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6. MULTIPLE-CORE ENCAPSULATION
6.6. Microencapsulation and Alginate

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6.6.1 Introduction

Currently, alginate beads represent a major entrapment system for cells. The method is very gentle, requiring no pH drop, no ionic strength or osmotic pressure changes, and no toxic cross-linker. It can be performed at room temperature. Since the first reference to this technology (1), thousands of papers have been published on its applications in food, biotechnology and biomedicine. A recent issue of Critical Reviews in Biotechnology (2) provides a series of reviews on cell immobilisation for food science, with a large section on alginate beads.

Cell entrapment in alginate beads provides both an immobilisation method and good cell protection. In particular, drying alginate beads with entrapped cells gives enhanced cell viability compared with free cells (3). Dry alginate beads can also be used for controlled release and protection against light and oxygen.

Alginate beads are mainly produced by dropping an alginate solution into a calcium chloride solution. However, there are several other methods that may be more appropriate for numerous applications. While not being exhaustive, this chapter will provide an overview of microencapsulation technologies based on alginate.

6.6.2 Alginate

Alginate is a polysaccharide, which is obtained by extraction from brown algae. Standford discovered and patented it in 1881. The alginate may constitute up to 40% of the dry matter of algae, mainly located in the cell wall and intercellular space. Alginate is extracted using solubilisation of alginate salts (Ca^{2+}, Mg^{2+} ...) by acidification with dilute acid (HCl) followed by alkalinisation (NaOH or Na_2CO_3). Sodium alginate salt is then precipitated by ethanol, acid or calcium chloride (4).

Alginate is a linear polymer of two epimers of uronic acids: β-D-mannuronic (M) and α-L-guluronic (G) (Fig. 6.6.1). They are polymerised as α or β (1->4)

glycosidic links (5). Alginate prepared from various sources may differ either in molecular weight or proportion of monomers (G fraction ranging from 10 to 80%). The length of the guluronic sequence is even more important, since it strongly influences gel formation (see below).

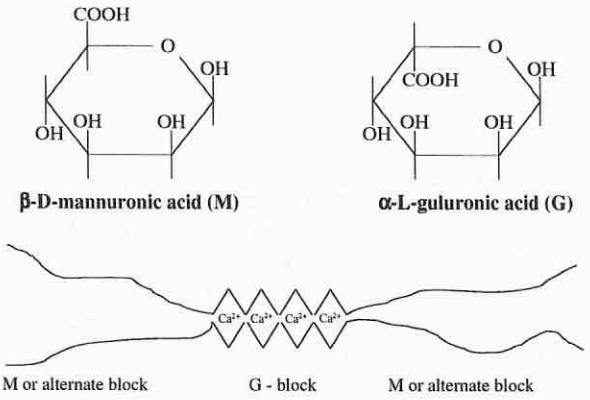

Fig. 6.6.1. Alginate monomers and gel structure

The viscosity of alginate is mainly determined by the molecular weight, ranging from 80 to thousands of mPa.s. The viscosity is slightly dependent on the composition, but it increases with decreasing ionic strength. It may increase drastically with even low concentrations of divalent cations.

Alginate has the capacity to bind di- and trivalent ions (except with Mg^{2+}), resulting in gel formation. Binding calcium, for example, is a co-operative mechanism and is quite stable against dissociation. This process is more effective when the alginate has longer G-blocks (5). Two consecutive guluronic monomers form a cavity, where the calcium can be bound through five links. Assuming several consecutive guluronics and two alginate chains in parallel, strong interchain binding results in a conformation called the "egg-box model" (Fig. 6.6.1). This configuration explains quite well co-operative binding and dissociation hysteresis of calcium by alginate. It also confers a higher mechanical strength on gel formed from alginate with a high guluronic content. In another way, a large hysteresis between association and dissociation of the calcium-

alginate complex leads to a non-equilibrium state in the gel, even after long incubation in a calcium chloride bath.

6.6.3 Alginate bead formation by external gelation

The most usual way to form alginate beads is to drop an alginate solution (1.5 to 4%) into a calcium chloride solution (50 to 100 mM). The beads are mainly formed instantaneously. However, they could evolve for up to 15 h (6). In most cases, the beads are maintained in calcium solution for only 15 to 30 min.

During gelation, shrinking of the droplets-beads can be observed, linked to formation of highly structured egg-box connections (4). This effect is a function of alginate and calcium concentrations, alginate structure and gelification time.

During the first stage of gelification, calcium is absorbed by the external layer of the alginate droplet, leading to some skin effects or at least alginate gradients. The final structure of the bead is determined by alginate and calcium gradients inside the beads. Many authors talk about inhomogeneous beads (7). To reduce this effect, one could use a higher calcium concentration or increase the ionic strength with sodium salt. The affinity of alginate for calcium is thus reduced, and calcium can penetrate more deeply into the droplet before being absorbed. Several authors have shown that this approach can be used to obtain mainly homogeneous beads.

One of the main concerns with alginate beads is their sensitivity to calcium chelators (such as phosphate). Physicochemical resistance of the beads may be increased by using a higher guluronic content and a longer G block length, higher calcium concentrations or replacement of calcium by barium or aluminium.

Another concern with this method is obtaining small beads at large scale. Simple dropping results in beads of 2 to 3 mm. Using additional drag forces (coaxial air flow, electrostatic potential) on a nascent droplet (Fig. 6.6.2), one could obtain small droplets (down to 100 µm) but with very limited productivity (30 ml/h) (8). Increasing the flow to give jet formation at the nozzle, and breaking the jet by nozzle resonance or a rotating cutter (Fig. 6.6.2), gives a higher flow rate (up to 30 litres/h for 3-mm beads) and a lower size distribution (5%) (9). Rotating devices have also been proposed to reach even higher flow rates (15 litres/h for 700-µm beads). For these systems, productivity is inversely proportional to the bead diameter (or square) and remains limited for small beads (less than 500 µm).

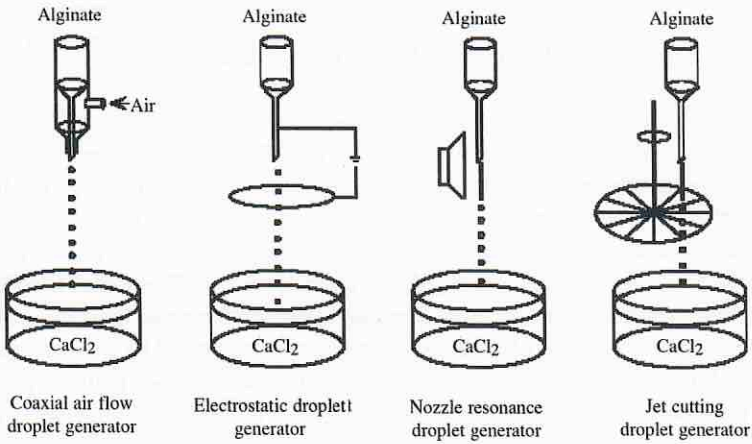

Fig. 6.6.2. Extrusion systems

6.6.4 Alginate hollow beads or microcapsules

Another problem for alginate beads is that an encapsulated material (especially cells) is often located in the surface layer (or even projects beyond the surface). Therefore, the material can be released easily from the beads. The main solution is to produce hollow beads or microcapsules.

The alginate beads can be coated with polymer membrane by suspending them in a polycationic solution (poly-l-lysine or chitosan at 0.1 to 0.2%) (10). However, the membrane is, in fact, formed by absorption of the polycations inside the external layer of the alginate beads, and is not a real coating of the beads. Therefore, cells that occur in the surface layer can still be released. Since the membrane has a molecular cut-off higher than 60,000 Daltons, a lot of enzymes can also easily escape.

To solve the problem of cell release, Moët & Chandon (Epernay, France) promoted a process of coextrusion. An (internal) alginate solution containing the cells is coextruded with an (external) alginate solution free of cells. The external solution coats the internal core before the droplets fall into a gelation bath, thus

preventing cell localisation near the surface. In this process, the double solution is extruded as a jet and cut by nozzle resonance. The technical (double nozzle, flow rates, etc.) and physical (viscosity, surface tension, etc.) conditions should be carefully set up to obtain spherical beads with a homogeneous external layer. Mainly, this is possible only for large capsules (3-mm), which is not a problem in the case of champagne secondary fermentation. This fermentation is quite slow (several weeks) and there is no mass transfer limitation.

Another method for obtaining hollow alginate beads is to extrude an inert polymer solution (for example dextran) containing calcium salt in a solution of alginate (11). By diffusing out of the droplet, calcium gelifies an alginate layer around the droplet. In such systems, the droplet viscosity must be larger than the receiving alginate bath viscosity.

The last method consists of extruding an alginate solution (2%) in a polycationic solution (chitosan, PPDMA, 0.2 to 1%) (12). A membrane is formed at the interface of the droplet. The membrane thickness may range from 10 to 50 µm. Such membranes can have a low molecular cut-off (a few thousand Daltons) and quite good mechanical resistance.

6.6.5 Alginate beads – preparation by internal gelation

To produce small beads (down to 20 µm) at a very large scale (cubic metres per hour), we have developed a process based on the direct release of calcium from the alginate droplet (13). Alginate (2%) is mixed with insoluble calcium carbonate (0.25%) at pH 7.5-8. The mixture (20-25 % v) is emulsified in an inert oil phase (75-80% v mineral or vegetable oil). Acetic acid (100 µl/100 ml, diluted in a small amount of oil) is added to the formed emulsion. The resulting acidification leads to the release of calcium and alginate droplet gelation. The alginate beads are then separated from oil and washed.

To obtain relatively low size distribution (down to 30 µm) and spherical beads, one should take care to use a very fine calcium salt powder (2 µm), calcium contre-anion with a double charge and with a dissociation constant 1.5 units lower than an initial pH (14). The pH drop must be at least two units. To avoid too large a pH drop, buffer should be introduced in the alginate while maintaining acetic acid quantity.

Alginate viscosity (or molecular weight) does not greatly influence the mean size, but lower viscosity leads to more narrow size dispersion. Using alginate with a high guluronic content, one can obtain stronger but larger beads and broader particle size distribution. This is mainly due to too early gelation. A compromise between strong beads and good size distribution must be found.

The process can be realised in batch systems with a turbine reactor. In this case, the emulsion should be mixed for 15 min before addition of the acetic solution. Moreover, Span 80 (1%) should be added to the oil prior to the alginate solution.

To form a better emulsion as a continuous process, one can use a static mixer (15). The static mixer consists of specifically designed pieces included in a tube (Fig. 6.6.3). By passing fluids through the static mixer, one can obtain good emulsification in a very short time (0.2 to 0.5 s). The alginate-calcium salt mixture is co-injected into the static mixer. Acidified oil is then injected into the circuit. Beads can be collected on a vibrated cloth strip with oil aspiration and washing water spray.

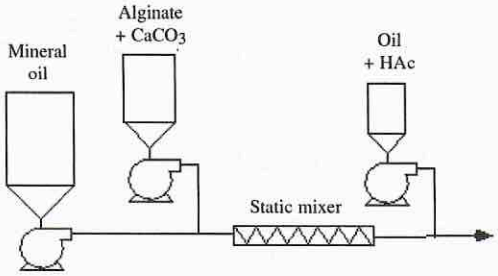

Fig. 6.6.3. Static mixers

We have tested a 1-cm diameter SMX mixer (Sulzer, Switzerland) and prepared 200- to 500-µm beads without adding any surfactant, using a flow rate of 24 to 60 kg/h. Extrapolation may be easily obtained by increasing the static mixer diameter, while maintaining the linear velocity in the static mixer (for 5-cm static mixer diameter, productivity can reach 1.5 tonnes of capsules per hour). Full-scale processing at an industrial level has not been fully tested to date.

6.6.6 Dry alginate beads

To allow easy transportation and storage, the alginate beads must often be in dry form. Moreover, protection of the encapsulated material (enzyme, vitamin, etc.)

against oxygen, denaturation or light can be ensured only in dry form. However, very little information is published on either direct production of dry beads or drying wet beads. Most research has been performed in too academic conditions (drying on a plate during 24 h) or for a specific industrial purpose. The following paragraphs summarise the limited background information collected by the authors.

Drying alginate beads is a double challenge: engineering (how to dry capsules efficiently) and biological (how to maintain high viability of cells or activity of the enzymes). We could even add a third question, which, for the most part, has no answer today: How can the beads be rehydrated? The biological question is quite a complex one (16). Cell survival, for example, is linked to both cell type and cell state. Some cells may have a higher viability if they were stressed before drying. Addition of some protectants, such as polyols, helps to maintain cell survival as well as the bead material composition and structure. Obviously, drying temperature and duration are very important. The process itself may play a role. Thus, there are many parameters that have been analysed only qualitatively until now. However, in the last few years, several groups have focused their efforts on these aspects. Therefore, one can expect new publications in the field in the near future.

There is a large volume of literature on drying, but it cannot really be applied to alginate bead drying. The beads can be successfully frozen and lyophilised (3) but this results in high porosity. Today, a lot of researchers deal with fluidised bed reactors. The beads are transferred in a tronc-conic reactor. To dry the capsules, hot air is injected by the bottom, ensuring at the same time, fluidisation and mixing. From preliminary experiments, it has been stated that the use of homogeneous beads with high alginate concentration will be easier and faster to dry. Homogeneity inside the beads reduces formation of a drying skin on the beads. Higher alginate concentration decreases the requirement for heat and limits the time of drying. The procedure should be conducted at relatively low drying air temperature (most encapsulated material being sensitive to temperature), which could be compensated partially by a higher flow rate. In any case, it is very important to keep a low product temperature.

Producing dry capsules directly may be performed by spraying sodium alginate solution in a drying tower. Particle coating can be obtained by spraying sodium alginate solution onto the particles in a fluidised bed. The usual rules of spray drying and spray coating can be used. However, if one wants to obtain an alginate gel structure after rehydration, it is necessary to bring the calcium source inside the coating, while avoiding gelation during spraying. There are no publications on this problem. One solution may consist of mixing alginate with

calcium carbonate. Rehydration may be performed either in acidic solution (pH lower than 6.5) or by adding a slow-release proton chemical to the alginate (17).

6.6.7 Conclusions

Alginate is quite an interesting material for encapsulation. It allows the preparation of beads or capsules in very gentle conditions. It is recognised as a safe and a food-grade material. The scope of applications is very large, although real industrial processes are still limited. The beads may not be stable in the presence of calcium chelatants. However, this constitutes a problem only in continuous fermentation. In other applications, such as controlled release, it may even constitute an advantage. Many technologies exist to apply alginate in encapsulated form but beads formed by external gelation are by far the most usual technique. Most of the literature refers to this method. The interest for other technologies needs to be enhanced.

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