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INDUSTRIAL SCALE ENCAPSULATION OF CELLS USING EMULSIFICATION/DISPERSION TECHNOLOGIES

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1. Introduction

Industrial scale immobilization of cells *via* entrapment within ionic or thermal gelling polymer systems, or *via* microencapsulation within semi-permeable membranes, necessitates the use of emulsification/dispersion steps to generate small and often micron-sized droplets, on a large scale. Individual droplets within the emulsion contain the biocatalyst, and lead to the final gelled microsphere or membrane-coated microcapsule. Widely used laboratory techniques involve the formulation of droplets and thus beads *via* individual droplet extrusion technologies, which are well suited to the small scale formulation of monodisperse beads and capsules, often in a millimetre size range. These techniques are poorly suited to very large scale formulation, particularly when droplets or microbeads are preferred, with diameters often extending to well under 500 μm [1]. Smaller diameter preparations are often desired to improve mass transfer characteristics, but droplet extrusion techniques become increasingly limited in productivity with decreasing bead size, particularly when using viscous polymer solutions.

Emulsion/dispersion systems overcome the limitations of single droplet extrusion methodologies, as they are generally not limited by scale, and control of the resulting microbead diameter is possible through selection of appropriate dispersion devices (vessels, baffles, impellers) and operating conditions (mixing rates, phase ratios, surfactants), leading to a wide range of possible bead diameters, extending from a few microns, up to within the millimetre size range. Scale is limitless, as vessel size becomes the design criterion.

There are five challenges associated with emulsion/dispersion systems:

- Challenge 1 involves the need for a non-toxic, biocompatible, non-aqueous dispersion phase, into which the gel sol is dispersed, droplets formed and

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gelation or polymerization initiated. Vegetable, mineral or silicone oils are commonly used.

- Challenge 2 requires that gelled beads, microspheres or microcapsules be separated easily and cleanly from the oil phase. The smaller the bead, the greater are the separation problems. As gelation polymers are generally hydrophilic, simple phase partitioning may be an easy and quick solution; however depending on the polymer, there may be some residual oil coating the microbeads or microcapsules, which is normally removed through washing with mild surfactant.
- Challenge 3 involves a better control over the microsphere size distributions, associated with emulsion/dispersion systems. While the operator has considerable flexibility over the control of mean bead diameter covering a considerable size range, thus far it has not been possible to produce monodisperse preparations of microspheres using emulsion technology. For some applications, this can be problematic, as a classification of the microspheres can lead to a serious reduction in yield, depending on the extent of the size dispersion.
- Challenge 4 is associated with the economic and environmental need to reuse, or recycle the oil phase used in the process. Disposal of large quantities of oil is costly, difficult and environmentally unacceptable.
- Challenge 5 relates to the ability to operate biocatalyst encapsulation operations as a continuous process. In fact, dispersion systems are readily adaptable to continuous operations, thus for the same level of productivity in comparison to a batch process, smaller scale of process equipment is required, and better uniformity in product quality possible.

The examples provided in this chapter, serve as case studies to illustrate several emulsion/dispersion systems used for cell encapsulation. The case studies are presented in the following order:

- simple thermal gelation
- thermal/ionotropic gelation
- continuous processing involving thermal/ionotropic gelation
- ionotropic gelation involving physicochemical reaction with membrane coating
- microencapsulation *via* interfacial cross-linking
- microencapsulation *via* interfacial polymerization reaction

All examples involve emulsion/dispersion systems. Detailed protocols are available in the references cited.

2. Thermal gelation of agarose microspheres containing recombinant *Saccharomyces cerevisiae* in bioconversion of fumarate to L-malate

Temperature setting gels are important for many applications in the food industry, because of their viscosifying and gel setting properties. Gel forming polymers are often heated to facilitate dissolution, then form a gel upon cooling. Most are familiar with the use of semi-solid agar medium, in the preparation of petri dishes for cell culture. Gel

beads can be formed using a similar principle, however because of the need to form small diameter spheres, it is necessary to either extrude the gel forming solution dropwise into a chilled bath, or alternatively, form an emulsion/dispersion of the gel forming sol, into warm vegetable oil, then initiate gelation by dropping the temperature of the emulsion. The faster the temperature drop to below the gel point, the more likely that the resulting gel beads are discrete and spherical. In addition, through careful control of the conditions under which the emulsion is prepared, it is possible to design microspheres with fixed mean diameter, and narrow size distribution. The biocatalyst is added to the gel forming solution prior to emulsification, and it is obvious that the cells would need to tolerate the elevated gel temperature until such time as the gel is set.

One particular encapsulation application [2] involves the emulsion/dispersion of agarose sol containing cells, into corn oil at a ratio of 1:4. As agarose forms a gel below 40°C, the emulsion was held at 40°C in a temperature regulated, water-jacketed mixing vessel, and gelation initiated by rapid cooling of the emulsion. After 15 min, gelled microspheres were partitioned into water, separated by filtration, washed to remove traces of oil and assayed. Agarose microspheres readily partitioned in the aqueous phase with essentially 100% recovery. There was no residual oil remaining with the microspheres, confirmed microscopically, and through a complete absence of oil film during subsequent repeated assay of the encapsulated cell activity. It was estimated that the microspheres contained 10% by weight cells. The diameter of the microspheres was controlled by varying the mixing rate during emulsification. Wide size distributions resulted, with standard deviations representing approximately 50% of the microsphere mean diameter. Recycle of the corn oil was not tested, but would be readily achieved as the oil was not modified during the encapsulation process.

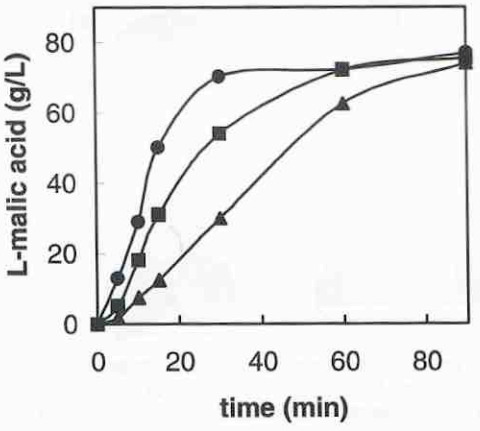

Figure 1. Bioconversion of fumaric to L-malic acid with an agarose-immobilized transformant yeast in 193 μm (●), 871 μm (■) and 2.4 mm (▲) diameter beads. Redrawn from Figure 5 in Reference [2].

The bioconversion of fumaric to L-malic acid was demonstrated with a recombinant *Saccharomyces cerevisiae* encapsulated in the agarose microspheres. Three bead mean

diameters were tested, 193 μm, 871 μm and 2400 μm. The rate of bioconversion increased with decreasing bead diameter as shown in Figure 1, explained by reduced mass transfer limitations. Similar bioconversion rates were observed between cells encapsulated within the smallest diameter microspheres (193 μm), to that of free cells. Based on regression analysis, it was shown that activities similar to that of free cells could be obtained with microsphere diameters less than approximately 300 μm. Stable activities over a 48 h period were observed with the encapsulated cells.

3. Thermal/ionotropic gelation in formulation of gellan gum microbeads containing gasoline-degrading microorganisms

Gellan gum is an interesting polymer for encapsulation, even though it has not received the attention of more widely available polysaccharides such as alginate and carrageenan. It is known to have superior rheological properties [3] leading to excellent mechanical and thermal stability [4], yet is probably more expensive due to it being produced *via* a fermentation process. Also in contrast to other ion-sensitive gelling polysaccharides (e.g. alginate), gellan-ion interactions are non-specific and gels can be formed with various cations [5]. The formation of gellan gum microspheres requires emulsion/dispersion, but in contrast to the case of agarose described above, gellan gum is a thermally gelling polysaccharide, but in addition requires cationic interactions. The following procedure is outlined to illustrate the principle [6].

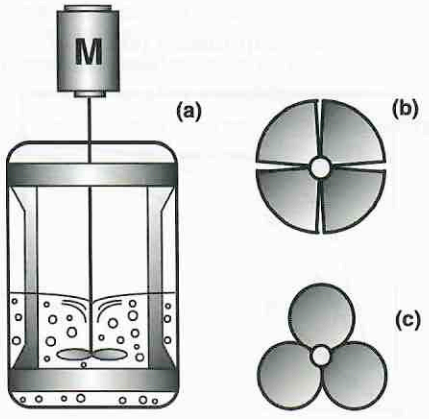

Figure 2. Design of baffled vessel and impellers used to form gellan gum microspheres by emulsification/internal gelation. Vessel is 10 cm wide, and quarter-circular paddle, and three-blade paddles are 5 cm diameter. (Figure courtesy of Dr. Peyman Moslemy)

The configuration of the baffled mixing vessel used to generate a gellan gum-canola oil emulsion under sterile and aseptic conditions is illustrated in Figure 2. The pre-gel solution consisted of 0.75% gellan gum in a solution of $CaCl_2$. A mixed bacterial culture was added to the sol at 45°C, and emulsified in warm canola oil facilitated with Span 80, an oil soluble surfactant. The temperature of the dispersion was quickly dropped to 15°C to initiate gelation, and the microbeads then partitioned into water, and washed

with sterile 0.1% Tween 80. Cell mass loading ranged up to 20 g_{cells} L^{-1} sol, and mean diameter and size distribution were determined as a function of emulsion mixing rate, gellan sol volume fraction, emulsifier concentration and emulsification time.

Microbead mean diameter was predetermined, within the range of 12 μm to 135 μm to enable hydraulic distribution through a granular matrix. Control of mean diameter was exercised by varying the mixing rate, emulsifier concentration and volume fraction of gellan gum in oil dispersion. Microbeads were spherical, and dispersions were unimodal and followed a log-normal distribution. One particular batch (0.75% gellan, 0.06% $CaCl_2$, 8 g_{cells} L^{-1} sol, 0.1% emulsifier, gellan gum/oil volume fraction of 0.143, emulsification time 10 min, and stirring rate of 4500 rpm) showed a mean diameter of 21 μm and a distribution size range from 16 to 34 μm (span of 0.2). Mean diameter decreased with increasing mixing rate and emulsifier concentration and as an ascending function of gellan gum volume fraction in the emulsion.

A pre-acclimated, mixed bacterial culture (MBC) was encapsulated for the bioaugmentation of a gasoline-contaminated aquifer. The carrier microbeads were to be sufficiently small to be transported through a granular soil matrix, using a formulation methodology that would be readily scaled to that required for environmental application. The objective of immobilization was to protect cells from biotic and abiotic stresses, including the inhibitory effects of the petroleum substrate. It was also desired to improve on the distribution of microorganisms through the subsurface environment, as free microorganisms tend to adhere to soil grains [7]

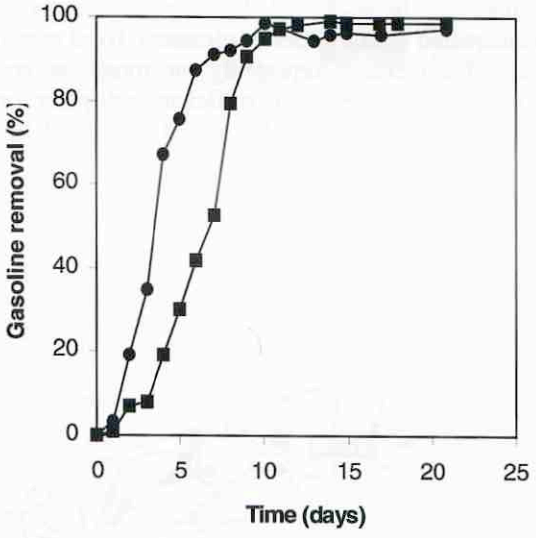

Figure 3. Biodegradation of gasoline by gellan gum encapsulated cells (●), and by free (■) mixed bacterial culture. The mean diameter of the microbeads was 23 μm. Redrawn from Figure 7 in Reference [6].

Encapsulated cells removed over 98% of gasoline hydrocarbons (Figure 3), and a reduced lag in comparison to free cells was explained by enhanced protection of the

cells by the encapsulating matrix. In this application, the oil phase following emulsion gelation could potentially be recycled in the process, as it had not been modified in any way. The fate of the surfactant added to facilitate emulsification is uncertain, but since it is oil soluble, it was assumed to have remained with the oil phase, thus additional surfactant would not need to be added for the second immobilization cycle.

4. Continuous process for the thermal gelation of κ-carrageenan microspheres containing brewer's yeast, using static mixer technology

κ-Carrageenan is a thermal setting gel, with a gel point temperature, dependent on the concentration of both the polymer solution, and that of the countervailing cation - KCl in this case. Lower gelation temperature carrageenans are increasingly available, and through appropriate control of the polymer and KCl concentration, are better suited to biocatalyst encapsulation. This particular example is being provided to illustrate a large-scale encapsulation process with interesting potential for continuous operation. The process was also conducted aseptically, demonstrating its potential in pure culture encapsulation.

The unit operation of interest in this process involves the use of static mixers. The mixers were used to carry out two functions, firstly to disperse the yeast paste into the viscous carrageenan sol, and secondly, to achieve the emulsion/dispersion of the yeast-carrageenan sol into vegetable oil, at a temperature above the gel-point of the polymer. The emulsion was subsequently quick-cooled, causing the carrageenan droplets to gel. Static mixers are constructed with a series of elements, fixed transversely within a pipe as shown in Figure 4. The elements repeatedly cut, rotate and recombine the flowing fluid, where the mixing energy generated is sufficient to disperse, or to emulsify viscous mixtures such as carrageenan and vegetable oil. It is desired that the more uniform shear, in contrast to a turbine impeller in a baffled reactor for example, promote an increasingly homogeneous dispersion [8] at flow rates typical of those encountered at the industrial scale (several to hundreds L h^{-1}). Control of microsphere properties is thus possible through a wide range of operating properties, including number of mixer elements, fluid velocity, mixer diameter, and carrageenan volumetric fraction in the dispersion.

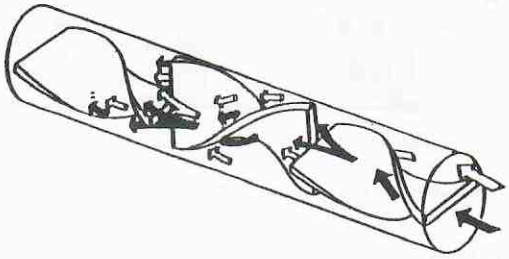

Figure 4. Design and operation of static mixer, used to disperse/emulsify two immiscible fluids. Note the static elements, which sequentially divide, rotate and recombine the flowing fluids, represented by black and white arrows.

Brewing operations have traditionally been carried out as batch processes, with fermentation times of typically 6-7 days. Thus economical, smaller scale, continuous processes for the primary and secondary fermentation stages are being examined. As alginate may be unstable in a wort-based medium, carrageenan has been considered as an alternative since potassium replaces calcium as the gelling ion. From a practical point of view, immobilized cells must be produced on a large scale and be stable for relatively long operational times, in the order of weeks or months. Mass transfer limitations are of critical interest [9], as it impacts on the flavour profile of the product.

Brewer's yeast was encapsulated in gelled κ-carrageenan microspheres [10] as illustrated in the process flowsheet shown in Figure 5. Carrageenan polymer and canola oil were pre-sterilized by heating, followed by cooling and holding at 40°C. Yeast was mixed with the carrageenan sol through the first static mixer, and the sol-yeast mixture was dispersed into warm oil *via* a second static mixer. The second mixer contained 12 mixing elements with a diameter of 12.7 mm. The resulting emulsion was rapidly chilled to 5°C, initiating gelation of the carrageenan droplets. Carrageen gel microspheres were then partitioned into cold sterile KCl solution (22 g L^{-1}). Process oil was then recycled back to the feed tank for reuse.

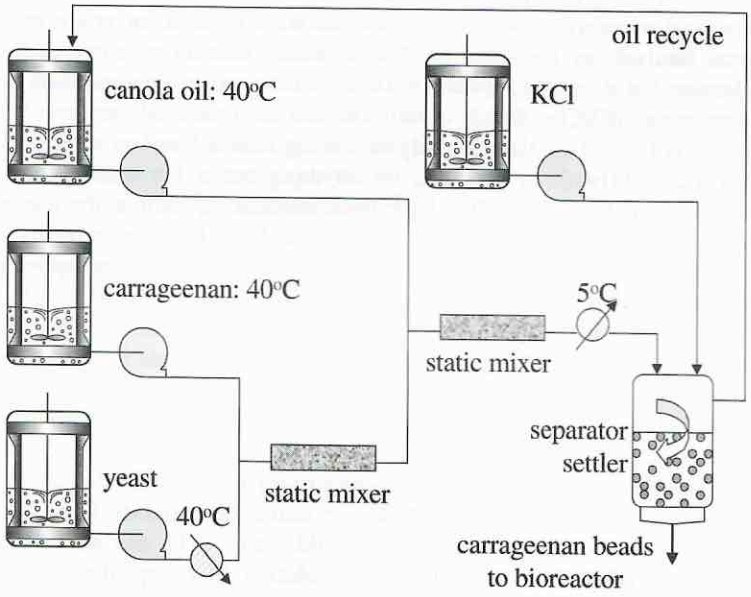

Figure 5. Flowsheet for the production of carrageenan microspheres containing brewer's yeast, in continuous process. Note the use of static mixers for both the dispersion of yeast into carrageenan sol, and for the emulsion/dispersion of the yeast carrageenan suspension into warm canola oil. Emulsified carrageenan sol is gelled by rapid cooling and partitioning into KCl solution.

Yeast loaded, carrageenan beads with a mean diameter of 1 mm and a coefficient of variability (standard deviation/mean diameter) of 40%, were then loaded into a 50 L

airlift bioreactor as illustrated in Figure 6 for carrying out a primary beer fermentation. A gas mixture of CO_2:air (98:2) was sparged to the reactor at a volumetric rate of 0.3 vvm. Beer wort sugars were consumed in 22 h, compared to a conventional fermentation with free cells, requiring 6 to 7 days. Measured flavour constituents and taste panel analysis found the flavour comparable to a conventional brew.

5. Emulsification/ internal gelation of alginate, forming membrane-coated microspheres in the encapsulation of *Lactococcus lactis*

Many biological encapsulants including living cells cannot tolerate the elevated temperatures typical of thermal gelation systems (40 to 60°C). Alginate is by far the most widely used encapsulation matrix, for several reasons. Gels can be formed at ambient temperatures and alginate polymer is inexpensive, widely available, biocompatible and forms reversible gels in the presence of multivalent cations such as Ca^{++} under gentle formulation conditions. Over the last decade, more suppliers of alginates are appearing in the market place, the quality of the polymer is improving, and alginates are now being sold partially or fully characterized in terms of its chemical and physicochemical properties and are available as food or medical grade material. The conventional method for formulating alginate beads containing entrapped cells is to extrude alginate sol dropwise, from a syringe needle into a gelation bath, containing soluble calcium salt ($CaCl_2$). Beads of uniform size are produced, however reduction in bead diameter is limited by the viscosity of the alginate sol and as a result, beads less than 500 µm are difficult to produce, on anything but a lab scale. Various simple modifications to this basic procedure have been introduced, such as the use of multiple needles, electrostatics, vibration, droplet propulsion from the needle tip by concentric airflow, and liquid jet cutters. Commercial encapsulators have now appeared on the market and appear popular amongst those working with these various droplet extrusion technologies. For beads in the millimetre size range, production rates in the order of 1 to 2 L h^{-1} are possible, and multiple needles permit small-scale industrial production, ranging to 10 L capacity. The most important limitation to these techniques is that they are still not suited to industrial scale application, particularly when beads within a sub-millimetre size range are desired.

Various attempts to use emulsion techniques to form ionic polysaccharide gel beads have been awkward or still require single droplet extrusion methods. For example, a hot carrageenan/oil emulsion was dropped into cold water [11] and an oil/alginic acid emulsion was extruded dropwise into $CaCl_2$ solution to encapsulate oil droplets in alginate [12].

Pelaez and Karel [13] proposed a method to form alginate gel slabs, in a procedure termed "internal gelation". The alginate sol was placed in a mold and gelled through the internal liberation of calcium ions from insoluble citrate complex *via* spontaneous breakdown of gluconolactone. The pH of the alginate would drop due to the slow formation of gluconic acid, leading to the liberation of soluble calcium from the insoluble salt. Alginate molds would set in 5 min. The extension of this technology to the formation of alginate beads or microspheres in an emulsion system is difficult, because of the inability to trigger gelation on command, the slow gelation rate leads to

aggregation due to slow gelation of the emulsified gel droplets, and the lack of control over pH in the gel.

Through a number of refinements to the internal gelation concept, adapted toward the production of gel microbeads, a novel procedure - termed "emulsification/internal gelation" was developed [14, 15, 16]. This procedure is illustrated in Figure 7 and involves the dispersion of cell encapsulant into alginate sol at a ratio of up to 1:1. Insoluble calcium salt - typically $CaCO_3$ - is then mixed into the alginate sol (500 mM Ca^{2+} equivalent, or 25 mM calcium salt). Various calcium salts may be used, although carbonate provides for near instantaneous release of soluble calcium with a slight pH adjustment in a neutral pH range, as shown in Figure 8, a range best suited to active biologicals. The alginate/calcium carbonate mixture containing cells is then dispersed into an oil phase (vegetable, mineral or silicone oil). Various mixing devices, typical of those used in food and chemical processing (see Figure 9), including static mixers, provide considerable level of control over droplet (microsphere) mean diameter and size distribution [15, 16]. Once the emulsion is formed, gelation is initiated by pH adjustment with an oil soluble acid, such as acetic acid. Approximately 0.66 mL of acetic acid is required per L of emulsion, assuming an alginate sol concentration of about 2% (pH change from 7.5 to 6). Gelation should be essentially instantaneous, otherwise aggregation of the gel microspheres may result. Following about 5 min of gelation, the emulsion is mixed gently with an aqueous phase, and microspheres partitioned from the oil dispersion. This is followed by filtration and washing with mild surfactant to remove residual oil. Recently, direct filtration of microspheres from oil was successfully attempted using fine wire mesh.

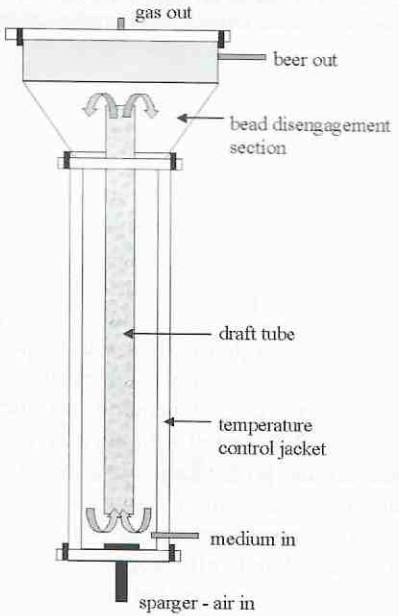

Figure 6. Gas-lift 50L bioreactor for continuous brewing process using carrageenan immobilized yeast. Figure adapted from Figure 3 in Reference [10].

An important aspect of this technique involves the need for calcium carbonate microcrystals, approaching a few microns in diameter. The smaller the calcium carbonate grain size, the more grains can be introduced within smaller and smaller microdroplets, and the faster is their dissolution rate. Resulting microspheres are thus more spherical, and less likely to aggregate. There should be no residual carbonate grains in the microsphere once gelation is complete.

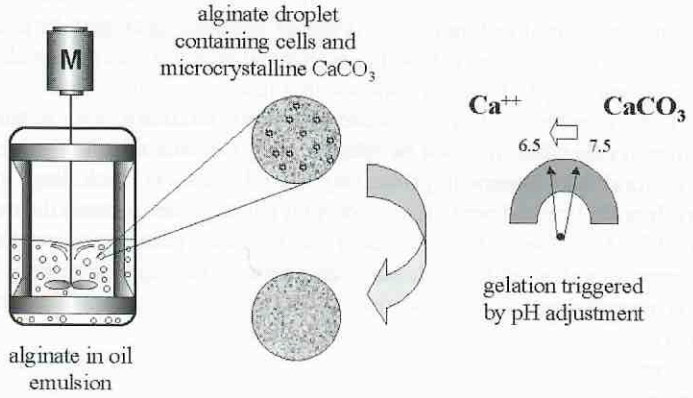

Figure 7. Cell encapsulation by emulsification/internal gelation of alginate. Alginate containing cells and calcium as insoluble microcrystalline salt is dispersed into vegetable oil. Calcium - Ca^{++} is released in situ by pH adjustment with an oil soluble acid - acetic acid, triggering instantaneous gelation, forming alginate microspheres.

Smooth, spherical microspheres with the narrowest size dispersion were produced using a low-guluronic acid and low-viscosity alginate, and carbonate as the calcium release vector. Microsphere mean diameters ranging from 50 to 1000 µm were produced, with standard deviations ranging from 35 to 45% of the mean [16].

The following is provided as an example of cell encapsulation using emulsification/internal gelation of alginate. *Lactococcus lactis* was encapsulated [17] in alginate microspheres for use in the diary industry for the manufacture of yogurt, fermented creams, and cottage cheese dressings. Over acidification can be avoided by removal of the encapsulated cells, facilitating their reuse, and extending the shelf life of the dairy product. Microspheres containing entrapped cells were spherical, with diameters extending down to 50 µm, depending on the formulation conditions. Resulting alginate microspheres were coated with poly-L-lysine, a polycationic polymer that forms a membrane coat around polyanionic alginate. Microspheres were immersed in 0.02% poly-L-lysine solution for 20 min to permit membrane formation. The purpose of the membrane was to limit the rate of release of cells from the encapsulation matrix. At high concentrations of encapsulated cells, approaching 10^9 cells mL^{-1} milk, similar rates of lactic acid production were observed between encapsulated cells, and controls consisting of an equivalent level of free cells.

This procedure has only to a limited extent been tested on a continuous basis, involving the use of static mixers. One difficulty, which would need to be overcome, requires the need to recondition the slightly acidified oil, prior to re-use. It may be that

residual acetic acid has been fully removed through aqueous phase partitioning during the microsphere separation step, but this has not as yet been tested. It is unlikely that the oil has been hydrolyzed to any extent by the gentle acid treatment, but again this question has not as yet been addressed.

In the application described above (encapsulation of lactic cultures within poly-L-lysine coated alginate microspheres), the alginate microsphere served as a core matrix around which a thin membrane coat was applied. In this section, a microencapsulation approach will be described, by which a membrane can be formed by the cross-linking of pre-formed polymer through an interfacial reaction. The resulting microcapsule consists of a liquid core, contained within a thin, semi-permeable membrane coat. Individual microcapsules are formed by the emulsion dispersion of liquid biocatalyst suspension into an appropriate solvent or oil phase, followed my membrane formation around the liquid microdroplets. Following membrane formation, the microcapsules are separated from oil suspension through aqueous phase partitioning, or filtration.

The key in encapsulating living cells by interfacial cross-linking, is to maintain a separation between the cells and the toxic cross-linking reagent. Glutaraldehyde is an obvious choice of cross-linker because it is oil soluble, but since it is also water soluble, it is difficult to exclude from the liquid microcapsule core. The following examples involve similar applications, but in both cases include the use of strictly oil soluble cross-linkers. The examples are interesting to examine, because in one case, it involves a cross-linking reaction with a protein (gelatin) and in the other case, it involves the cross-linking of a polysaccharide (chitosan). Both of these materials are of interest for use in food and pharmaceutical products. The technique is easily adapted for use in other cell immobilization applications.

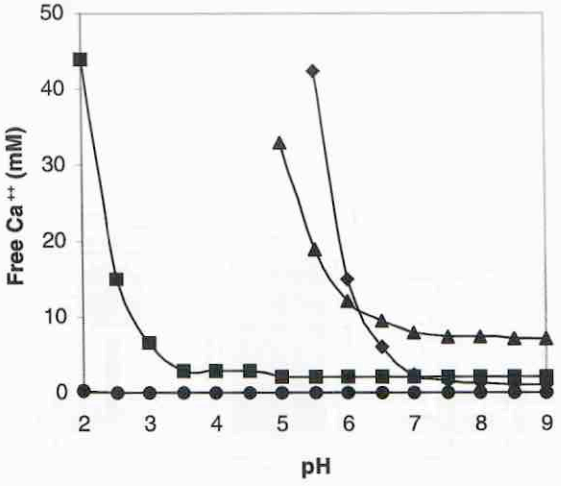

Figure 8. Free calcium concentration at different pH levels for various calcium salts. Redrawn from Figure 6 in Reference [16]

6. Microencapsulation of lactic cultures by interfacial cross-linking reaction involving pre-formed biopolymer

The bioencapsulation of *Lactococcus lactis* within cross-linked gelatin membranes was achieved as follows. Culture, suspended in 24% gelatin solution, was emulsified into either sunflower oil or silicone oil, facilitate by a surfactant [18]. Mixing was provided by a sheet lattice impeller as illustrated in Figure 9. The ratio of gelatin to oil was 1:10. The cross-linker, toluene-2,4-diisocyanate, was added to yield a final concentration of 34 mmol dm^{-3} in the oil. After 15 min reaction, the oil was decanted, and the microcapsules filtered and rinsed with water. Microcapsule diameters were 124 ± 74 and 271 ± 168 μm when formulated in sunflower and silicone oil respectively. Activity of the microencapsulated cells was determined by pH reduction in milk in repetitive sequential fermentations. Loss of activity, in comparison to the free cell controls, was realized immediately after microencapsulation, but was quickly recovered by cell growth within the microcapsules. Acidification rates similar to those achieved with cells immobilized in alginate beads, were measured by the third sequential fermentation.

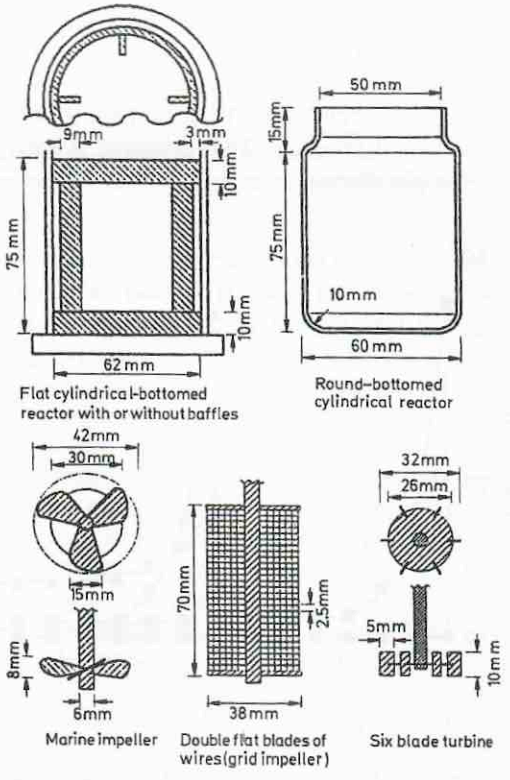

Figure 9. Design of baffled vessel and impellers used to emulsify/disperse aqueous polymer solution containing the biocatalyst, into an oil phase.

In a related study [19], *Lactococcus lactis* was microencapsulated within chitosan membranes, cross-linked with a variety of oil soluble cross-linkers. The procedure involved suspending cells into a 4% chitosan solution, followed by emulsion/dispersion into an organic phase in a ratio of 1:5, facilitated by means of Span 85 surfactant. Various organic phases were tested, including cyclohexane, or sunflower, canola or mineral oil. The emulsion was generated using a frame lattice impeller for 1 to 5 min, followed by the addition of terephthaloyl chloride, glutaraldehyde or hexamethylene diisocyanate as cross-linkers. Following membrane formation, microcapsules were separated by settling and decantation of the oil, and filtered and washed with Tween 20 solution. Microcapsule diameters depended strongly on the choice of oil used, with the oil viscosity being the determining parameter. As a result, mean diameters covered a broad range, from 100 μm to 1 mm. Lower viscosity solvents facilitated microcapsule recovery, while the more viscous oils were more problematic, particularly for the smaller diameter microcapsules. Chitosan cross-linked with hexamethylene diisocyanate or glutaraldehyde formed strong membranes, and resulting microcapsules had a narrow size distribution with a mean diameter of 150 μm. Some loss of acidification activity was observed upon microencapsulation due to the toxicity of the cross-linker, but activity recovered during sequential batch fermentations to levels equivalent to that observed with free cells.

Detailed procedures and a description of the chemistry behind the cross-linking reactions involving chitosan and various oil soluble cross-linkers, can be found in Reference [20]. The recovery and reuse of the oil phase in these procedures may be achieved by minimizing residual cross-linker. Given the high reactivity of these reagents, it is assumed that the cross-linker has been fully consumed by the reaction, but this would need to be carefully verified.

7. Microencapsulation by emulsification and interfacial polymerization reactions

In the procedure described above, pre-formed polymer is cross-linked at the droplet surface or interface, giving rise to a cross-linked membrane coat. An alternative is to carry out an interfacial polymerization reaction in an emulsion system, whereby one of the polymerization monomers is water soluble, and thus present in the aqueous phase microdroplet, and the other polymerization monomer is oil soluble, and thus present in the oil dispersed phase. Interfacial polymerization is initiated by addition of the oil soluble polymer, or by addition of a polymerization catalyst or initiator. In this case, a synthetic polymer coat is formed around the emulsified droplet, which upon separation from the oil phase, gives rise to membrane bound microcapsules. Strong and stable membranes are formed, which because of the wide range of possible polymers that can be used, provide for considerable range of compatibilities and permeabilities.

A well-known example of an interfacial polymerization reaction involves the formation of nylon or polyamide membrane microcapsules. The procedure involves the use of a diamine base (*e.g.* 1,6-hexamethylene diamine) solubilized in the aqueous internal phase, which is dispersed into an oil or solvent phase, to which is added a dichloride (e.g. sebacoyl chloride). The resulting polymerization reaction gives rise to a thin polyamide film (nylon-6,10), coating the liquid microdroplet [21].

The difficulty with this procedure lies in the extremes of pH that are encountered, solvent toxicity if an organic solvent is used as the continuous emulsion phase, and potential toxicity of the monomers themselves. Various modifications to the procedures have been introduced to minimize toxic effects such as through improved control of pH, the use of alternative monomers or various fillers, and non-toxic vegetable oils.

In our experience, and for the reasons described, interfacial polymerization reactions are problematic when the objective is to microencapsulate living cells. The potential is interesting however, but the procedures should be approached with caution. It should be mentioned that this procedure has been successfully applied to the microencapsulation of active and stable enzyme preparations such as urease [22,23].

8. Summary and conclusions

The objective of this review has been to provide a perspective on emulsification/dispersion technologies which are available to immobilize living cells and other biocatalysts through entrapment or microencapsulation protocols. The resulting product is a gel bead or microsphere, which may or may not be membrane coated, or a membrane coated microcapsule containing a cell suspension in a liquid core. The objective is to formulate these active cell preparations on an industrial scale, with a careful control over size - with mean diameters potentially extending into a micron size range. Enormous flexibility in the selection and control of mean microsphere or microcapsule diameters is exercised through the control of emulsification parameters. However, emulsions lead to size dispersions, so attention must be paid to the manner in which the emulsions are generated so as to exercise as high a level of control over the size dispersion as is possible. The key benefit then is in the ability to generate microspheres down into a micron size range, on virtually any industrial scale that could be contemplated. In fact, emulsification/dispersion systems are the only entrapment or microencapsulation protocols available for industrial scale application, in a microsphere or microcapsule size range under 500 μm. The methodologies are also readily extended to continuous processing, under aseptic conditions.

Several examples have been provided involving the encapsulation of living cells. These procedures are adaptable to the encapsulation of other cell lines.

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