous solutions, providing an alternative to physico-chemical technologies for waste water treatment. The cost of the gel matrix, however, represents a serious drawback for the large-scale development of such immobilized-cell systems. Furthermore, biocatalyst particles must display sufficient mechanical stability to accommodate the various stresses to which they will be subjected in bioreactors, e.g. hydrodynamic shear stresses and collisions between particles. The effects of such stresses on biocatalyst particles (abrasion, fatigue) largely depend on the mechanical properties of
the polymer matrix. They depend on the size, shape, and density of the particles (Dos Santos et al., 1997) but also of their roughness, hardness, elasticity and degree of homogeneity.

Pectin extracted from sugar beet pulp may represent a cost-effective immobilization matrix alternative to commercial algal alginate and citrus pectin. Sugar beet pectin (SBP), hydrolysed by a simple alkaline process, is able to form gels (at pectin concentrations of 2% w/v or higher) in the presence of divalent cations (Harel et al., 1998). SBP gel beads, either sterile (cell-free) or loaded with viable cells of *Pseudomonas putida*, have been tested previously for cadmium accumulation (Harel et al., 1997): their metal ion binding capacity compared to that of algal alginate gel particles.

The present work is a first approach to appraise the ability of SBP gel to accommodate stresses in bioreactors. We investigated the mechanical behavior of SBP gel membranes under uniaxial compression. Several rheological parameters (e.g. fracture stress and energy) were calculated from stress–strain curves and compared to those of citrus pectin and algal alginate gel samples.

2. Materials and methods

2.1. Sugar beet pulp and polysaccharides

Sugar beet pulp was supplied by a sugar refining company (Générale Sucrerie, Nassandres, France). Pectin extraction (acid hydrolysis of raw material) and alkaline hydrolysis were performed as described previously (Harel et al., 1998). Sodium alginate and citrus pectin were purchased from Prolabo (Rhone Poulenc Ltd, Manchester, England) and SKW Biosystem (Carentan, France), respectively.

2.2. Preparation of calcium alginate and pectate gel membranes

Disc-shaped polysaccharide gel samples were prepared according to the procedure used routinely in our laboratory (Jouenne et al., 1993). An aqueous solution of polysaccharide (2% w/v) was poured into the circular opening of a Plexiglas plate fastened to another, removable plastic plate. The cylindrical cavity was covered with a microporous membrane (HA filter from Millipore, Freehold, NJ, USA; pore size, 0.45 µm; porosity, 79%) that was held in position by a third, perforated plastic plate. The whole structure was immersed for 2 h in a stirred aqueous solution of calcium chloride (0.3 M) to allow gel formation by CaCl₂ diffusion through the microporous membrane. An homogeneous disc-shaped gel layer (thickness, 0.3 cm; surface, 10 cm²) could then be removed from its housing.

A set of about 25 identical gel discs was constructed for each polysaccharide. Before the compression experiments, gel discs were stocked in CaCl₂ solution at 4°C for 24 h and washed by immersion for 12 h in distilled water at ambient temperature. To study the influence of conservation in distilled water on the mechanical behaviour of SBP gels, the duration of this immersion period was extended to 16 or 35 days.

2.3. Measurement of fracture properties and data treatment

Stress–strain curves of polysaccharide gels were determined at room temperature using an uniaxial compression machine (Series 4300 from Instron, Canton, MA, USA). This apparatus consists of two stainless-steel flat discs (20-cm diameter), one of which is fixed while the other can be moved vertically. The force necessary for maintaining constant moving speed of the mobile piece (2.54 mm min⁻¹) was recorded as a function of time until fracture of the tested gel sample occurred. Data were collected and treated using a specific software (Instron Series IX, Version 1.16) that calculated stress (N m⁻²) by dividing the applied compression force by the area on which this force acted and expressed the dimensionless strain as the ratio of the change in gel membrane thickness to the initial membrane thickness. Fracture stress, strain at fracture, fracture energy and Young's modulus in compression were determined from the resulting stress–strain curves (Dos Santos et al., 1997) as described below.
3. Results

Fig. 1 shows a typical stress–strain curve of SBP gel where the different rheological parameters are specified. The coordinates of the point in the curve at which fracture of the gel sample occurred (i.e. maximum of the stress–strain curve) gave the stress and strain at fracture. The total energy (addition of flow and fracture energies) required for fracture was given by the area below the curve until the fracture point. The Young’s compression modulus (ratio of stress to strain at small deformation) was calculated from the initial slope of the curve.

Fig. 2 shows stress–strain curves of the three tested gels. These curves were quite similar, i.e. strain increased with stress until fracture of the gel slab occurred. Nevertheless, a flow phenomenon was observed prior to fracture for pectin gel membranes. The average values of the main rheological parameters, evaluated from these curves as described above, are summarized in Table 1. Table 2 gives the rheological parameters of SBP gel after immersion over varying times in distilled water.

4. Discussion and conclusion

The rheological parameters determined for the different gel matrices could be analyzed in terms of material resistance, e.g. the gel strength increased with the fracture stress (Leenen et al., 1996). Energy values reflected viscoelastic phenomena that occurred during the compression tests.

According to the fracture stress values (Table 1), pectate gel membranes showed less resistance to stress than alginate ones, citrus pectate gel displaying more resistance than SBP gel. The strain at fracture was more important for SBP gel than for citrus pectate gel, however, highlighting a more viscous rheological behavior of SBP gel. This was confirmed by the values of Young’s modulus but also by the analysis of flow energies.

The mechanical behavior of a polymer matrix first depends on its monomeric composition and sequential arrangement, but also on the macromolecular chain size and the nature of intermacromolecular links. The macromolecular characteristics (intrinsic viscosity, weight–average molecular weight) of SBP are lower than those of commercial citrus pectin (Harel et al., 1998) due
Table 1
Rheological parameters of polysaccharide gel samples a

<table>
<thead>
<tr>
<th>Gel sample (2% w/v)</th>
<th>Fracture stress × 10^{-4} (N m^{-2})</th>
<th>Fracture strain (N m^{-2})</th>
<th>Young’s modulus (J m^{-3})</th>
<th>Fracture energy (J m^{-3})</th>
<th>Flow energy (J m^{-3})</th>
<th>Total energy (J m^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal alginate</td>
<td>244 ± 19</td>
<td>0.554 ± 0.031</td>
<td>228 900 ± 26 900</td>
<td>243 100 ± 23 900</td>
<td>–</td>
<td>243 100 ± 23 900</td>
</tr>
<tr>
<td>Citrus pectate</td>
<td>48.3 ± 4.5</td>
<td>0.304 ± 0.023</td>
<td>338 400 ± 29 000</td>
<td>28 800 ± 26 000</td>
<td>4400 ± 300</td>
<td>33 200 ± 2500</td>
</tr>
<tr>
<td>SBP</td>
<td>17.0 ± 1.2</td>
<td>0.462 ± 0.027</td>
<td>92 400 ± 14100</td>
<td>11 600 ± 1300</td>
<td>3500 ± 300</td>
<td>15 100 ± 1200</td>
</tr>
</tbody>
</table>

a Results were expressed as mean ± standard deviation (n = 25).
Table 2
Effect of conservation in distilled water on the rheological characteristics of SBP gel samples

<table>
<thead>
<tr>
<th>Sample age (days)</th>
<th>Young's modulus (N m⁻²)</th>
<th>Fracture energy (J m⁻³)</th>
<th>Flow energy (J m⁻³)</th>
<th>Total energy (J m⁻³)</th>
<th>Flow percentage b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>92 400 ± 14 100</td>
<td>11 600 ± 1300</td>
<td>3500 ± 300</td>
<td>15 100 ± 1200</td>
<td>23.2</td>
</tr>
<tr>
<td>16</td>
<td>77 100 ± 5600</td>
<td>12 100 ± 700</td>
<td>13 000 ± 500</td>
<td>25 100 ± 1700</td>
<td>51.8</td>
</tr>
<tr>
<td>35</td>
<td>56 300 ± 8 900</td>
<td>3300 ± 200</td>
<td>12 600 ± 1100</td>
<td>15 900 ± 600</td>
<td>79.2</td>
</tr>
</tbody>
</table>

a Results were expressed as mean ± standard deviation (n = 25).
b 100 × flow energy/total energy.

to pectin chain depolymerization that occurs during alkaline hydrolysis (Kim et al., 1978a,b) and unfavorably influences the mechanical properties of calcium pectate gels (Gemeiner et al., 1991). The flow energy reflected the cold drawing of macromolecular chains when pectate gels were compressed prior to fracture. This parameter represented 23% of the total energy for Ca-SBP gel but only 13% of the total energy for Ca-citrus pectate gel (Table 1). This difference means that the polymer lattice of Ca-SBP gel is weaker than that of Ca-citrus pectate gel displaying a more viscoelastic behavior. Flow energy was negligible for Ca-alginate gel (Table 1) that accommodated the stress as an elastic material.

Long term storage in distilled water affected the mechanical resistance of SBP gel. We observed (Table 2) a decrease in Young’s modulus values during storage, together with an increase in the flow percentage reflecting an enhancement of the fluage phenomenon. This evolution of rheological parameters indicated a loss in elasticity of gel membranes, i.e. a plasticization phenomenon that probably resulted from gel swelling in water. After immersion in water over 16 days, the flow energy reached a limit value corresponding to the highest volume of water absorbed by the polymer matrix. Swelling induced heterogeneities inside the gel structure: fracture always starts at such irregularities (Dos Santos et al., 1997). After exposure to water for 35 days, however, the fracture energy had drastically decreased, highlighting the loss in gel strength. Like carrageenan and alginate gels (Leenen et al., 1996), pectate gels would in the long-term dissolve in media that do not contain counterions (Ca²⁺). Nevertheless, storage of SBP gel samples in CaCl₂ solution is likely to decrease the metal-binding efficiency of the gel matrix owing to competition for the metal-binding sites of the gel (Dronnet et al., 1996). Furthermore, the long-term exposure of cell-loaded SBP particles to CaCl₂ solution might be detrimental to cell activity.

In conclusion, the relatively low mechanical resistance of SBP gels would seriously impede their long-term utilisation in bioreactors for the biological treatment of wastes (e.g. metal biosorption). Moreover, cell-loaded SBP gel particles may suffer from the growth of immobilized organisms leading to an increase in internal pressure, cell leakage and mechanical disruption of the gel surface (Dos Santos et al., 1997). Hardening treatments of Ca-pectate gels using glutaraldehyde or polyethyleneimine are possible (Tomáška et al., 1995), but they are harmful to viable cells. From these elements, cell-loaded SBP gel particles may be more useful in other areas of environmental relevance where immobilized cells are used as inocula (e.g. soil bioremediation or biofertilisation).

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References