Chapter 2
Bead Formation, Strengthening, and Modification

2.1 Introduction

This chapter provides a brief description of the typical materials used today for bead formation and their limitations. These include gel beads prepared from agar/agarose, κ-carrageenan, alginate, and celluloses after special dissolution, chitosan, and to a lesser extent polyacrylamide and other synthetic polymers. Every section includes a short description of the natural or synthetic polymer followed by a description of the crosslinking agents used for both the creation and strengthening of several types of beads. In many cases, special methods to modify the porosity of the formed beads are also described. The chapter also covers procedures for the construction of different forms of carriers—from cylindrical to almost perfectly spherical—by changing both molds and the medium into which the molten or dissolved hydrocolloid, polymer or preparation/mixture is dropped or transferred. Information can also be found on means of dropping, changing drop size and distribution, and liquid sprays. The chapter also includes more than a few examples of these beads’ uses. For the most part, however, uses are covered in detail in other chapters of this book, devoted to the beads’ major applications in the food and biotechnological fields (Chapter 4), in medicine (Chapter 5), for drug delivery (Chapter 8), in agriculture (Chapter 9), and in environmental fields (Chapter 10). Applications of unique carriers, such as dried beads and liquid-core capsules, are described in Chapters 6 and 7.

2.2 Entrapment

Cells are entrapped by gels that permit the diffusion of small molecules, both substrate and product, at rates that are adequate for the cells’ viability and functioning. In general, there are two types of cell entrapment. The first includes preparations in which cell viability is the primary concern. Viability is defined here as the cells’ ability to increase in size and undergo nuclear and, where possible, cytoplasmic division (Tampion and Tampion 1987). If this occurs, intensification of the process after immobilization can be expected, i.e., the loosely entrapped cells will proliferate to very high densities, dependent upon the suitable addition of culture medium. Thus,
the cell density in the matrix can be higher than that generated in free culture. The second type of immobilization involves entrapment of non-viable cells. The loss of viability may be intentionally induced or occur as a result of the techniques and materials used for the entrapment. In some cases, this provides an operational benefit since permeability barriers are removed and competitive biocatalytic pathways are destroyed. Of course, another option is to use activated supports that will couple covalently with purified enzymes, but this is beyond the scope of our discussion and in general, entrapment substances are less costly (Tampion and Tampion 1987).

2.3 Single-Step Methods

The major types of entrapment have been thoroughly reviewed (Cheetham 1980; Bucke 1983; Mattiasson 1983; Nussinovitch 1994, 1997). One of the most frequently used methods is single-step entrapment. This involves the simple gelation of macromolecules by lowering or raising temperatures, using hydrocolloids such as agar, agarose, κ-carrageenan, and chitosan and proteins such as gelatin and egg whites. Although quite simple to achieve, these preparations commonly suffer from heat damage and low mechanical strength. An alternative single-step method consists of ionotropic gelation of macromolecules such as alginate and low-methoxy pectin by di- and multivalent cations, but these systems suffer from low mechanical strength and breakdown in the presence of chelating agents. Jen et al. (1996) provide a summary of the hydrogels used for cell immobilization. They also review current developments in the immobilization of mammalian cells in hydrogels and discuss hydrogel requirements for use in adhesion, matrix entrapment and microencapsulation, the respective processing methods, and existing applications (Jen et al. 1996).

2.3.1 Agar

Agar was discovered in Japan in the mid-seventeenth century (Yanagawa 1942; Hayashi and Okazaki 1970; Matsuhashi 1978). One of the sources for traditionally manufactured agar in Japan is the thalloid alga Gelidium, with as many as 124 local species (Segawa 1965; http://en.wikipedia.org/wiki/Gelidium). The members of this genus are known by a number of common names. Specimens can reach ~2–40 cm in length; branching is irregular or occurs in rows on either side of the main stem, and the alga produces tetraspores. As stated, many of the algae in this genus are used to make agar (Guiry and Guiry 2008). An additional resource is the genus Gracilaria, which became significant after the discovery of alkali pre-treatment (Funaki 1947; Matsuhashi 1972; Armisen and Kain 1995; Murano and Kaim 1995). Gracilaria is a genus of the red algae (Rhodophyta) which is noted for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish (Davidson 2004). Various species in this genus are cultivated in the developing world, including Asia, South America, Africa,
and Oceania (Steentoft and Farham 1997; http://en.wikipedia.org/wiki/Gracilaria). Numerous reviews contain information on the collection and processing of agar seaweed, the gelation mechanism, the effects of adding other materials on agar properties, and its applications, and the interested reader is referred to these references (Nussinovitch 1997). Agar is unique among gelling agents in that gelation occurs at temperatures below the gel’s melting point (Nussinovitch 1997). Agar produces rigid gels at a concentration of \(~1\%\) (w/w) (Davidson 1980; Nussinovitch 1997). The sol sets to a gel at about 30–40°C. After setting, the gel maintains its shape. Self-supporting shapes are formed with 0.1% agar (the rest being water). In the past, the word “brittle” best described agar gels; today, however, elasticity or rigidity can be achieved using different agars. The gel is melted by heating to \(~85–95°C\) (Nussinovitch 1997). Agar is used by microbiologists as an inert growth support because it is resistant to degradation by most microorganisms (Tampion and Tampion 1987).

To achieve entrapment by agar, a solution of 2–4% gum is prepared. Preference may be given to preparing the agar in a medium that is suitable for the particular cells being entrapped. To avoid damaging the entrapped cells, the temperature of the agar solution is reduced as close as possible to its setting point. It is important to note that the concentrated cell suspension is not preheated but added to the gum solution during mixing (Tampion and Tampion 1987). Brodelius and Nilsson (1980) reported slight heat damage to plant cells during a preparation procedure at 50°C. The shape can be custom designed by using a mold, by cutting, or via the use of various techniques and apparatuses. A sheet or slab with a predetermined thickness can be cut up into smaller pieces. Cylindrical beads can be produced by using a perforated Teflon mold with 3 mm diameter holes (Brodelius and Nilsson 1980). Spherical beads can be produced by either dropping the molten preparation into ice-cold fluid or pouring into a preheated vegetable oil to produce an emulsion that is cooled to 5°C with stirring (Wikstrom et al. 1982). Instead of injecting drops of warm agar solution into a cold oil bath, the warm solution may be dripped onto the surface of a cold oily medium. A particularly efficient process involves mechanically dispersing the warm solution in a cold immiscible oil or the like using an agitator. The rate or degree of agitation determines the size of the resultant gel beads (Delrieu and Ding 2001). Another report describes dropping agar solution at 45–50°C into an ice-cold mixture of toluene and chloroform (3:1, v/v), followed by washing with phosphate buffer and air-drying to produce further mechanical strength (Banerjee et al. 1982). Centrifugation may also be involved. In this case, the agar solution (or bacteriological medium that contains agar and is mixed with bacterial inoculum) is pipetted at \(~50°C\) into mineral oil and stirred for 6 min at room temperature. The oil is cooled to 4°C with continuous stirring for 20 min, and then the oil–agar mixture is centrifuged at 4000 rpm for 20 min to sediment the beads (http://pen2.igc.gulbenkian.pt/cftr/vr/f/bragonzi_establishment_pseudomonas_airway _chronic_infection_agar_bacteria.pdf). Preferred agar beads are complexes of a continuous phase of agar gel in a self-supporting solid or semi-solid form with a restraining polymer. Entrapped in and dispersed randomly throughout each agar bead is a water-soluble, preferably polar restraining, polymer, preferably a
quaternized cationic polymer such as polyquaternium or steardimonium hydroxyethylcellulose. Various active agents may be bound to the restraining polymer, for example, ascorbic acid, lactic acid, or papain (Delrieu and Ding 2001). It is important to note that today, agar and other beads can be purchased from various companies, which offer a wide range of activated agar and agarose beads for cosmetics, personal care, and neutraceutical, affinity chromatography, and immobilization processes. Furthermore, to support the customer with a reliable product, these companies have total control over the supply chain, from collecting the seaweed, to having a profound knowledge of the raw materials, through the final manufacture of the finest beads.

### 2.3.2 Agarose

Two groups of polysaccharides—agarose, the gelling component which is an essentially sulfate-free, neutral (non-ionic) polysaccharide (Fig. 2.1), and agaropeptin, a non-gelling ionic (charged) polysaccharide—are contained within the agar extract (Nussinovitch 1997). The basic sugar units of agarose (which consist of a linear structure with no branching) are D-galactose, 3,6-anhydro-L-galactose, and D-xylose. The percentage of agarose in agar-bearing seaweed is 50–90 (Araki 1937). Agarose gel is formed by lowering the temperature of the heated agarose to under 40°C. Its melting point is $\sim 90^\circ$C and its molecular weight $10^5$ Da. Agarose is more costly to use than agar, except in biotechnological applications as a base material for electrophoresis and as a filter for gel filtration (Osada and Kajiwara 2001). Low-melting agarose is used in a manner analogous to agar. There are many reports of using agarose as an entrapment medium, for example, for the entrapment of *Escherichia coli* and anucleate minicells produced by a mutant with defective cell division (Khachatourians et al. 1982). Special grades of agarose with lower gelling temperatures are used for the immobilization of *Catharanthus roseus* (Brodelius and Nilsson 1980). Not only bacteria are entrapped in agarose: examples include the photosynthetic alga *Chlorella vulgaris* ( unicellular strain from 5 to 10 $\mu$m in size, a known source of chlorophyll and a resource for the production of biodiesel) and the blue-green bacterium *Anacystis nidulans* (Wikstrom et al. 1982) in research designed to study the oxidative deamination of amino acids. Such deamination can be fortified by co-immobilization of *C. vulgaris and Providencia* sp. PCM 1298.

Agarose can be formed in the shape of threads, for example, in the immobilization of hepatocytes (Foxall et al. 1984; Farghali et al. 1994). The immobilized

![Fig. 2.1 Structure of an agarose polymer](http://en.wikipedia.org/wiki/File:Agarose_polymere.svg)
preparation can be looked upon as an intermediate step between the animal model (either whole or isolated perfused liver) or subcellular organelles and solubilized enzymes. Even though immobilization in beads and hollow fibers is also possible, the thread technique is simple and can be employed for many other cells, such as Sertoli cells [i.e., a “nurse” cell of the testes, part of the seminiferous tubule which is activated by follicle-stimulating hormone (FSH) and has an FSH receptor on its membranes], in just a few hours (Foxall et al. 1984; Farghali et al. 1993). The method involves the isolation of rat hepatocytes and mixing the agarose solution at 37°C with the cells at a density of 4–5 × 10^7 cells/ml. The threads are prepared by passing the agarose-cell mixture through cooled tubing, a step which can also be performed manually. The agarose solidifies and entraps the cells. The formed threads are compressed into a column for further experimentation and can be used as small research bioreactors (Gillies et al. 1993) and in nuclear magnetic resonance studies of cells (Caraceni et al. 1994). Agarose can be used not only for manufacturing beads for cell encapsulation but also as a bead coating (Jain et al. 2008). Coating is performed by rolling solid beads (agarose, collagen–agarose, or gelatin sponge–agarose combinations) in ∼5–10% agarose, contacting the rolled beads with mineral oil and then washing the oil from the beads. Such beads, containing secretory cells, can be transplanted into mammals to treat a condition caused by impaired secretory cell function (Jain et al. 2008).

2.3.3 κ-Carrageenan

Agar, furcellaran, and three types of carrageenan (κ, ι, and λ) make up the family of gums derived from red seaweed (Nussinovitch 1997). Carrageenans are linear polysaccharides composed of alternating β(1-3)- and α(1-4)-linked galactose residues. The basic repeating unit is carrabiose (a disaccharide). The (1-4)-linked residues are commonly, but not invariably, present as 3,6-anhydride. Variations in this basic structure can result from substitutions (either anionic or non-ionic) on the hydroxyl groups of the sugar residues and from the absence of a 3,6-ether linkage (Stasney 1990). Carrageenans are soluble in water at temperatures above 75°C. Sodium salts of κ- and ι-carrageenans are soluble in cold water, whereas salts with calcium and potassium exhibit varying degrees of swelling but do not dissolve completely. λ-Carrageenan is fully soluble in cold water (Nussinovitch 1997). Gel formation may be likened to crystallization or precipitation from solution. Both κ- and ι-carrageenans require heat for dissolution. After cooling and in the presence of positively charged ions (e.g., potassium or calcium), gelation occurs (Nussinovitch 1997). Gel preparation involves dispersing the κ-carrageenan plus the salt (potassium chloride) in distilled water and heating to ∼80°C, then adding the water evaporated during the heating process and slowly cooling to room temperature to induce gelation (Nussinovitch 1997). Maximum strength is achieved for 1, 2, and 3% κ-carrageenan gels at ∼1.5% potassium chloride (Nussinovitch et al. 1990). In another study dealing with the gelation of κ-carrageenan in the presence of potassium chloride (Krouwel et al. 1982), it was claimed that carrageenan gels
(beads) are superior to agar and inferior to calcium alginate. A comparison between κ-carrageenan and calcium alginate as entrapment media revealed a small advantage of the former over the latter (Grote et al. 1980).

Since gel formation is thermally reversible, immobilized carrageenan preparations are not well suited to higher temperature applications (Guiseley 1989). Moreover, the gel-inducing reagent’s concentration might influence the success of the process: in very small quantities, the preparation may not be stable, whereas in excess it might inhibit some enzyme activity (Chibata et al. 1986). In addition to extrusion through an orifice or hollow needle (dripping method), dispersion in liquid or air is possible and shapes such as cubes, beads, or membranes can be produced. After its manufacture, κ-carrageenan bead strength can be increased by treatment with chlorohydrins, diepoxides, glutaraldehyde, tannin, or polyamines (Chao et al. 1986). The concentrations used should be considered in light of the possible damage to the viability/activity of the cells or enzymes (Chibata et al. 1987). Addition of Al$^{3+}$ cations is another way in which such beads can be strengthened (Sanroman et al. 1994).

A mixture of *Saccharomyces cerevisiae* and carrageenan was pumped into a 2% (w/v) potassium chloride solution (Wang and Hettwer 1982). The immobilized preparation contained ten times more cells than the free cell suspension. The immobilized cells reached a stationary-phase plateau at a higher cell density in about twice the time taken by the free yeast cells. An increase in bead size from 3.5 to 5.5 mm had no effect on the final concentration of the cells. Further study revealed that inclusion of 5% (w/v) tricalcium phosphate (hydroxyapatite) (Fig. 2.2) crystals at sizes of up to 30 $\mu$m in the carrageenan gel can delay the dramatic drop in viable cell counts that are usually observed after 30 h (Wang and Hettwer 1982). At low pH, rapid dissolution of the crystals occurred, inducing better growth due to increased porosity, while at natural pH, better growth due to pH-value regulation was detected. When 10% (w/v) tricalcium phosphate was included, the settling velocity of the beads doubled (Wang and Hettwer 1982). Those authors also suggested separating old, less productive beads from the younger ones due to their differences in density resulting from dissolution of the included salt. An unexplored option is the questionable interaction between the tricalcium particles and the yeast within the gel as contributors to the adsorption support. Wang et al. (1982) claimed that increasing the potassium chloride concentration not only reduces cell leakage from the κ-carrageenan beads but also reduces yeast viability. κ-Carrageenan beads can be used to immobilize a very large number of species, with almost no restrictions (Mattiasson 1983). Immobilized *Saccharomyces carlsbergensis* produced ethanol continuously for over 3 months, at close to the

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**Fig. 2.2** Structure of tricalcium phosphate  
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theoretical conversion yield (Wada et al. 1980). A different approach to using \( \kappa \)-carrageenan was presented by Grote et al. (1980). They added \textit{Zymomonas mobilis} to 2\% (w/v) \( \kappa \)-carrageenan at 47–50\(^\circ\)C to coat Raschig rings in a rotating flask. The rings were packed into a column and stabilized by 0.75\% (w/v) potassium chloride in a 15\% (w/v) glucose solution. The continuous operation was found to be successful although a 30\% reduction in activity after 1 month was reported, in addition to a reduction in the void space of the column reactor (Grote et al. 1980).

Another report discussed combining locust bean gum and \( \kappa \)-carrageenan to immobilize \textit{Penicillium urticae} conidia for production of the antibiotic patulin (Fig. 2.3). Germination and growth took place at 28\(^\circ\)C for 36 h, giving better results in comparison to the free cells; this was also manifested by an extension of their half-life from 6 days (free cells) to 16 days for the entrapped preparation (Deo and Gaucher 1983). In addition to the production of antibiotics, immobilization of \textit{Trichoderma viride} in \( \kappa \)-carrageenan matrix has been used for the production of extracellular enzymes (Frein et al. 1982).

Crosslinked carrageenan beads can be used as a controlled-release delivery system. The influence of bulk carrageenan and crosslinker concentrations on bead size was studied to evaluate the mechanism of crosslinking between epichlorohydrin (a well-known crosslinker for polysaccharides) and the polysaccharide. The conditions were optimized with macroparticles (3.1 mm in diameter) for a better understanding of crosslink density and its effect on the morphology and surface topography of the bead (Keppeler et al. 2009). Low epichlorohydrin concentrations led to unstable and weak beads with uneven, cracked surfaces. The optimum crosslinker concentration, which resulted in smooth and stable gel beads, was applied to microparticles (76 \( \mu \)m in diameter). The swelling/shrinking behavior of these crosslinked microsponges in saline solution showed great potential for their application as delivery systems in food or pharmaceutical products (Keppeler et al. 2009).

2.3.4 Alginates

Alginates are perhaps the most studied and recognized component for entrapment. Alginic acid (Fig. 2.4) is a linear copolymer composed of \( \Delta \)-mannuronic acid (M) and \( \Lambda \)-guluronic acid (G) (Whistler and Kirby 1959; Hirst and Rees 1965). Regions can consist of one unit or the other, or both monomers in alternating sequence, i.e., M blocks, G blocks, or heteropolymeric MG blocks, respectively. More information on their structure can be located elsewhere (Nussinovitch 1997;
Phillips and Williams 2000). Alginate forms gels with a number of divalent cations (McDowell 1960). For food and biotechnological purposes, calcium is particularly suitable because of its non-toxicity. In poly-G segments (with chain lengths of over 20 residues), enhanced binding of calcium ions occurs and a cooperative mechanism is involved in the gelation. Crosslinking takes place via carboxyl groups by primary valences and via hydroxyl groups by secondary valences (Nussinovitch 1997). Coordinate bonds extend to two nearby hydroxyl groups of a third unit that may be in the same molecular chain, thereby retaining the macromolecule’s coiled shape (Glicksman 1969), or in another chain, resulting in the formation of a huge molecule with a three-dimensional net-like structure (Glicksman 1969). Entrapment by alginate is a mild, safe, and simple method which is generally suitable for immobilizing any type of cell (i.e., bacteria, yeast, fungi, higher plant cells, animal cells, and even embryos) while retaining maximal biocatalytic flexibility.

When Chlorella was immobilized in alginate beads (Danity et al. 1986), importance was placed on stabilizing the gel structure and minimizing growth where prolonged use was required. An increase in gel strength can be achieved by using aluminum nitrate (trivalent cations) (Rochefort et al. 1986). Many procedures have been suggested to scale up the use of alginate beads, for instance, a rotating nozzle ring which sprays the gum solution–bacterium mixture into rotating vessels containing crosslinking solution (Matulovic et al. 1986). This apparatus is capable of producing beads of the requested size in large quantities per unit time. Another large-scale approach was proposed by Rehg et al. (1986), who used a dual fluid atomizer in which sodium alginate solution droplets were sheared off the tips of hypodermic needles into calcium chloride solutions to produce beads with an average diameter of 1 mm (Rehg et al. 1986).

Bead manufacture is not restricted to spherical shapes. For example, when Z. mobilis cells were immobilized in their late exponential stage (Grote et al. 1980), a syringe was used to inject the alginate-cell suspension into the gaps between perforated plates in a column reactor, which was filled with 0.75% (w/v) calcium chloride, forming what they described as fiber-like masses. Maximal ethanol production was obtained by diluting the substrate (glucose) and production continued for 800 h (Grote et al. 1980). Alginate beads of 1 mm in diameter were used to immobilize Z. mobilis, which attained a very high cell density. The reactor was operated continuously for up to 168 h. The fermentation ability was boosted by
strengthening the beads with calcium chloride during the operation (Margaritis et al. 1981).

It is well known that other divalent cations, such as Cu\(^{2+}\) or Pb\(^{2+}\), have a greater affinity to alginate than Ca\(^{2+}\). However, toxicity limits their use in many fields. Instead of Ca\(^{2+}\), the use of Ba\(^{2+}\) for the immobilization of yeast cells (Chen and Huang 1988) was reported, and Cu\(^{2+}\) has been used for the immobilization of phenol oxidase (Palmieri et al. 1994). A differently shaped immobilization system for ethanol production by \textit{S. cerevisiae} was produced by gelling alginate with calcium ions directly in a reactor around a regular pattern of rods. When the rods were removed, a gel block with internal flow channels was formed for easy liberation of the gas produced during the fermentation (Johansen and Flink 1986). Production of beads with controllable sizes in the range of 0.5–3.0 mm by a suitable apparatus was reported by Klein and Kressdorf (1983). Haggstrom and Molin (1980) immobilized both vegetative cells and spores of \textit{Clostridium acetobutylicum} in alginate beads for the production of acetone–butanol–ethanol. Spores were activated by heating the beads to 95\(^\circ\)C, in order to induce germination, prior to washing in non-growth medium. Anaerobic conditions were achieved by flushing with nitrogen gas. Use of a continuous stirred tank fermentor to produce isopropanol–butanol–ethanol by immobilization of \textit{Clostridium beijerinckii} allowed for several rounds of reuse, although sudden and sometimes rapid losses in activity occurred after 15–27 days (Krouwel et al. 1983). A simple glass packed bed column was used for continuous and batch-recycled fermentation of 4.8\% (w/v) glucose by \textit{Lactobacillus delbrueckii} entrapped in alginate beads. After 40 h at 43\(^\circ\)C, 97\% of the theoretical yield was obtained. The operation could be run for 55 days with only a slight reduction in activity at first batch reuse (Stenroos et al. 1982).

Alginate can be used as a matrix for the immobilization of fungal hyphae. Pellets (2 mm) of the fungus \textit{T. viride} were immobilized and the activity of β-glucosidase was investigated using cellbiose and salicin as substrates (Matteau and Saddler 1982). The β-glucosidase activity had a half-life of over 1000 h at 50\(^\circ\)C, based on 340 h of continuous operation. To produce fragments of mycelium for immobilization, Livernoche et al. (1981) placed 2 cm diameter glass balls inside flasks of the fungus \textit{Coriolus versicolor} on a shaker. The resultant fragments could easily be incorporated into alginate gel beads. Royer et al. (1983) reported on hyphal outgrowth from beads that contained viable mycelial fragments. In addition to bacterial, yeast, and fungal preparations, higher plant cells have also been immobilized in alginate. Entrapped cells of \textit{Daucus carota} retained their ability to biotransform digitoxigenin and remained viable 24 days (Jones and Veliky 1981). Cell clumping is a problem which can be eliminated by allowing regrowth of viable cells entrapped in alginate beads (Morris and Fowler 1981). Alginate is a convenient medium for the encapsulation of mycobacteria (Brink and Tramper 1986) and the marine alga \textit{Dunaliella tertiolecta} (Grizeau and Navarro 1986). Shapes other than beads or threads can be used: one report describes the use of a calcium alginate film formed on a stainless steel mesh and immobilizing the bacteria \textit{Lactococcus} for both milk acidification and inoculation. The productivity of such preparations depends on the ratio between the surface area of the immobilized
biocatalyst and the bioreactor volume (Passos and Swaisgood 1993; Passos et al. 1994).

2.3.5 Chitosan

Commercial chitosan is derived from the shells of shrimp and other sea crustaceans. Chitosan is manufactured by deacetylation of the \(N\)-acyl group by heating chitin in a highly concentrated (40%) alkali solution or heating powdered chitin in fused calcium hydroxide at 180°C for 30 min. Chitosan is positively charged and soluble in acidic to neutral solution, with a charge density that depends on pH and the percentage degree of deacetylation (DA value). It binds to negatively charged surfaces. Chitosan is biocompatible and biodegradable. Purified chitosans are available for biomedical applications (Shahidi and Synowiecki 1991). Chitosan and its derivatives have been used in non-viral gene delivery for the transfection of breast cancer cells; with approximately 50% degree of trimethylation, the derivative is most efficient at gene delivery (Kean et al. 2005). Chitosan is a polycation that can be crosslinked with multivalent anions. This option can be used to prepare beads.

A chitosan–glycerol–water gel or gel-like membrane, useful as a carrier for medications to be applied to wounds, was prepared by dissolving chitosan in an acid–water–glycerol solution which when neutralized forms a gel upon standing (Jackson 1987). A typical simple example of gel formation was provided with chitosan tripolyphosphate and chitosan polyphosphate gel beads (Mi et al. 1999). Chitosan gel beads could also be prepared in an amino acid solution at about pH 9, despite the requirement for a pH above 12 for gelation in water (Kofuji et al. 1999). pH-sensitive hydrogels were also synthesized (Qu et al. 1999a, b) by grafting \(D\),\(L\)-lactic acid onto the amino groups in chitosan without catalyst. Enzymatic reactions might also lead to gels (Chen et al. 2003). Glutaraldehyde was used as a crosslinking agent for chitosan. A semi-interpenetrating network was synthesized with poly(ethylene oxide) and chitosan and crosslinked with glyoxal (Khalid et al. 1999). Advances in the field of chitosan gelation promote biomedical applications that use microgel or nanogel particles for drug delivery (Oh et al. 2008). Uses include, for example, mixing \textit{E. coli} cells into a chitosan acetate solution. The solution was dropped and left in 1.5% (w/v) sodium polyphosphate at pH 5.5 for 30 min. It was then transferred to polyphosphate at pH 8.5 for shrinking inducement. This preparation was used to study the tryptophan synthetase activity of the bacterium (Vorlop and Klein 1981). Of course drying can result in further strengthening of the beads. When dry, these beads do not decompose in the presence of phosphates as alginates do. Kluge et al. (1982) extended the afore-described work to the fungus \textit{Pleurotus ostreatus}. Pellets composed of mycelium disrupted in dilute sodium chloride were washed and immobilized in chitosan. Polyphosphate was the best crosslinker for enzyme retention. Stocklein et al. (1983) demonstrated the efficiency of working with chitosan for conversion of phenylalanine to tyrosine by a \textit{Pseudomonas} sp.
2.3.6 Cellulose

Cellulose is the most plentiful organic substance in nature, making up about one-third of the world’s vegetative material (Zecher and Van Coillie 1992). Cellulose content in wood and cotton is \(~40–50\%\) and \(85–97\%\), respectively (Ott 1946; Glicksman 1969; Whistler 1973). Cellulose (Fig. 2.5) is a linear polymer of \(\text{D-}\)glucose monomers joined by \(\text{D-}\beta(1,4)\) linkages, built from repeating units of cellobiose. The nature and structure of these molecules facilitate the formation of crystalline regions with consequent rigidity and strength (Ward and Seib 1970; Whistler and Zysk 1978). The degree of polymerization (DP) of cellulose depends on its origin (Krassig 1985; Zecher and Van Coillie 1992). The polymer has a maximum of three degrees of substitution (Greminger and Krumel 1980; Zecher and Van Coillie 1992). Products with a wide range of functional properties can be created by controlling the degree and type of substitution (Whistler and Zysk 1978). Extensive intra- and intermolecular hydrogen-bonded crystalline domains cause cellulose to be insoluble in water. Manufacture of water-soluble cellulose derivatives starts with a preformed polymer backbone of either wood or cotton cellulose (Greminger and Krumel 1980). Cellulose can be converted to a soluble compound via its derivatization and disruption of hydrogen bonds (Zecher and Van Coillie 1992). Cellulose derivatives are prepared by reacting alkali cellulose with either methyl chloride to form methylcellulose (MC), propylene oxide to form hydroxypropylcellulose (HPC), or sodium chloroacetate to form sodium carboxymethylcellulose (CMC). In the latter case a side reaction, the formation of sodium glycolate, also occurs (Stelzer and Klug 1980). Mixed derivatives such as methyl hydroxypropylcellulose (HPMC) can be formed by combining two or more of these reagents. Of the many possible derivatives investigated and manufactured, CMC, MC, HPMC, and HPC are utilized in the food industry in addition to modified forms of cellulose, which have been found to have useful functional hydrocolloidal properties and significance in several food applications. CMC is the most important cellulose-derived hydrocolloid for viscosity-forming applications, and it is used for its ability to react with charged molecules within specific pH ranges (Ganz 1966; Hercules Inc. 1978; Stelzer and Klug 1980). HPC, a non-ionic cellulose ether, is soluble in water below \(40^\circ\text{C}\) and in polar organic solvents such as methanol, ethanol, and propylene glycol (Butler and Klug 1980).

Microcrystalline cellulose has thickening and water-absorptive properties. MC and HPMC are soluble in cold water but insoluble in hot water. Upon heating such
a solution, gel structures can be formed at gelation temperatures ranging from 50 to 90°C (Dow Chemical Co. 1974; Greminger and Krumel 1980; Aqualon Co. 1989; Zecher and Van Coillie 1992). Solution viscosity decreases with increasing temperature to the thermal gel point, then rises sharply to its flocculation temperature (Zecher and Van Coillie 1992). Gels formed as a result of phase separation are susceptible to shear thinning. If the temperature is lowered, the original solution is restored. The thermal gel point is influenced by the type and degree of substitution. Flocculation temperature is influenced by the concentration of salts (decrease) and alcohols (increase) (Zecher and Van Coillie 1992). Microcrystalline cellulose gels are highly thixotropic and have a finite yield value at low concentrations. The formation of a network by solid-particle linkage is responsible for the produced yield value and elasticity. If shear is applied, the gel shears and thins. Resting allows the gel to reform a network. The addition of CMC reduces the thixotropic character of the gels and results in reduced yield values. Gum addition changes the rheological behavior of microcrystalline cellulose gels. Temperature has a small effect on the viscosity of microcrystalline cellulose dispersions (Nussinovitch 1997).

Cellulose is not soluble in water but dissolves in organic liquids (Tampion and Tampion 1987). Cellulose beads were produced by using N-ethyl pyridinium chloride and dimethyl formamide for polymer dissolution. *Actinoplanes missouriensis* was added, followed by dropping into water to produce the entrapping beads. Glutaraldehyde was used as a crosslinking agent to prevent cell leakage. Glucose isomerase retained 40–60% of its original activity before the immobilization and preparation, with a half-life of 45 days (Linko et al. 1977). Cellulose acetate was also used, but without much additional benefit (Sakimae and Onishi 1981). Cellulose can be used to create solid fibers for entrapment purposes. Wet spinning of fibers can be achieved with standard equipment (Dinelli 1972). Fibers produced from cellulose acetate were successful in entrapping *E. coli*, which exhibited 80% penicillin acylase activity when compared to free cells (Dinelli 1972). If fibers were heavily loaded, the bacteria showed reduced activity. By reducing solvent damage, better results may be achieved. Another approach, using cellulose acetate in acetone mixed with *E. coli* for the impregnation of cotton cloth, was reported. Optimal aspartase activity can be obtained by controlling porosity and degree of cell loading (Joshi and Yamazaki 1986).

### 2.3.7 Proteins

#### 2.3.7.1 Collagen

Collagen is a natural animal protein. It is derived from connective tissues such as skin and cartilage (Tampion and Tampion 1987). It is manufactured by using organic solvents to extract the collagen’s mixed materials, followed by a water wash and extraction in a dilute salt. The process involves using acid or alkali, which is then removed by enzyme interactions leaving the collagen as the non-solubilized material (Osada and Kajiwara 2001). Another procedure involves degrading fresh skin
extract in 0.06 M citric acid buffer (pH 4). After dialysis, extraction is performed in 0.5 M hydrogen phosphate-2-sodium. The precipitate obtained in the dialysis is again extracted using 0.2 M citric acid buffer (pH 3.8). Dialysis of this extract produces a recycled collagen (Osada and Kajiwara 2001). The hydrogel produced from collagen is a physical gel. It is formed when the concentration of the collagen solution is increased. When boiled for a long time in water, dilute acid or dilute alkali, it changes into a gelatin made of protein derivative (Osada and Kajiwara 2001). Collagen is widely used as a support for enzymes. It is often cast into a membrane form and stabilized with glutaraldehyde (Tampion and Tampion 1987). The first coagulation with the immobilized cells is probably due to hydrogen bonding forces and possibly an adsorption process in which the lysine residues of the collagen participate (Cheetham 1980). However, for stabilization, the dominant step is the extensive covalent bonding by glutaraldehyde. This step must be temporally controlled since excessive exposure can damage cell function. Successful entrapment of eight bacteria, *Aspergillus niger*, mammalian erythrocytes, and chloroplasts in a patented collagen system was reported by Vieth and Venkastsbramanian (1979). The method is based on casting the membrane from tanned collagen and winding with inert plastic spacer material. The invention has not raised a great deal of interest due to the extensive use of gelatin.

Spherical microcarriers can be used for cell culturing since they provide large surface areas for cell growth. They can be manufactured from polysaccharides, gelatin, or collagen (Cahn 1990; Altankov et al. 1991). The matrix formed by collagen has the unique characteristic of being macroporous and contains a fibrous microstructure appropriate for cell ingrowth (Langer and Vacanti 1993). Collagen microcarriers are generally prepared from a suspension of crude collagen extract. Spherical beads can be structured by discharging a suspension of collagen fibers into liquid nitrogen, followed by dehydration and crosslinking of the gel beads with formaldehyde or glutaraldehyde vapors (Yannas and Kirk 1984; Dean et al. 1989). These processes employ harsh conditions and do not appear to be suitable for the entrapment of cells in situ for subsequent cell culturing (Tsai et al. 1998). Alginate beads can be formed under mild conditions by contacting with calcium ions (Nussinovitch 1997), and this gelling property was made use of by preparing spherical gel beads of collagen/alginate: droplets of a mixture containing collagen (1.07–1.90 mg/ml) and alginate (1.2–1.5% w/v) were discharged into a 1.5% (w/v) calcium chloride solution at 4°C (Tsai et al. 1998). Collagen in the gel beads was reconstituted by raising the temperature to 37°C after alginate had been liquefied by citrate. Scanning electron microscopy of the beads revealed the characteristic fibrous structure of collagen. To demonstrate the application of this new technique in cell culture, GH3 rat pituitary tumor cells were entrapped and cultured in these gel beads (Tsai et al. 1998).

**2.3.7.2 Gelatin**

Gelatin is a natural organic protein that can be used to create physical hydrogels. The gelatin is manufactured by refining processed collagen. It has a molecular weight
of 100,000–250,000 (Osada and Kajiwara 2001). Gelatin is sold as a whitish to light yellow, flavorless, and odorless powder. It does not dissolve in cold water, but swells to between 5 and 10 times its size. It dissolves in warm water and becomes a homogeneous sol. Upon cooling, an elastic gel is formed. After dissolution, a clear solution is achieved, and the created gel is clear or semi-clear. An increase in concentration makes the gel stronger (Osada and Kajiwara 2001). Gelatin is used as a thickener and gelling agent for photography, cosmetics, and the food industry (Michon et al. 1997). It is an important wall material in the production of pharmaceutical microcapsules that enclose an active agent (Vandelli et al. 2001). Other uses for bacterial encapsulation, adhesives, and emulsion films are common.

The use of gelatin (10% w/v) was reported as a solution for the suspension of cells of *Arthrobacter* strain X-4 which were pre-adapted to xanthine before the immobilization, since the preparation was designed to generate high xanthine oxidase activity. Crosslinking by glutaraldehyde, freeze-drying, and then milling produced a powder with particles of 0.5, 0.7, and 1.0 mm nominal diameter (Tramper et al. 1979). Gelatin was reported as a suitable immobilization medium for *S. cerevisiae*, which was capable of sustaining more stable invertase activity than free cells, although the fermentation of glucose or sucrose was not possible (Parascandola and Scardi 1981). Gelatin and its copolymers with agarose and alginate, all crosslinked with glutaraldehyde, were used for the immobilization of *C. roseus*. The glutaraldehyde had an adverse effect on cell growth, respiration, and selectivity of the permeable membrane (Brodelius and Nilsson 1980).

Control over microcapsule size and size distribution has several important implications for controlled-release drug delivery (Berkland et al. 2001). More than a few methodologies for microcapsule preparation exist, including precipitation, spraying, phase separation, and/or emulsion techniques. Microchannel emulsification is a novel technique for preparing water-in-oil and oil-in-water emulsions (Kawakatsu et al. 2001; Sugiura et al. 2001). The microchannel plate has uniform microsized channels fabricated on a single-crystal silicon substrate using photolithographic and etching processes. Emulsions with a relative standard deviation of approximately 5% have been effectively prepared by applying this method (Iwamoto et al. 2002). Gelatin microbeads with a narrow size distribution were prepared by microchannel emulsification. An average particle diameter of 40.7 μm was prepared using this technique. Gelatin microbeads dispersed in isooctane after overnight gelation had smooth surfaces, with an average particle diameter and relative standard deviation of 31.6 μm and 7.3%, respectively (Iwamoto et al. 2002). The dried gelatin microbeads could be thoroughly resuspended in isooctane after overnight gelation had smooth surfaces, with an average particle diameter of 15.6 μm and a relative standard deviation of 5.9%. Such a procedure is promising for the creation of monodispersed microbeads (Iwamoto et al. 2002). It should be emphasized that other proteins can be used for immobilization, and the protein need not have the ability to form a gel on its own. However, in such cases, glutaraldehyde should be used as the crosslinking agent.
2.3.7.3 Hen Egg White

Egg white is the common term for the clear liquid (albumen) in the egg. It consists mainly of $\sim 15\%$ proteins dissolved in water. Egg white constitutes about two-thirds of the total egg’s weight, excluding the shell, with water accounting for ca. 90$\%$ of this weight. The remaining weight of the egg white comes from proteins, trace minerals, fatty materials, vitamins, and glucose (McGee 2004). Egg white contains approximately 40 different proteins, among them ovalbumin, ovotransferrin, ovomucoid, ovoglobulin G2, ovoglobulin G3, ovomucin, lysozyme, ovoinhibitor, ovoglycoprotein, flavoprotein, ovomacroglobulin, avidin, and cystatin. At 62–65°C, ovotransferrin—the most heat-sensitive protein in the egg white—starts to denature and the egg white begins to set. At 80°C, the main protein ovalbumin denatures. Denaturation and rearrangement at 80°C causes the egg white to firm up (McGee 2004).

Hen egg white can be used for entrapment after being crosslinked with glutaraldehyde. Free cells of Calderiella acidophila—a thermoacidophilic Archaebacterium capable of growth at 87°C at pH 3.0—were mixed with liquid egg white at 0°C and glutaraldehyde in phosphate buffer was added. This was followed by vacuum rotary evaporation at 60°C, grinding, and washing (De Rosa et al. 1981). The studied activity of $\beta$-galactosidase was $\sim 30$ times greater than that of comparable free cells. The matrix can be rendered magnetic by the addition of 0.3–0.7 $\mu$m diameter magnetite particles (De Rosa et al. 1981). Egg white contains large quantities of lysozyme, which is capable of lysing many species of bacteria. This ability was utilized by D’Souza et al. (1983) to continuously self-sterilize an entrapment matrix with E. coli as the immobilized cells and Micrococcus lysodeikticus as the contaminant. During the 7 days of the study, lysis of about 30$\%$ of the cells was observed. The use of fresh egg whites instead of purified lysozyme was reported by Mattiasson (1979) to be less expensive. A thorough consideration of the glutaraldehyde concentration and conditions used is crucial to the efficiency of the process (D’Souza et al. 1983).

2.3.8 Synthetic Polymers

2.3.8.1 Polyacrylamide

The polymer polyacrylamide is manufactured from acrylamide subunits that can be readily crosslinked. Acrylamide must be handled very carefully to avoid toxic exposure. Polyacrylamide (Fig. 2.6) is not toxic; nevertheless, non-polymerized acrylamide can be present in the polymerized acrylamide. It is therefore recommended that polyacrylamide be handled with caution. In its crosslinked form, it is a soft gel that can be utilized in polyacrylamide gel electrophoresis and in manufacturing soft contact lenses. In its straight-chain form, it is also used as a suspension agent and thickener. Polyacrylamide gels are cast into slabs or blocks and mechanically converted into small particles after polymerization (Tampion and Tampion 1987).
The polymerization conditions and their influence on the retention of \(\beta\)-glucosidase in an immobilized *Alcaligenes faecalis* preparation were studied by Wheatley and Phillips (1983). Highest retention of activity was observed under reaction conditions in which the maximal temperature did not rise above 40\(^\circ\)C and its rise and fall were most rapid (Wheatley and Phillips 1983). Complete loss of viability of polyacrylamide-entrapped plant cells was reported by Brodelius and Nilsson (1980). Cells of *Corynebacterium dismutants* entrapped in polyacrylamide showed improved thermostability. Synthesis of alanine was highest in comparison with \(\kappa\)-carrageenans or absorption to DEAE (DE52) cellulose (Sarkar and Mayaudon 1983). Advantages of polyacrylamide over polymethacrylamide or polyepoxide for the entrapment of *E. coli* in beads for the production of tryptophan were reported by Bang et al. (1983). Suzuki and Karube (1979) studied the production of penicillin by *Penicillium chrysogenum* after its immobilization in polyacrylamide and alginate. Alginate exhibited poor mechanical properties while in polyacrylamide, the cells showed lower initial activity. To overcome the cell damage caused by the entrapment, organisms can be developed that are better suited to the immobilization than species taken directly from conventional production systems (Tampion and Tampion 1987).

2.3.8.2 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol (PVA) is an odorless and non-toxic water-soluble synthetic polymer. PVA has a melting point of 230\(^\circ\)C and 180–190\(^\circ\)C for the fully hydrolyzed and partially hydrolyzed grades, respectively. It decomposes rapidly above 200\(^\circ\)C as it can undergo pyrolysis at high temperatures. It has excellent film-forming, emulsifying, and adhesive properties. Phosphorylated PVA can be used for the immobilization of bacterial and yeast cells or activated sludge due to its non-toxicity and low cost (Arigo et al. 1987; Hashimoto and Furukawa 1987; Shindo and Kamimura 1990; Myoga et al. 1991; Wu and Wisecarver 1992). Spherical PVA beads are produced by crosslinking with saturated boric acid solution (for a short time to eliminate damage to the immobilized microorganisms), followed by esterification of the PVA with phosphate for further solidification. A pH range of 4–6 is suitable for both bead formation and strength. Stability and strength are better maintained at higher pHs. In the case of PVA-immobilized denitrifying sludge, the beads were observed to be stable over long periods (Chen and Lin 1994; Lin and Chen 1995). To change the poor gas permeability of PVA gels, sodium alginate is added to the PVA solution and the saturated boric solution includes calcium chloride for further crosslinking of the alginate. Later, this is removed by using the phosphate
solution, thus a more porous structure and phosphorylation are achieved simultaneously (Chen and Lin 1994; Lin and Chen 1995). The water-purification capacity of a polyvinyl(alcohol) (PVA) gel bead filtration system using photosynthetic bacteria was studied (Jeong et al. 2009). Long-term goldfish-rearing experiments were conducted using four different types of aquarium systems. Prominent decomposition of organic matter in the aquarium tank containing the PVA system, as well as less turbid aquarium water and more active goldfish, was observed. In addition, use of the PVA gel beads resulted in almost complete denitrification, even after 6 months of goldfish rearing. The results of this study indicate that this immobilized photosynthetic bacterial system has the potential for use as a component in circulating filtration systems (Jeong et al. 2009).

### 2.3.8.3 Other Synthetic Polymers

Other synthetic polymers can be used for cell entrapment, including copoly(styrene-maleic acid), polyethylene glycol (PEG) methacrylate, methoxypolyethylene glycol methacrylate (MPEGMA), PEG dimethacrylate, polyisocyanates, and polyurethane. Many researchers use polyacrylamide as their first choice of synthetic polymer due to the poor performance of many of the others (Tampion and Tampion 1987). Klein et al. (1979) compared the potential ability of many synthetic polymers to form an ionic network. MPEGMA polymerization by radiation was studied (Fujimura and Kaetsu 1982). Cell damage from this process can be reduced by using $0.5 \times 10^4 \text{ rad/h}$, $\sim 100$ times lower than the initial radiation dose. Other beneficial actions included lowering the temperatures to $-24^\circ C$ and $0^\circ C$ and using the monomer of MPEGMA, which is somewhat less toxic than that of 2-hydroxyethyl methacrylate (HEMA) (Fujimura and Kaetsu 1982). Entrapment of enzymes and cells in poly(HEMA) has been reported in many instances, a few examples being the entrapment of phosphatase (Cantarella et al. 1988), $\beta$-glucosidase (Alfani et al. 1987), $\beta$-fructofuranosidase, glucose oxidase, and cells of *S. cerevisiae* (Cantarella et al. 1989).

In contrast to conventional entrapping biopolymers (e.g., agar, alginate, and carrageenan), polyurethane gels demonstrate increased mechanical stability (Fukui et al. 1987); however, the isocyanate prepolymer is toxic (Klein and Wagner 1983). Blocking the isocyanates (by reaction at room temperature of the prepolymer with NaHSO$_3$) to form the polycarbamoyl sulfonate (PCS) propolymer could help solve this problem (Vorlop et al. 1992). The relationship between PCS gelation time and pH was studied (Muscat et al. 1996). At pH 8.5 and room temperature, gelation took on the order of seconds, while at pH < 5.5, it took up to 10 h. Diffusion coefficient values for a PCS hydrogel of $0.50–1.45 \times 10^{-5} \text{ cm}^2/\text{s}$ for the substances glucose, ethanol, nitrate, and nitrite ion at $25^\circ C$ were observed. These values were in the same range as those observed for calcium alginate (Daynes 1920). Substances with molecular weight $>67,000$ did not diffuse through the PCS hydrogel (Muscat et al. 1995). Entrapping nitrifying bacteria (*Paracoccus denitrificans*) in PCS versus calcium alginate beads showed almost the same activity (Muscat et al. 1995). Another study (Wilke et al. 1994) reported that during the immobilization,
reversible deactivation of \textit{P. denitrificans} occurs. \textit{S. cerevisiae} entrapped by PCS versus calcium alginate beads was reported to have similar activities directly after the immobilization. PCS hydrogel membranes were suitable for cell and enzyme immobilization (Kotte et al. 1995; Muscat et al. 1995).

Polyisocyanates have also been reported for the immobilization of \textit{E. coli}, with the option of using either gels or foam beads. The foamed material is produced in stirred liquid paraffin. The foam’s volume increases during the production process due to the inclusion of carbon dioxide bubbles. Low-density rigid foams are favored (Klein and Kluge 1981). Polyurethane foam was used to immobilize \textit{C. roseus} cells. Isocitrate dehydrogenase and cathenamine reductase activity was detected in the foamed preparation by dimethyl sulfoxide (Felix and Mosbach 1982).

### 2.4 Two-Step Methods

Two-step methods may be used if they provide the manufacturer with improved processes or products or the possibility of overcoming evident problems. A procedure to create a more porous structure with desirable elastic behavior and mechanical stability throughout handling was reported by Klein and Eng (1979). They mixed epoxy resin reagent and curing agent with \textit{E. coli} cells in aqueous medium. Alginate was then mixed in, followed by the traditional dropping into a calcium chloride bath to produce alginate beads. Following polycondensation of the resin, the beads were air-dried and the alginate dissolved in a phosphate buffer to produce swelling of the beads and a porous product. These moieties were advantageous over those obtained by single-step production with epoxy resin followed by grinding. The latter method did not sustain yeast cell viability and therefore, the improved two-step method was proposed (Klein and Kressdorf 1982). The selected types of resin and curing agent were those with low contents of low molecular weight components. Their 15 min reaction was followed by the addition of the alginate–yeast solution. The rest of the procedure was similar to that described previously. The results demonstrated an alcohol production activity of 21% of the original free cells and an option for more stable beads that can be successfully reactivated (Klein and Kressdorf 1982).

Three methods of alginate stabilization were proposed by Birnbaum et al. (1981). Alginate beads were treated with polyethyleneimine-HCl by infiltration of their structure for 24 h, and 1% (v/v) glutaraldehyde was applied at pH 7.0 for 1 min, followed by a wash in water. Although the glutaraldehyde was toxic to the immobilized yeast (\textit{S. cerevisiae}) and ethanol production was reduced, the activity still remained considerable and the method was less costly than other proposed processes. A second method suggested the activation of alginate with a mixture of \textit{N}-hydroxysuccinimide and 1-ethyl-3-(3-dimethyl-amino-propyl)-carbodiimide for 1 h. The cells were added to this mixture and beads were produced by the traditional dropping of solution into a calcium salt bath (Birnbaum et al. 1981). The third suggested procedure involved the addition of sodium meta-periodate to half of the alginate for 1 h. The cells were mixed with the other, untreated half and then the two halves were mixed together and beads formed. Curing for 1 h was followed by
treatment with polyethyleneimine-HCl and a water wash. The mechanisms underlying these methods are not fully known, but they may involve some ionic or covalent binding of the cells themselves or, in the second and third procedure, direct coupling to the alginate (Birnbaum et al. 1981).

Alginate (2% w/v) and gelatin (20% w/v) were mixed with yeast cells (S. cerevisiae and Saccharomyces uvarum) before dropping into a calcium chloride solution. After formation of the beads, phosphate buffer was used to leach out the alginate and the gelatin beads were stabilized with glutaraldehyde. Since the latter is toxic, maintaining its concentration under 0.015 M results in enhancing fermentation rates (SivaRaman et al. 1982). Another method to enhance the mechanical properties of κ-carrageenan beads was reported by Chibata (1979). They were treated with hexamethylenediamine (HMDA) and glutaraldehyde, both at 85 mM. As a result, the half-life of E. coli aspartase activity was extended to 680 days. Other hardening agents included glutaraldehyde alone or persimmon tannin. Higher productivity (15-fold) was reported for HMDA + glutaraldehyde-hardened κ-carrageenan beads in comparison to polyacrylamide (Chibata 1979).

2.5 Cell Immobilization by Electrostatic Method

Electrical fields can be used to produce micron diameter beads (Goosen et al. 1986; Bugarski et al. 1993, 1994a, b; Goosen 1994; Sun 1994). The degree of applied electrical potential helps control bead size. During extrusion of a fluid through a positively charged stainless steel needle using a syringe pump, and upon electrification at a voltage <30 kV with low current (<0.4 mA), a charge is induced on the liquid’s surface and mutual charge repulsion results in an outwardly directed force, producing a liquid spray. The drops are of various sizes and are emitted over a wide range of angles. If low-viscosity alginate is sprayed into a calcium chloride solution (contained in a grounded plate), the spontaneous crosslinking reaction produces alginate beads. Controlling liquid pressure, applied voltage, electrode spacing, and charge polarity is important for the production of regular and periodic spraying (Bugarski et al. 1993, 1994a, b; Sun 1994).

References


References


References


