CELL IMMOBILIZATION FOR THE DAIRY INDUSTRY

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Introduction

Lactic acid bacteria (LAB) are widely used in the production of fermented dairy products such as cheeses, yoghurts and creams because of their technological, nutritional and eventual health properties. The production of organic (mainly lactic and acetic) acids and the resulting acidification is essential for the production, development of typical flavour and preservation of these products. The transformation of lactose by lactic cultures improves the digestibility and various metabolic and enzymatic activities of LAB lead to the production of volatile substances, which contribute to flavour, aroma and texture developments in fermented dairy products. Probiotics are defined as microbial cells which transit the gastrointestinal tract and which, in doing so, benefit the health of the consumer (Stanton et al 2001). Among these micro-organisms, LAB and especially lactobacilli and bifidobacteria are already used in many probiotic dairy products.

Culture production and milk fermentations are traditionally carried out in batch bioreactors using freely suspended microbial cells. However, recent researches on immobilized cell (IC) technology applied to LAB and probiotic cultures have emphasized the importance and interest in this new technology. In particular, cell immobilization has been shown to offer many advantages for biomass and metabolite productions compared with free-cell (FC) systems such as: high cell density and very high volumetric productivity, reuse of biocatalysts, high process stability (physical and biological) over long fermentation periods, retention of plasmid-bearing cells, improved resistance to contamination, uncoupling of biomass and metabolite productions, stimulation of production and secretion of secondary metabolites and physical and chemical protection of the cells.

Other complementary strategies to fermentation, based on cell protection, have been tested to increase sensitive LAB, and particularly bifidobacteria, survival in products and eventually in the human intestine. These methods use cell encapsulation to provide a physical barrier against the stressful environmental conditions. This short review presents recent developments in the immobilized cell technology with LAB and probiotic bacteria and application in foods, with a focus in the dairy area. For additional information, readers are invited to consult two recent reviews on this topic (Doleyres and Lacroix 2004; Lacroix et al 2004).

Immobilization techniques

Different methods (physical entrapment in polymeric networks, attachment or adsorption to a preformed carrier, membrane entrapment, microencapsulation) have been used for immobilizing LAB. The purpose of these techniques is either to retain high cell concentrations within the bioreactor or to protect cells from a hostile environment. For industrial applications in the food industry, the carrier material must be non-toxic, readily available and affordable. It should also lead to high-cell loading and the cells should have a prolonged viability in the support.

For food applications, the most widely used immobilization technique is the entrapment of cells within a food-grade porous polymeric matrix (Table 1). In many applications,

controlled-size polymer droplets are produced using extrusion or emulsification, under mild conditions. Thermal (κ-carrageenan, gellan, agarose, gelatin) or ionotropic (alginate, chitosan) gelation of the droplets are used to produce spherical gel biocatalysts. These polymers are readily available and widely accepted for use as additives in the food and particularly dairy industry. Gel entrapment is a relatively simple method resulting in usually spherical beads with diameters ranging from 0.3 to 3.0 mm with high biomass concentration. A careful selection of polymer composition is necessary to achieve high mechanical stability of gel biocatalysts during long-term fermentation, according to the conditions of the fermentation (Artignan et al 1997). However, the large scale production of beads under aseptic conditions necessary for food applications still remains an important issue for the industrialization of immobilized cells in the food sector.

Table 1: Lactic acid bacteria immobilization by entrapment techniques

Support	Species	Maximum cell concentration[a]	Reference
Ca-alginate	*Lactococcus lactis* ssp.	$2.0 \; 10^{11}$ cfu ml^{-1}	Prévost and Diviès 1992
Ca-alginate	*Lactococcus lactis* ssp.	$3.8 \; 10^{11}$ cfu g^{-1}	Prévost and Diviès 1992
κ-carrageenan – LBG[b]	*Lactobacillus casei*	$5.1 \; 10^{11}$ cfu ml^{-1}	Arnaud et al 1992
κ-carrageenan – LBG[b]	*Lactococcus lactis* ssp. (three strains)	$1.3 \; 10^{11}$ cfu g^{-1}	Lamboley et al 1997
Gellan gum	*Bifidobacterium longum*	$6.8 \; 10^{10}$ cfu g^{-1}	Doleyres et al 2002

[a] cfu ml^{-1} or g^{-1} of support

[b] LBG = locust bean gum

Applications of immobilized cell technology

BIOMASS PRODUCTION

In this application, methods are used to promote cell release from gel beads that occurs spontaneously as microcolonies form near the surface of the biocatalysts. In some applications, such as entrapped biomass production, cream fermentations and metabolite productions, cell release is not desirable. Steps for reducing FC levels have been presented by Champagne et al (1994). However, repeated use of beads tends to increase the level of FC, and although different treatments have been tried, the rate of cell release is only reduced during the early stages of the fermentation (Champagne et al 1994; Klinkenberg et al 2001).

The release of cells growing in the peripheral layer of highly colonized gel beads can be used to efficiently produce biomass in the bulk liquid medium. This cell release activity can be used for producing single or mixed strain cultures and to continuously inoculate food liquids to process fermented foods such as fermented milk products.

Lactic acid bacteria starter

The LAB are largely used in single and mixed cultures for the production of cheeses, fermented milks, yoghurts, and cultured butter. The main objectives of producing concentrated starters are to obtain a high number of living cells containing the necessary

enzymes to function effectively during manufacturing of cultured milk products, and also to maintain a given strain balance in mixed starter cultures. Although lactic starters are traditionally produced by batch fermentation, the accumulation of metabolic end products limits cell growth. The use of continuous fermentations may overcome this limitation, but they are more susceptible to contamination and loss of plasmid-mediated characteristics and they rapidly lead to the disappearance or domination of strains in a mixed-strain continuous fermentation (D'Angio et al 1994; Gilliland 1985). One promising alternative is the use of IC technology to produce mixed lactic starters in continuous fermentation (Lamboley et al 1997). The high IC concentration results in a very high productivity and decreases contamination risks due to the high dilution and inoculation rates provided by cell release from beads. Immobilisation also improves plasmid stability (D'Angio et al 1994; Huang et al 1996).

The production of mixed-strain mesophilic lactic starters was studied during continuous fermentations of SWP, with three strains of lactococci separately immobilised in κ-carrageenan/LBG gel beads in a stirred tank reactor (Lamboley et al 1997). The process showed a high biological stability and cell productivity (maximum cell productivity of 5.3 10^{12} cfu l^{-1} h^{-1}) over the tested period exceeding 50 days. By varying pH, dilution rate (D) and temperature (T), a large range of strain ratios could be obtained, while starter activity remained constant. The microbiological and mechanical stabilities of gel beads, that are critical properties of an IC process for industrial applications, were shown during prolonged fermentations (7 weeks).

Probiotic cultures

Probiotics are now used to prepare a large variety of pharmaceuticals and fermented milk foods, such as fresh cheeses, fermented milks and health supplements. However, many probiotic cultures, such as bifidobacteria, are fastidious and non-competitive bacteria that are very sensitive to environmental parameters, such as oxygen and acidity, and require complex and expensive media for propagation, with the addition of growth-promoting factors, due to their stringent growth requirements (Ibrahim and Bezkorovainy 1994).

Our recent data on IC technology clearly showed that this approach can be used to continuously and stably produce mixed-strain starters containing fastidious and non-competitive micro-organisms, such as bifidobacteria, with a high volumetric productivity and high biomass concentrations in the outflow of the continuous fermentation, even at high dilution rates exceeding the specific growth rate (Doleyres et al 2004a). The production of a mixed lactic culture containing *Lactococcus lactic* ssp. *lactis* biovar *diacetylactis* MD and *Bifidobacterium longum* ATCC 15707 was studied during a 17-day continuous IC culture at different temperatures between 32 and 37°C. The two-stage fermentation system was composed of a first reactor (R1) containing cells of the two strains separately immobilized in κ-carrageenan/LBG gel beads and a second reactor (R2) operated with free cells released from the first reactor. The system allowed to continuously produce a concentrated mixed culture with a strain ratio whose composition depended on temperature and fermentation time. A stable mixed culture (with a 22:1 ratio of *L. diacetylactis* and *B. longum*) was produced at 35°C in the effluent of R2, whereas the mixed culture was rapidly disbalanced in favour of *B. longum* at a higher temperature (37°C) or *L. diacetylactis* at a lower temperature (32°C).

Cell growth in gel beads is limited by diffusional limitations of both substrates and inhibitory products, in this case lactic and acetic acids. This leads to the development of steep inhibitory product, pH and biomass gradients in colonized beads which can induce a non-specific stress adaptation of IC. Data from Doleyres et al (2004b) showed that IC technology combined with long-term continuous culture can be used to efficiently produce, in a one step process, cells with enhanced tolerance to different environmental stresses. In addition, cells produced by

continuous IC cultures, which are in exponential or early stationary growth phase, exhibited both a high viability and metabolic activity compared with starving cells produced by conventional batch cultures.

The IC technology can be used to continuously and stably produce mixed-strain starters, eventually containing fastidious micro-organisms, such as bifidobacteria, with a high volumetric productivity and high biomass concentrations in the outflow of the continuous fermentation, even at high dilution rates exceeding the specific growth rate.

PREFERMENTATION OF MILK

Starter culture preparation is of paramount importance in the manufacture of fermented dairy products. Any failure in the starter preparation will, in most cases, lead to detrimental effects on quality, affecting the appearance, texture and flavour of the end product. Traditionally, batchwise processing has been used, but increasing demand for these products has resulted in a large increase in size of fermentation tanks.
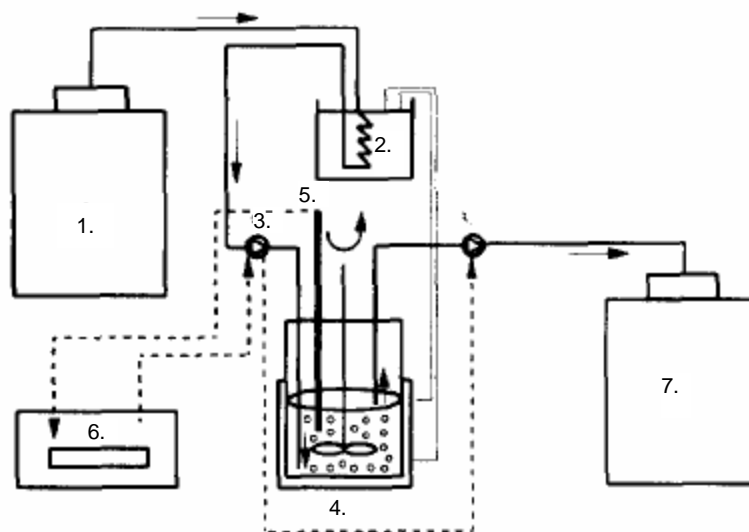
Figure 1: Schematic diagram of the apparatus for continuous inoculation and prefermentation of milk: 1, refrigerated milk storage tank; 2, thermostated water batch; 3, peristaltic pump; 4, continuously stirred tank reactor with lactic acid bacteria immobilized in gel beads; 5, pH probe; 6, pH controller; 7, storage tank of preacidified and inoculated milk (from Sodini-Gallot et al 1995).

The continuous inoculation-prefermentation of milk for yoghurt production in a stirred tank reactor by separately entrapped cells of *Lactobacillus delbruekii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* in Ca-alginate gel beads was the first studied dairy application of IC (Prévost and Diviès 1987; 1988a; 1988b). In this system, incoming milk was used to control pH in the IC reactor (Figure 1). The milk feed rate was therefore a direct function of the acidification activity in the fermenter. With a pH set at 5.7, the residence time of milk in the reactor was very short, 8-9 minutes, and lactic acid production was increased 3.3-fold compared with free-cell mixed cultures (Prévost and Diviès 1988a). The ratio cocci:rod remained stable in the prefermented milk during the 10 day culture, equal to 1 which is the optimum value for yoghurt manufacture. The high cell confinement due to entrapment might explain the high cell and lactic acid production in the bioreactor (Prévost and Diviès 1988b). This technology allowed a reduction in fermentation time by

approximately 50 and 20% compared with freeze-dried strains and a liquid yoghurt culture, and the resulting yoghurt qualities were evaluated as satisfactory (Prévost and Diviès 1988b).

The inoculation-prefermentation of milk for fresh cheese production with a mixed culture of entrapped in κ-carrageenan/LBG gel beads was also extensively studied by our group. For example, the total fermentation time to produce the fresh cheese curd (pH 4.8) was considerably reduced by up to more than 50% compared with the traditional industrial process (Sodini et al 1995; 1997), or by 10-15% of that of batch fermentation under optimum laboratory conditions (Sodini et al 1998). This fermentation time reduction was explained by the high inoculation level and lower pH of prefermented milk compared with the classical batch fermentation. Moreover, cells released from gel beads are exponentially growing cells with no lag time compared with freeze-dried or bulk starter cultures used in cheese manufacture. The very high production rate (a reactor of 100 liters can produce daily over 100,000 liters of prefermented milk inoculated with 1-2 10^8 cfu ml^{-1}, or 1 million liters of milk inoculated at the usual rate used for fresh cheese production, i.e. approx. 10^7 cfu ml^{-1}), the microbial stability and the extensive reduction of fermentation times are among the major advantages of this new process.

METABOLITE PRODUCTION

Industrial cheese manufacturing yields large volumes of whey as a by-product. In many plants, the valuable whey proteins are concentrated by ultrafiltration and incorporated into cheese or dried and sold as food ingredients. The ultrafiltration step yields large volumes of low-value whey permeate, which has limited uses. Due to its high lactose (about 50 g l^{-1}) and mineral contents (9 g l^{-1}), whey permeate may be used as a culture medium for the production of lactic starter cultures or metabolites. The immobilized cell technology has been studied by our group for production of different metabolites and functional ingredients from LAB.

Lactic acid production

Lactic acid is widely used as an acidulant and preservation agent in foods and as a precursor for production of emulsifiers, such as stearoyl-2-lactylates, for the baking industries (Vickroy 1985). New applications such as its use as a monomer for production of biodegradable plastics and as an environment-friendly chemical and solvent will increase future lactic acid demand (Datta et al 1995). Whey permeate must be supplemented with a source of metabolizable nitrogen and vitamins, such as yeast extract (YE), in order to satisfy nutritional requirements of LAB. However, the cost of YE contributes largely to lactic acid production costs (Tejayadi and Cheryan 1995) and, therefore, minimizing YE addition is an important goal for process optimization.

High lactic acid productivities and long-term stability (over 100 day periods) have been obtained during continuous IC fermentation of YE-supplemented whey permeate by *Lb. helveticus* immobilized in κ-carrageenan/LBG gel beads, but with limited conversion of lactose (Norton et al 1994a). Complete sugar conversion was only obtained when an additional FC reactor was placed in series with the IC reactor (Norton et al 1994b; Figure 2). In this two-stage IC/FC process, an overall lactic acid productivity of 13.5 g l^{-1} h^{-1} was reached during continuous culture in whey permeate containing 10 g l^{-1} YE, with 1 g l^{-1} residual sugar at an overall dilution rate of 0.27 h^{-1}. The authors also concluded that operation at low yeast extract levels (1-3 g l^{-1}), or using periodic addition of higher YE concentrations, could lead to a cost-effective operation. The productivity of a two-stage IC reactor (19-22 g l^{-1} h^{-1} with low residual sugar) largely exceeded that measured for the two-stage IC/FC process (Schepers et al 2004) and for FC batch cultures (approximately 2.0 g l^{-1} h^{-1}) with the same strain and experimental conditions (Schepers et al 2002). Higher productivities in SWP, with

10 g.l^{-1} YE, and high sugar utilization close to 100%, were only measured in cell-recycle processes, with 35 g l^{-1} h^{-1} for *Lb. helveticus* [9] and and 85 g l^{-1} h^{-1} for *Lb. bulgaricus* (Mehaia and Cheryan 1986). However, the industrial application of membrane reactors is largely limited by high capital investment, complex maintenance of the sophisticated equipment and membrane fouling, particularly with whey permeate, which results in a rapid decrease of process performance (Tejayadi and Cheryan 1995).
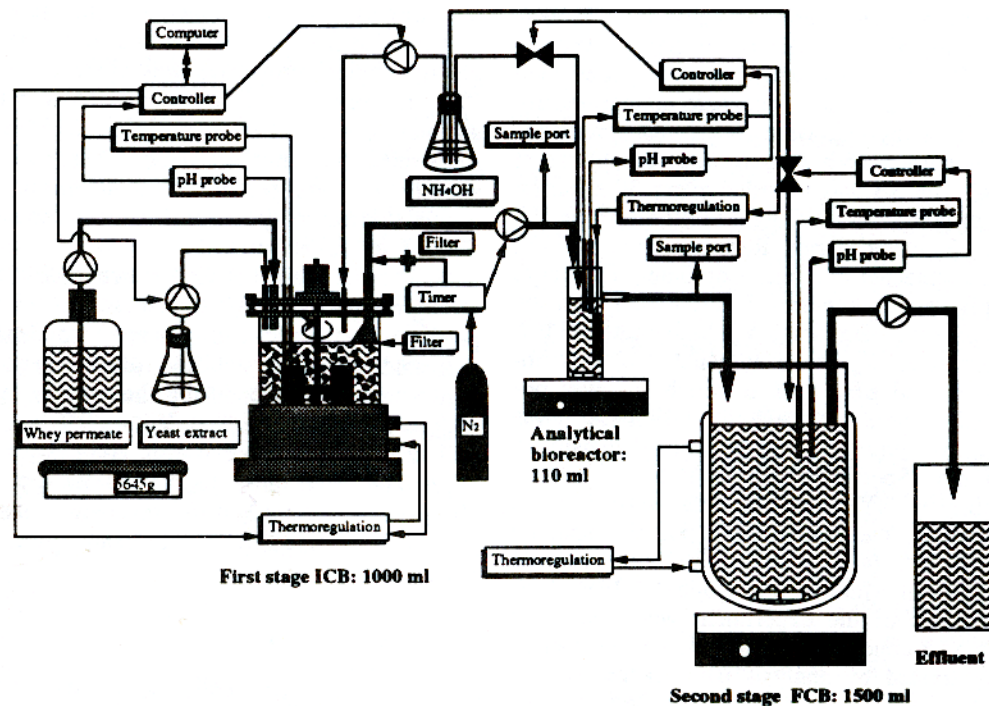
Figure 2: Experimental set-up of the continuous two-stage fermentation process, including an intermediate analytical bioreactor. ICB = immobilized cell bioreactor; FCB = free cell bioreactor (from Norton et al 1994b).

Exopolysaccharide production

Exopolysaccharide (EPS) produced by LAB have received increasing attention in recent years. They play an important role in the manufacturing of fermented dairy products such as yogurts, drinking yogurts, cheeses, fermented creams and milk-based desserts. The EPS are very attractive for use as food additives because of their contribution to the texture, mouthfeel, taste perception and stability of the final product. They may act as texturizers and stabilizers and so decrease syneresis and improve product stability. Furthermore, EPS may contribute to human health as prebiotics or due to their antitumor, antiulcer, immunomodulating or cholesterol-lowering activities (Ruas-Madiedo et al 2002). However, compared with dextran-producing or Gram-negative EPS producers, the low production of EPS by most LAB is a constraint for their commercial use as food additives (De Vuyst and Degeest 1999).

Very little research has been done on cell immobilization for EPS production. In our group, Bergmaier et al (2003) used the mucoid properties of *Lb. rhamnosus* RW 9595M for cell immobilization by adsorption on solid porous supports (ImmobaSil®). The production of EPS was investigated during pH-controlled IC repeated-batch cultures in SWP. A high immobilized biomass of 8.5 10^{11} cfu ml^{-1} support was measured after colonization by DNA analysis. During repeated IC cultures, a high EPS concentration (1750 mg l^{-1}) was measured

after four cycles for a short incubation period of 7 h. The high biomass in the IC system increased maximum EPS volumetric productivity (250 mg l^{-1} h^{-1} after 7 h culture) compared with FC batch cultures (110 mg l^{-1} h^{-1} after 18 h culture corresponding to maximum EPS concentration of 1985 mg l^{-1}).

A 38-day continuous fermentation was carried out in a two-stage bioreactor under the same conditions, with a first fermentation stage containing immobilized cells and a second fermentation stage in series that was continuously inoculated by cells released from the first reactor (Bergmaier et al 2004). Despite very high biomass concentrations in both reactors, the average total soluble EPS production of the fermentation system was very low (138 mg l^{-1}), with no effect of temperature, dilution rate and carbon to nitrogen ratio, compared with that measured in batch cultures with FC (2350 mg l^{-1} after 18 h incubation) or IC (1750 mg l^{-1} after 7 h) and continuous culture with FC (1808 mg l^{-1} at a dilution rate of 0.3 h^{-1}). This result was explained by important changes in cell morphology and physiology, and the formation of very large aggregates containing very high cell and insoluble EPS concentrations.

These data show the high potential of the strain, *Lb. rhamnosus* RW9595M, and of IC technology for the production of EPS as a functional food ingredient. In addition, the production of insoluble EPS allows an easy recovery of the product and the aggregates containing high EPS and viable cell concentrations could have interesting applications as symbiotic product, combining both probiotic and prebiotic activities.

Bacteriocin production

Bacteriocins are ribosomally-synthesized, extracellularly released proteins or protein complexes with a bactericidal activity against closely related bacteria, and for some bacteriocins, against a wide range of Gram-positive bacteria (Jack et al 1995). In recent years, there has been considerable interest in bacteriocins produced by LAB, and for diverse applications such as biopreservatives, to improve quality and innocuity of food products. However, the major limiting factor in using bacteriocins as food preservatives is their low yield during production.

Cell immobilization has been used to increase cell density for bacteriocin production in supplemented whey permeate medium. A very high nisin Z production (8200 IU ml^{-1}, with 1 IU = 1 international unit of nisin = 0.025 μg pure nisin) was measured in the broth after 1-h cycles during repeated-cycle pH-controlled batch (RCB) cultures with *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* UL719 immobilized in κ-carrageenan/LBG gel beads in SWP, with a corresponding volumetric productivity of 5730 IU ml^{-1} h^{-1} or approximately 150 mg l^{-1} h^{-1} (Bertrand et al 2001). This productivity is much higher than maximum nisin productivities reported in literature (approximately 500 IU ml^{-1} h^{-1}) or maximum productivities obtained with the same strain UL719 for FC batch cultures (850 IU ml^{-1} h^{-1}), and FC (460 IU ml^{-1} h^{-1}) or IC (1760 IU ml^{-1} h^{-1}) continuous cultures. The stability of RCB cultures was demonstrated for twenty-four and thirty-six 1-h cycles carried out over 3 and 6-day periods, respectively. A similar IC-RCB fermentation was successfully used to produce a high concentration of pediocin by *P. acidilactici* UL5 (Naghmouchi, 2003).

Microencapsulation

Probiotic bacteria are "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma 1998). To produce therapeutic benefits, the minimum suggested level of viable probiotic bacteria in a food product should be ≥ 10^7 cfu ml^{-1} or g^{-1} of a product at the time of consumption (Adhikari et al 2000). Despite the importance of viability of these beneficial organisms, several surveys have

shown large fluctuations and poor viability of probiotic bacteria, especially bifidobacteria, in yogurt preparations which is the main probiotic carrier (Schillinger 1999). Acidity, pH, concentration of lactic and acetic acids, hydrogen peroxide, and dissolved oxygen content have been identified to have an effect on viability during manufacture and storage of yoghurt (Dave and Shah 1997). Moreover, because viable and biologically active microorganisms are usually required at the target site in the host, it is essential that the probiotic be able to withstand the host's natural barriers against ingested bacteria.

A microcapsule consists of a semipermeable, spherical, thin, and strong membrane surrounding a liquid core, with a diameter varying from a few microns to 1 mm (Gonçalves et al 1992). Different materials have been used for microcapsulation of LAB and probiotic cultures (Table 2), although many of these technologies do not fall within the strict definition of microencapsulation, but rely on gel entrapment techniques. One important challenge for cell encapsulation is the large size of microbial cells (typically 1-4 μm) or particles of freeze-dried culture (more than 100 μm). This characteristic limits cell loading for small capsules or, when large size capsules are produced, can negatively affect the textural and sensorial properties of food products in which they are added. In almost all cases, gel entrapment using natural biopolymers such as calcium alginate, κ-carrageenan and gellan gum has been favoured by researchers. However, although promising on a laboratory scale, the developed technologies for producing gel beads still present serious difficulties for large-scale production of food-grade microencapsulated microorganisms, such as low production capacity and large bead diameters for the droplet extrusion methods or transfer from organic solvents and large size dispersion for the emulsion techniques, and difficulty to maintain aseptic conditions.

Encapsulation of probiotic cells has been suggested by several authors to enhance cell resistance to freezing and freeze-drying. A higher stability was reported for *Lb. acidophilus* and *B. longum* immobilized in alginate beads compared with FC during storage of frozen dairy desserts (Shah and Ravula 2000) and for *B. longum* in ice cream (Sheu et al 1993). In this latter study, the addition of cryoprotective agents (glycerol and mannitol) in the alginate solution increased the protective effects of immobilization and gave survival rates as high as 90% compared with only 40% for FC. In addition, an optimum capsule size between 30 and 100 μm was suggested for food applications, since a smaller diameter resulted in less cell protection and a larger diameter gave texture and sensory defects in the products. However, ambiguous data were reported for immobilization and freeze-drying, with a positive effect on cell viability for *L. lactis* (Champagne et al 1992) and *Lb. plantarum* (Kearney et al 1990) immobilized in Ca-alginate, but no effect on *B. longum* in κ-carrageenan/LBG (Maitrot et al 1997).

Cell encapsulation also improved probiotic viability in fermented dairy products. Immobilization of *B. longum* in κ-carrageenan (Adhikari et al 2000), *B. bifidum* and *B. infantis* in Ca-alginate (Hussein and Kebary 1999), and *B. infantis* in gellan-xanthan beads (Sun and Griffiths 2000) allowed to maintain high-cell concentrations during 5-week storage of yoghurt, with no change in sensorial properties (Adhikari et al 2000). Picot and Lacroix (2002) studied the encapsulation of *B. breve* and *B. longum* as freeze-dried or fresh cultures in water-insoluble food-grade microcapsules produced by emulsion and/or spray-drying, using milk fat and/or denatured whey proteins as immobilisation material. Viable counts of *B. breve* entrapped in whey protein microcapsules using this method were significantly higher than those of FC after 28 days in yoghurt stored at 4°C (+ 2.6 log cycles), but no effect was observed for *B. longum*. The encapsulation of *B. bifidum* in κ-carrageenan beads maintained cell viability for as long as 24-weeks of cheddar cheese ripening, with no negative effects on texture, appearance and flavor (Dinakar and Mistry 1994).

Table 2: Microcapsules with potential use in the dairy industry.

Matrix	Species	Reference
Alginate	*Lactobacillus plantarum*	Kearney et al 1990
Alginate	*Lactobacillus delbruekii* ssp. *bulgaricus*	Sheu et al 1993
Alginate	*Bifidobacterium bifidum* *Bifidobacterium infantis*	Hussein and Kebary 1999
Alginate	*Bifidobacterium longum*	Lee and Heo 2000
Alginate	*Lactobacillus acidophilus* *Bifidobacterium longum*	Shah and Ravula 2000
Alginate	*Lactobacillus acidophilus* *Bifidobacterium lactis*	Fàvaro Trindade and Grosso 2000
κ-carrageenan	*Bifidobacterium bifidum*	Dinakar and Mistry 1994
κ-carrageenan	*Bifidobacterium longum*	Adhikari et al 2000
κ-carrageenan LBG	*Bifidobacterium longum*	Maitrot et al 1997
Gellan/xanthan	*Bifidobacterium infantis*	Sun and Griffiths 2000
Phtalate cellulose acetate	*Bifidobacterium pseudolongum*	Rao et al 1989

Different encapsulation methods exhibited protective effects on cell viability during *in vitro* tests simulating gastric or intestinal digestion. Exposure to a simulated gastric juice at pH 2.5 caused the FC viable count to drop from $1.2 \ 10^9$ cfu ml^{-1} to undetectable levels in 30 min, while the IC viable count decreased by only 0.67 log cycle within the same period (Sun and Griffiths 2000). Lee and Heo (2000) reported that survival of *B. longum* ATCC 15707 immobilized in calcium alginate beads in simulated gastric juices and bile salt solution was better with higher gel concentrations, with an effect of bead size. Very large beads (i.e. > 2 mm) provided more protection for *B. longum* ATCC 15707 in simulated gastric juices and bile salt solution. Spray drying encapsulation in whey-protein suspension protected *B. breve*, but not *B. longum*, during and after sequential exposure to simulated gastric and intestinal juices (+ 2.7 log cycles) compared with FC (Picot and Lacroix 2002).

Different data obtained for the protective effects of immobilisation might be due to strain-specific effects of immobilisation, but also to differences in methodology of the different studies since numerous experimental factors may affect cell survival. In particular, the technological properties of cells should be taken into account in the selection of encapsulation method, such as heat resistance for spray-dray encapsulation.

Conclusions

Many advantages have been demonstrated for IC systems that may be applied to LAB and probiotic bacteria in the dairy and starter industries. However, no industrial application has emerged yet. This can be explained by important changes in the equipment and management of production, due to the switch from batch to continuous fermentations. In addition, highly qualified employees are certainly required to operate more complex processes with IC and continuous or repeated batch cultures that can run over 24 h daily periods. Application of this

research could be particularly important for the production of probiotic bacteria, functional dairy products containing high concentrations of viable bacteria and bioingredients from LAB with important functional properties for use in foods and health. Immobilization can efficiently protect cells, making this approach potentially useful for delivery of viable bacteria to the gastrointestinal tract of humans via dairy fermented products. It may be anticipated that application of IC technology in the dairy sector will begin with these special cultures which are difficult to propagate and use with the traditional culture techniques, and which are used to produce high-value dairy products with positive effects on consumers' health.

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