Chapter 13
Immobilization of Cells and Enzymes for Fermented Dairy or Meat Products

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13.1 Introduction

Historically, we can find fermented products in almost all cultural backgrounds around the world. Notably, there are many different milk or meat-based foods and this chapter will focus on them (Kosikowski 1982; Wood 1998). Cheese, yoghurt, sour cream, kefir, or cultured butter are probably the most common fermented dairy products, but many regional varieties exist (Farnworth 2004). Fermented meats are typically found as dry sausages (Lüke 1998). Yeasts are mostly involved in the manufacture of bread and alcoholic beverages, which are basically cereal- or fruit-based products. In fermented meat and milk, the main microorganisms used are the lactic acid bacteria (LAB). Yeast and molds are rather involved in ripening. Therefore, the LAB will constitute the main focus of this chapter.

In addressing the potential of immobilized cell technology (ICT) or immobilized enzyme technology (IET) in dairy and meat fermentations or enzymatic processes, we will consider a wide scope of "immobilization" procedures. Therefore, for the purpose of this manuscript, ICT and IET will include adsorption of enzymes or bacteria on surfaces as well as microentrapment or encapsulation in particles of various natures.

In the dairy and meat sectors, lactic starters are now inoculated, which plays a major role in standardizing characteristics of a product. As a rule, starter cultures are added to milk or meat as free cells. However, there are two instances where encapsulated cultures are used commercially (production of LAB and yoghurt), and many potential applications have been proposed. One aim of this chapter is, therefore, to present the current practices with respect to immobilized LAB as well as opportunities for expanded use.

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In the last 15 years, probiotic cultures have also been added to foods for their health benefits. Probiotics can be described as "live microorganisms which, administered in adequate amounts, confer a beneficial physiological effect on the host" (Araya et al. 2002). Adding probiotic bacteria to foods presents a variety of challenges (Champagne et al. 2005). Similarly, protective cultures may be used to improve the safety of a product and its shelf life by competitive exclusion (Hammes and Hertel 1998; Eie et al. 2007). How immobilization (or encapsulation) can be helpful in improving the stability of probiotics and protective cultures in fermented foods will be examined. Please see Chap. 10 also for more information on the microencapsulation of probiotics.

Although the LAB constitute the cornerstone of dairy and meat fermentations, some processes also include enzymes. Rennet and lipases in cheese manufacture represent the best examples. As for the LAB, the enzymes are typically added in free form. But there are circumstances where encapsulation prior to use in the manufacturing process can be very useful and this will also be addressed.

It must be mentioned that many fermentation processes of meat or dairy by-products have been developed, notably, whey fermentation for bioproducts (for example, ethanol or organic acids). ICT has extensively been used for the production of such bioproducts (Norton and Vuillemard 1994). However, these fermentations are designed to generate specialty products or ingredients, and the milk or dairy components are not typically reintroduced into the fermented product. Since there are no fermented foods involved in such applications, this chapter will not address ICT/IET of this type of specialty ingredients in great detail.

High fructose corn syrup and amino acids are the best known examples where IET is used commercially in food processes (Cheetham 1988; see also Chap. 14 of this book). In the dairy sector, enzymes with potential applications include catalase, glucose oxidase, alkaline phosphatase, lysozyme, glucose isomerase, lactoperoxidase, superoxide dismutase, and sulfhydryl oxidase (Fox 1993; Lee 1996), but the principal immobilization of lactases, lipases, and proteinases will be discussed.

Several free enzymes are utilized for meat applications (Table 13.1), but the use of encapsulated enzymes in fermented meat products is largely under-studied. On the other hand, (micro) encapsulation techniques are used for other purposes (Table 13.2). The use of bromelain and papain for meat tenderization is well documented but to our knowledge, none are used encapsulated (Toren 2007). Several enzymes, notably proteinase and lipase have also been tested for the acceleration of dry fermented sausage production but again as free molecules (Naes et al. 1994; Blom et al. 1996). It is nonetheless interesting to mention here that even before muscle is converted to meat, enzymes are added to animal feeds to improve their nutritional and metabolic efficacy. Enzymes used for that purpose represent 6% of the whole enzyme market (Anonymous 2003). The use of phytase to reduce phosphorous emission in the environment from animal feces has been commercially used notably in pig and fish production (Haefner et al. 2005). Although phytase must be released in the gut to be effective, some are encapsulated to provide a protective effect during the processing steps (Benchabane et al. 2004).

Immobilization requires a specific processing step which adds to the costs. Therefore, there must be a clear advantage to carry out immobilization of the bioactive components.

Table 13.1 Examples of enzymes used in various meat related applications

Application	Enzymes	References
Tenderization	Bromelain Papain Ficin Actinidin	TenderIn™ World Technology Ingredients Toren (2007)
Restructured meat and improvement of homogenate gel microstructure	Fibrinogen Trombin Transglutaminase Tyrorinase	Fibrimex®, ex Sonac Loenen Tseng et al. (2006) Lantto et al. (2007)
Fish protein solubilization	Bacterial whole cells	Venugopal (1994)
Enzymatic additives for better feed conversion	Phytase Amylase Fibrozyme® Vergpro®	Anonymous (2003)

Table 13.2 Examples of microencapsulation technologies used for various meat processing applications

Materials	Technology	Matrix	Purpose	References
Meat pigment	Spray-drying	Carbohydrate	Color retention	Shahidi and Pegg (1991)
Volatile meat flavor	Entrapment	Starches	Flavor retention	You-Jin et al. (2003)
Acidulant, antioxidant, salts	Spray-drying or fluidized bed spray-coating	Lipid based coating; cores of variable composition	Direct acidification	Lemay et al. (2002b), Meatshure®, ex Balchem Corporation
Antimicrobials		Various packaging film	Safety and shelf life improvement	Kerry et al. (2006)
Bacteriophage		Under patent application	Safety by controlling pathogens in animal through feed	Murthy (2007) GangaGen Life Sciences Inc.

It can be an economic advantage or it can be that the ICT/IET solves a problem which is encountered when using free cells or enzymes. Having this in mind, the current chapter will not be subdivided into various classes of dairy products or enzymes, but rather on the basis of problems which are solved by ICT/IET or by advantages which are provided by the technology.

13.2 For the Production/Inoculation of Lactic Cultures

The application of ICT for the production of concentrated cultures is indirectly linked to milk or meat fermentations because it initially targets the supplier of cultures used at the dairy or meat processing plants. Indeed, fermented meat and dairy

industries tend to rely on specialized suppliers for their cultures. At these suppliers' production plants, many technological steps in the production process of the lactic cultures are detrimental to the cell viability – oxygen during fermentation, centrifugation or filtration pressures, freezing, or drying. Furthermore, some processing steps require expensive equipment (centrifuges, filtration units, and freeze-drying units) that ought to be optimized. One of the two known current industrial applications of ICT is the production of lactic/probiotic concentrated cultures in order to address these problems. Basically, cells are micro entrapped into gel particles and added to the growth medium (Fig. 13.1). Two techniques for the immobilization of lactic cultures in gels are available, i.e., extrusion and emulsion techniques, and a description of the two is presented in Table 13.3. If one wished to (1) carry out the entrapment procedure directly into the fermentation medium, (2) remain on a small scale, or (3) carry out a coating step with chitosan, then the extrusion method is best. Otherwise, the emulsion technique seems more appropriate.

The cells grow within the gel particles and the concentrated biomass is obtained simply by recovering the particles. Populations close to 10^{11} CFU/mL of gel are obtained with mesophilic organisms (Champagne et al. 1992), particularly if fermentations conducted under continuous culture technology are practiced (Prevost and Divies 1987), but are slightly lower with thermophilic starters (Table 13.4). Once the ICT cultures are recovered, they can be sold to food processors and used in the microencapsulated form in the fermented meat or milk products.

However, there are also disadvantages to this biomass production technology for the culture suppliers. A bead-production step must be added in the concentrated starter/probiotic production process. Secondly, overall yields can be lower (Table 13.4). With *Lactococcus lactis*, the total bead-entrapped population in the fermentor

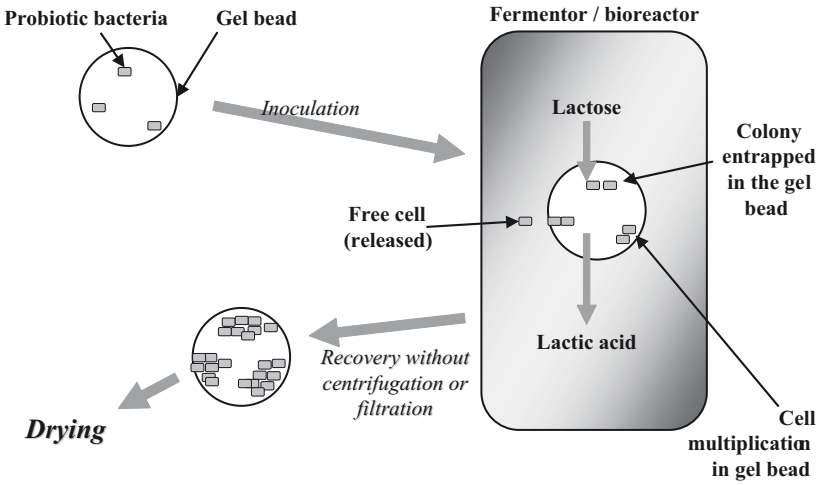

Fig. 13.1 Production of concentrated suspensions of lactic or probiotic cultures in gel beads (Champagne 2006)

Table 13.3 Comparative features of extrusion and emulsion techniques for gel entrapment for biomass production of lactic cultures (adapted from Krasaekoopt et al. 2003)

Parameter	Extrusion	Emulsion
Polymers	Alginate, carrageenan, whey protein	Alginate, carrageenan, agar, gelatin
Technical feasibility	Difficult to scale up	Easy to scale up
Simplicity	High	Low
Done in the fermentation medium itself	Yes	No
Add a coat to the bead	Yes	May be difficult
Survival of microorganisms	5–95%, depending on pH of $CaCl_2$ solution	80–95%
Size of bead	Gravity only: 1–5 mm. Electromagnetic, or vibrating systems, rotating disk: 100 μm to 1 mm	25 μm to 2 mm

Table 13.4 Production of *Lactobacillus rhamnosus* by ICT in alginate beads

Treatment	Content in fermentor	Population (CFU) per mL or g	Total population in fermentor (CFU/L)
Free cell-control	1,000 mL	3.0×10^{9}	3.0×10^{12}
Immobilized cell system			
In the beads (immobilized)	50 g	3.0×10^{10}	1.5×10^{12}
In the broth (free cells)	950 mL	1.1×10^{9}	1.0×10^{12}

Data taken from Champagne et al. (2007)

represented only 25% of that obtained in free-cell fermentation produced in similar conditions and, in the ICT system, free cells represented about half of the population in the fermentor (Morin et al. 1992). This was also observed with thermophilic cultures (Champagne et al. 1993). Third, some lactobacilli do not grow well in alginate gels (Lamboley et al. 2003).

A feature specific to cultures grown in gel beads is that most of the biomass is located on the surface, principally because of mass-transfer limitations of substrates and fermentation products. Cells are therefore released from the beads into the surrounding medium (Champagne et al. 1993). Although this feature is undesirable when the ICT process is used for biomass production, as described above for the industrial manufacturers of starters/probiotics, it becomes desirable when, for example, a dairy plant wishes to use the system for continuous inoculation of milk.

This property led to the second ICT starter process – continuous inoculation of milk. In this inoculation approach, the dairy-based substrate is continuously injected in a bioreactor containing gel particles harboring the bacterial cultures

(Lamboley et al. 2001). As cells are released from the particles, the medium exiting the bioreactor becomes strongly inoculated. After an adaptation period of a few days, the continuous inoculation by multiple-strain mesophilic cultures is achieved, and strain ratios have been shown to be stable up to 53 days (Lamboley et al. 1997). The same phenomenon was demonstrated with yoghurt cultures (Prevost and Divies 1985). Thus, an important feature of this ICT process is standardization of strain ratios in milk inoculation. This obviously is very helpful in ensuring reproducible fermentation rates and constant quality of the finished product.

The strain ratios of a mesophilic cheese mixed starter cultures can be adjusted by changing the pH of the medium, the dilution rate of milk, the bead content in the bioreactor or the incubation temperature. In response to pH or temperature parameters, the strain ratios in the ICT system followed patterns similar to free-cell cultures. Thus, lowering the pH of the medium below 6.0, and increasing the incubation temperature above 32°C, tends to be more detrimental to *Lactococcus lactis* ssp. *cremoris* than to *Lactococcus lactis* ssp. *lactis* (Lamboley et al. 1997). The great advantage of this technique is that bulk starter preparation can be eliminated. Thus, the ICT technique is a much more economical method for milk inoculation than the traditional starter technology preparation processes, and even more economical compared to the direct inoculation procedure with frozen or freeze-dried cultures. Furthermore, the technique can potentially be used to inoculate milk with probiotics such as bifidobacteria (Doleyres and Lacroix 2005).

However, an important disadvantage of the continuous inoculation ICT technique is the potential attacks of the released cells by bacteriophages. Indeed, bead-entrapped cells in the bioreactor are protected against phages (Steenson et al. 1987), but not the free cells released into the milk. Milk for cheese making is not sterilized and bacteriophages from raw milk can survive pasteurization. Therefore phages in raw milk can contaminate the bioreactor and implant themselves (Macedo et al. 1999). Hence, in considering the application of ICT for milk inoculation, a certain number of parameters must be examined. First, the technology appears to be better adapted to yoghurt manufacture, rather than cheese production, because (1) the yoghurt milk is heated at temperatures which destroy bacteriophages prior to fermentation, and because (2) the thermophilic cultures used in yoghurt processing are simply less prone to bacteriophage attack than mesophilic LAB used in cheese making. Another aspect to consider is the problem related to the storage of the beads when the plant is not manufacturing fermented milks. Data show that the beads can be stored for 48 h at 4°C in peptone water, but a reactivation/stabilization period of 4 h is recommended. Finally, many studies examining the ICT continuous inoculation process were carried out in supplemented whey media, and it is to be expected that the use of different medium might influence the behavior of the cultures.

ICT-based continuous inoculation of milk can actually reduce the overall fermentation times. In yoghurt and cheese making batch processes, milk is typically inoculated between 1 and 5×10^7 CFU/mL. In ICT bioreactors designed for continuous milk inoculation, the dilution rate (D) can be adjusted to generate much higher free cell populations in the effluents than the populations initially found after inoculation

in batch processes. Thus, for a yoghurt culture, an effluent population of 1×10^8 CFU/mL can be achieved at $D = 10\ h^{-1}$ (Prevost and Divies 1988b) and even higher populations are reached at lower D values (Prevost and Divies 1988a). Obviously, higher inoculation rates results in shorter fermentation times (Prevost and Divies 1985). The same strategy was applied to a mesophilic starter culture, resulting in a 50% shorter fermentation times for a fresh cheese (Philadelphia, "fromages frais") or sour cream type fermentations (Prevost and Divies 1987).

It is noteworthy that the ICT continuous inoculation technique not only serves to stabilize strain inoculation, but the cells released from the beads have different properties and physiology compared to free cells. It is known that cells growing at high cell density in biofilm have increased resistance to adverse environmental conditions (Bower and Daeschel 1999). ICT cultures are exposed to comparable high cell density environments. Hence, it can be hypothesized that cells of LAB released from the beads should be less sensitive to a variety of residual antibiotics or sanitizers in milk than are the traditional free cells (Doleyres and Lacroix 2005). This property has not yet been exploited and deserves further examination.

13.3 For Shorter Fermentation Times

13.3.1 Immobilized Cells

As mentioned previously, ICT inoculation can reduce the fermentation time in fermented milks. Contrary to the dairy sector, there is little research on encapsulation of starter, probiotic or protective cultures in meat fermentation (Työppönen et al. 2003; Saucier and Champagne 2005). As a rule, the specific acidifying rate of free cells is higher than that of ICT cultures in alginate gel beads (Champagne et al. 1992) because of mass transfer limitations. However, there is one instance where this was not observed. Kearney et al. (1990) carried out meat fermentation with free and immobilized cultures of *Lactobacillus plantarum* and *Pediococcus pentosaceus*. The fermentation time required to obtain a pH of 5.0 was reduced from 45 to 28 h with the alginate-encapsulated cells. The authors suggest that the greater fermentation performance of the ICT culture was linked to the microenvironment enabling greater availability of nutrients (i.e., skim milk) and more protective hydration conditions within the beads. It is well known that the composition of the hydrating medium will influence the viability of the cultures (De Valdez et al. 1985). Since dry sausage formulations contain salts and other antimicrobial agents which are detrimental to the viability of the cells, it is not surprising that the microenvironment in the gel particles during hydration might be more favorable, for greater viability, than in the batter itself as free cells. Furthermore, a drying process, to various water activity levels, is often carried out after the fermentation step to improve shelf life.

13.3.2 Immobilized Lactase or Proteases

Lactase (β-D-galactosidase) converts lactose into glucose and galactose. The major applications of lactase have been in fluid and powdered milk to alleviate lactose maldigestion and to improve milk sweetness (Lee 1996). In Asia where lactose intolerance is predominant, lactase treatment is compulsory to manufacture yogurt or cheese. Lactose is also a hygroscopic sugar that has a strong tendency to absorb flavors and odors and causes many defects in refrigerated foods such as crystallization in dairy foods, development of sandy or gritty mouth feel (Panesar et al. 2006).

With respect to fermented milks, in addition to its health benefit for the populations which do not digest lactose well, lactose hydrolysis would have the following benefits: (1) faster acidification rates by LAB (Gaudreau et al. 2005), (2) higher viable counts (Shah et al. 1997) and (3) synthesis of oligosaccharides as prebiotics (Hung et al. 2001).

Various systems have been designed for immobilized lactase: permeabilized cells in alginate beads (Genari et al. 2003), immobilization on cellulose (Roy and Gupta 2003), boronate- or chelate-epoxy beads (Pessela et al. 2003) or chitin beads (Illanes et al. 2000), as well as corn grits (Siso et al. 1994).

Although numerous hydrolysis systems have been investigated, only a few of them have been scaled up with success. Centrale del Latte of Milan, Italy, utilized the SNAM Progetti technology process which makes use of a neutral lactase from *Saccharomyces* (*Kluyveromyces*) *lactis* entrapped in cellulose triacetate fibers. Sumitomo Chemical, Japan, has developed an immobilized β-D-galactosidase preparation of fungal origin on the rugged surface of an amphoteric ion-exchange resin of phenol formaldehyde polymer and this technology was used by Drouin Cooperative Butter Factory for producing pasteurized milk and hydrolysed whey (Honda et al. 1993). A rotary column reactor has been developed by Snow Brand's factory that could be used as a stirred tank reactor and a packed bed reactor, since this apparatus may be used in both types of processing. Pasteurization of immobilized β-D-galactosidase using glycerol or propylene glycol was effective without inactivation of β-D-galactosidase. Major problems associated with the immobilized enzyme system still are microbial contamination, protein adherence and channeling. Therefore, for long-term operations, using immobilized enzymes, periodic washing and medium pasteurization are indispensable during operation.

In summary, in deciding if IET should be adopted for a process of lactose hydrolysis of milk used for lactic fermentations, one must ascertain if at least one of the following benefits can be obtained: less lactose maldigestion problems, higher acidification rates, higher populations and increased oligosaccharides levels. However, since scale up presents many challenges, the IET process has only been commercially applied to unfermented milk(s).

Proteinase treatment of milk has also been shown to be beneficial to the subsequent acidification rate in yoghurt cultures (Hemantha-Kumar et al. 2001). However, no proteolytic IET are reported in the literature for specific improvements in fermented milk(s).

Protease immobilization could also be used for a continuous coagulation process. The major proteinase used in dairy is rennet. Immobilization techniques have been investigated for recycling rennet, other milk clotting enzymes and microbial chymosin. Cheryl et al. (1975) used a continuous coagulation system for milk using pepsin and rennet in porous and alkylamine glass incorporated into a fluidized-bed reactor with the overall superior performance. Other continuous cheese-making process using an immobilized rennet enzyme reactor through a spiral flow path has been patented (Goldberg and Chen 1989). In this process, spiral flow path is formed by a spirally wound microporous sheet containing the immobilized rennet. Anprung et al. (1989) found that the most active immobilized rennin resulted from using 50-mesh river-bed sand as a carrier by covalent bonding that retained 82.6% of its original activity after 4 months.

13.4 For Accelerated Flavor Development

Two immobilized systems are available: attenuated cells and encapsulated enzymes. Both are available on the market and potential users must contact suppliers for benefits and limits.

Since LAB contain the main enzymes for cheese ripening and lactose hydrolysis, they are a logical source for "encapsulated" enzymes. However, adding more starter cells affects the technological process (acidification rate, final pH) which can be undesirable. One option is to reduce the acidification property (attenuation) of the cultures while maintaining the enzymatic components active. Contrary to proteinases which are located at the cell surface, peptidases are intracellular constituents. The literature shows that the extent of LAB autolysis affects the levels of various peptides in cheese extracts (Azarnia et al. 2006). Therefore, attenuated cultures can be seen as adsorbed or encapsulated enzymes, a sophisticated form of IET and its success requires two components: (1) a process to control the acidifying ability of the cultures, and (2) the autolysis ability to release the enzyme into the cheese matrix during ripening. Experience shows that attenuating the cultures in itself is difficult (Law 1999), and autolysis is variable as well (O'Reilly et al. 2002). Nevertheless, some of these problems have been overcome and there are commercial products in the market (Law 1999).

Since many reactions are required to generate cheese flavor, it is unlikely that a single enzyme can possibly generate all the flavor compounds required for a typical cheese flavor profile. Consequently, the development of an adequate flavor might require microencapsulation in multi-enzymes systems (Azarnia et al. 2006). Peptidases seem to be critical components in the effectiveness of these accelerated ripening systems, but proteinases can also be present (Kheadr et al. 2000). The addition of unencapsulated proteases for ripening directly into the milk has two major drawbacks: (1) proteolysis begins too early which can affect gel structure or yields, and (2) it is estimated that 95% of the added enzyme is actually lost in

Table 13.5 Some enzyme encapsulation techniques for accelerated cheese ripening

Encapsulation matrix/ technology	Effectiveness/benefit	References
Liposomes – phospholipids	– Pure lipids may be too expensive for economic viability – Prevented bitter off-flavors	Law (1999) Kheadr et al. (2000), (2003)
Gum beads composed of alginate, carrageenan, pectin, etc.	– 50% encapsulation efficiency, 90% recovery in cheese – Increased proteolysis but no effect on sensory	Kailasapathy and Lam (2005) Anjani et al. (2007)
High melting point milk fat particles prepared by spray coating	– 40% encapsulation efficiency, 74% recovery in cheese – Slower enzyme release than gums	Kailasapathy and Lam (2005)
Water-in-oil emulsions	– 80–90% encapsulation efficiency – Eightfold increase in diacetyl concentration during ripening	Magee and Olson (1981)

the whey (Law 1999). This has led to a number of studies which have examined encapsulation for accelerated cheese ripening (Table 13.5). Microencapsulated peptidases seem particularly promising (Kailasapathy et al. 2006). Although, some microencapsulation methods of cheese ripening have been effective, their industrial use remains limited because other approaches are available to cheese manufacturers, such as inoculation with specialty cultures (Azarnia et al. 2006).

Lipases are added into many type of cheeses (e.g., Romano, Provolone, Parmesan, Feta) to modify flavors. They have traditionally been added as free enzymes. However, as for ripening enzymes, losses occur in the whey. As mentioned previously, many reactions are involved in the development of Cheddar cheese flavor; one research team in particular has added microencapsulated lipase in combination with various proteases in order to generate multiple flavor elements (Kheadr et al. 2003). In general, cheeses made with a mixture of microencapsulated lipase and bacterial proteases were preferred to control cheeses. Even microencapsulated lipase alone benefits Cheddar cheese ripening (Kheadr et al. 2002).

13.5 For Protection During Food Processing

13.5.1 For Protection of Cells During Fermentation and Drying in Specialty Sausages

Probiotic bacteria are mostly added to dairy products, but there are novel applications for these cultures, and addition to dry sausages has been proposed (Työppönen et al. 2003). In one study, free or alginate-microencapsulated cells of *Lactobacillus*

reuteri were added to the meat batter and viability followed during processing. Following the fermentation and drying periods, free cell counts dropped by 2.6 log units, whereas alginate-microencapsulated *L. reuteri* were reduced by approximately 0.5 log (Muthukumarasamy and Holley 2006). Furthermore, no significant difference in sensory quality was found between the control and sausages containing either unencapsulated or microencapsulated *L. reuteri*. This is another example where one of the concerns of the use of ICT cultures, i.e., negative impacts on sensory properties, did not occur. Unfortunately, while micro-encapsulation increased survival of *L. reuteri* and *Bifidobacterium longum*, it reduced their inhibitory action against *Escherichia coli* O157:H7 (Muthukumarasamy and Holley 2007).

13.5.2 For Enhanced Survival of Cells to Heating

In dairy processes, starters are always added to milk after heat treatment if one is applied. In meat fermentation, the raw batter is not heat treated prior to fermentation. So, the meat must be of excellent microbial quality in order to have an optimal control of the fermentation process. In fresh cheeses, a typical pasteurization treatment is applied (73°C for 15 s), but in yoghurt, the heat treatment is usually above 85°C. Free cells of LAB cannot survive these processes, so attempts were made to improve heat resistance using encapsulation techniques. Mandal et al. (2006) showed that *Lactobacillus casei* cells microencapsulated in alginate particles were more resistant to heat processes at 55–65°C. It was also found, in a MRS broth acidified to pH 5.0, that LAB encapsulated in alginate beads were more resistant to a heat treatment of 55°C for 15 min than were free-cells (Lemay et al. 2002a). Limited data suggest that microencapsulation of LAB in spray-coated particles could even be better than alginate beads; Goulet and Wozniak (2002) report enhanced survival of spray-coated lactobacilli to heat treatment at 50°C for up to 7 h in a simulated dry food process.

All these data point to enhanced survival of ICT or encapsulated cultures to moderate heating treatments. In practice, however, this enhanced survival is still not sufficient to enable inoculation prior to the higher heat treatment (73–95°C) used in dairy processes. With respect to meats, unexpectedly, when alginate-microencapsulated *Lactobacillus casei* were added in an acidified chicken meat model, the improved resistance conferred in the broth model did not extend to the solid matrix (Lemay et al. 2002a). In a more recent study, the efficacy of alginate-microencapsulated LAB to survive various heat treatments applied in sausage processing was confirmed by Muthukumarasamy et al. (2006). These data demonstrate the potential of microencapsulation in gel particles for enhanced survival of lactic culture to a thermal processing step, but that the food matrix itself can affect the results.

13.5.3 For Enhanced Survival of Cells to Freezing

Fermented milks can be frozen and are termed “frozen yoghurt” or “frozen desserts.” In some instances, probiotic bacteria are included in the dairy mix. Freezing LAB or probiotics in unfermented media generates losses in viability ranging from

10 to 90% as a function of strain and conditions (Champagne et al. 2005). Thus, in deciding if ICT is useful one must examine the stability of the selected probiotic strain. If viability losses are greater than 90% (1 log) then ICT cultures become economically advantageous. The high total solids level in ice cream mix including fat (emulsion) may provide some protection to the cells (Kailasapathy and Sultana 2003). However, viability losses are greater with fermented products (Laroia and Martin 1991). Thus, a 1 log reduction in *Bifidobacterium longum* viable counts was observed following freezing in a low acid yogurt (pH 5.85) but a 2 log reduction was recorded when the pH was 4.47 (Modler and Villa-Garcia 1993). Hence, the low pH of fermented milks enhances the detrimental effect of freezing.

Many studies in various dairy desserts show that probiotics microencapsulated in alginate or carrageenan beads have lower viability losses following freezing (Sheu and Marshall 1993; Shah and Ravula 2000). This is particularly evident when cryoprotectants, such as glycerol, are added in the gel particles (Sheu et al. 1993). Microencapsulation enables the use of such protective compounds in the beads, without having to add them to the ice milk mix, where they would most likely affect sensory properties. Therefore, the microenvironment provided by beads seems to be a critical aspect for a successful application and this is particularly the case with low pH fermented products.

Unfortunately, this approach also has its drawbacks. With large beads, an effect on the texture of the fermented foods is to be expected which, in a number of applications, is undesirable. In frozen desserts, it was found that gel particles had to be 30 μm in diameter or less in order to avoid a detectable effect on texture (Sheu et al. 1993). Grinding the ICT beads after a freeze-drying step could generate such particle sizes (Lemay et al. 2002a) but the effect on sensory properties requires further investigation.

13.5.4 For Protection Against Bacteriophage Attack

Bacteriophages are viruses which attack specifically prokaryotic cells including lactic cultures. Extensive bacteriophage development during a lactic fermentation significantly affects acidification rates and can even provoke a complete stop in acid production (Fig. 13.2). Therefore, they are a constant concern in cheese making plants. At the dairy plant level, means to protect starter cultures against bacteriophage infection typically include plant design and production set up (preventing cheese whey from contaminating milk), starter tank design and operation, culture rotations, use of direct vat set (DVS) cultures and sanitation. It was shown that LAB in alginate beads are protected from bacteriophages (Steenson et al. 1987) and that acidification is maintained in phage-contaminated milk (Fig. 13.2). Interestingly, phage counts increased during the fermentation, presumably on released cells or on those growing at the surface of the beads. Although, this ICT property would theoretically be of great value to the industry, commercial application is still lacking. This might be due to the lower specific activity of ICT cultures (Champagne et al.

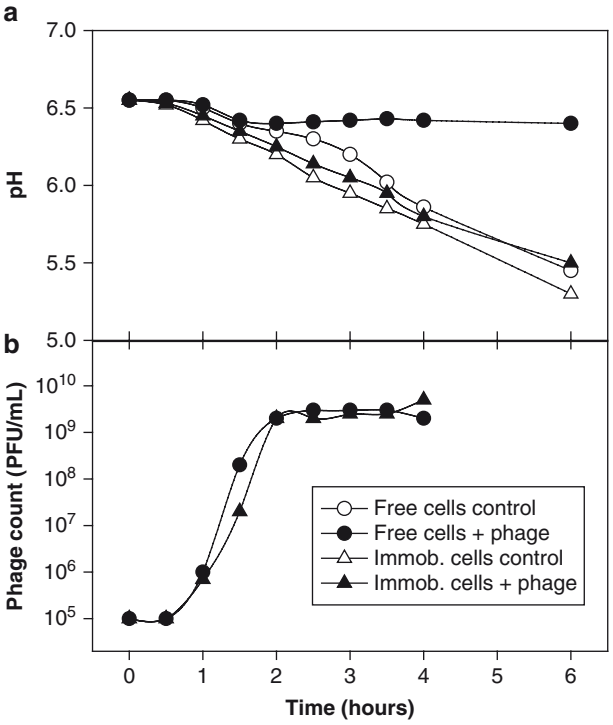

Fig. 13.2 Effect of microentrapment of lactococci into alginate beads and of bacteriophage contamination of the bioreactor on (**a**) milk acidification, and (**b**) bacteriophage growth in the system (Champagne et al. 1992)

1992) or to the potentially detrimental effect on texture resulting from the presence of the beads. This being said, the data showing phage protection were obtained on rather large beads (1–2 mm in diameter) and it remains to be determined if the smaller particles could also enable satisfactory acidification in the presence of phages. Reducing particle size diminishes the benefits of microencapsulation on survival to freezing (Sheu et al. 1993). So, it can be questioned whether the same would occur with phage protection.

All data on phage protection have been obtained with alginate as the matrix. In some countries there might be legislations that prohibit the use of non-dairy ingredients. In this instance, extrusion particles made from whey protein instead of alginate (Reid et al. 2005) may be considered.

In fermented meats, bacteriophages are not so much of a concern mainly because the phage particles do not spread easily in a solid matrix and therefore limits the viral infection. Hence, the benefit of ICT towards phage attacks would not be of great value in meat products.

13.6 For Improved Stability of Cells During Storage

Although attractive, the trend of adding probiotic cultures to fermented milk is facing several challenges including maintaining the viability of the cells in the product and in the gastrointestinal tract (Muthukumarasamy et al. 2006; see also Chap. 10). The same statement applies to protective cultures used to improve food safety by means of competitive exclusion and production of inhibitory compounds (ex. bacteriocin). Since viability of probiotics is considered critical in their functionality and because there are numerous reports of their viability losses in fermented dairy products (Champagne et al. 2005), many studies have examined the benefits of microencapsulated probiotic cultures during the storage of fermented milk products and passage into the gastrointestinal tract (Muthukumarasamy et al. 2006). With respect to enhanced stability, the ICT benefits are not convincing in frozen products (Sheu et al. 1993) or cheese (Gobbetti et al. 1998; Kailasapathy and Masondole 2005). As for survival to the freezing process itself, in deciding if ICT is useful one must examine the stability of the given probiotic strain. If viability losses are greater than 90% (1 log) then ICT cultures become economically advantageous. However, a very different picture is obtained in yoghurt-like products. Although there are discrepancies, most teams report increased viability of probiotics when they are microencapsulated in gel beads (Adhikari et al. 2000; Anjani et al. 2004). It was assumed that microencapsulated cells were better protected against acidity, but Talwalkar and Kailasapathy (2003) observed that the benefits of microentrapment only occurred when there is oxygen present in the medium. Microencapsulation thus appears to provide a microenvironment having reduced oxygen levels, which prevent viability losses to oxygen-sensitive strains (Talwalkar and Kailasapathy 2004). Data from McMaster et al. (2005) also show an enhanced degree of oxygen tolerance by bifidobacteria in gel beads. Thus, ICT is clearly beneficial in fermented milk products to improve storage stability of probiotic and protective cultures.

In one study, the addition of probiotic cultures either in the free or encapsulated state did not significantly affect appearance and color, acidity, flavor and after taste of the yogurts during storage. There were, however, significant differences in texture (smoothness) of the yoghurts (Kailasapathy 2006). In one current commercial ICT application, encapsulated bifidobacteria are added as beads into yoghurt. They can be easily detected both visually and in the mouth feel. In this application the use of capsules is clearly stated on the label, and ICT almost appears as a marketing benefit for product differentiation.

13.7 For the Inhibition of Undesirable Flora

Catalase decomposes hydrogen peroxide into oxygen and water. In some processes, hydrogen peroxide is added to raw milk to activate the lactoperoxidase-thiocyanate preservation system (Boots and Floris 2006). Catalase is then added to remove the residual peroxide. It would therefore seem logical to have an

immobilized enzyme system to remove peroxide from milk in a continuous fashion.

Lysozyme is marketed to prevent clostridia overgrowth and gas production in a certain number of ripened cheeses, particularly the "swiss-type" varieties. It also has potential for the preservation of other foods including meats (Bower et al. 1998).

Although microencapsulated antimicrobials are used in bakery, to our knowledge, no immobilized catalase or microencapsulated lysozyme has been used for these purposes so far. Should the lysozyme negatively affect the lactic cultures, then spray-coating with a fat which would only melt at a cooking stage of the cheese making process could be considered. Spray-coating is typically carried out by spraying a coating material (e.g., fat-based) in a fluidized bed system on a core powder containing the cell or enzyme components (Fig. 13.3). It is not cell immobilization process as such, but rather an encapsulation technology. In some applications, it is more appropriate to use coated bioactives than to use microentrapped or immobilized ones.

13.8 Conclusions

It can be seen that immobilization of cells and enzymes has the potential to address many problems in food processing. However, particular conditions are often required before one can consider ICT or IET and a summary of those which were presented throughout the chapter is presented in (Table 13.6). Some processes are already used

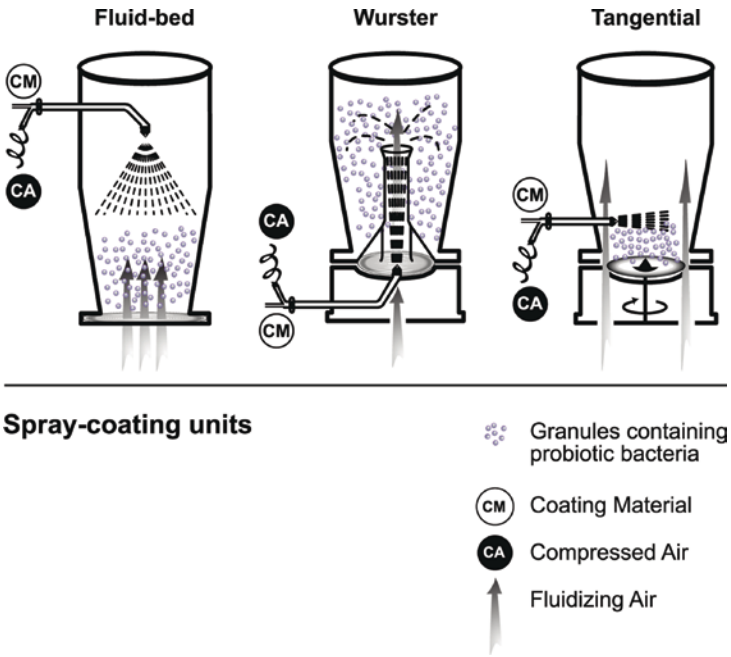

Fig. 13.3 Various systems which enable the spray-coating of cultures in a fluidized bed system

Table 13.6 Conditions to choose ICT or IET bioactives over free-cell of free enzymes in fermented dairy or meat products

Application	Condition
All applications	Encapsulation or immobilization adds to the cost. There must be one of the following benefits: – Protecting the stability ingredient – Enhanced functionality of the ingredient – Enhanced retention of the ingredient in the end-product – Product differentiation
Biomass production of starter cultures	– A strain is difficult to produce with traditional processing methods – ICT confers a functionality (protection against phages or GI conditions, stability during storage)
Continuous inoculation of milk with starter or probiotic cultures	– Milk is heated to destroy phages (ex. yoghurt) – Cultures are less prone to phage attack (processes which do not generate whey, thermophilic cultures) – Specialty cultures are to be added (probiotics) – Few production interruptions occur (weekends) – Need for cultures having specific functionalities generated by immobilization
Meat fermentations	– With alginate particles
Attenuated cells for flavor enhancement	– Cultures must autolyse in the given cheese matrix (process, pH, salt level etc.) to release enzymes
Survival to heating	– For foods that are heterogeneous in appearance or texture – Encapsulation by spray-coating is more efficient than immobilization into alginate bead for this application – Encapsulation by spray-coating with a fats having high melting point temperatures is preferable if one whishes release of the contents at a given temperature
Protection against bacteriophage attack	– With whey-based particles – In ripened cheeses because beads can dissolve
Survival to freezing Stability of probiotics during storage	– If a strain shows unacceptable viability losses (>1 log) to the freezing process or and/or over the storage period
Inhibition of undesirable flora	– Spray-coated with fats in fluidized bed systems with compounds which enable a triggered delivery of the compounds at a late stage of processing

commercially, particularly with flavor acceleration, stability of probiotics and protective cultures in foods. In the future, it can be expected that technological applications of ICT or IET will increase, as novel benefits will also appear. Thus, immobilization (encapsulation) of probiotics may not only have technological benefits, but may also improve the functionality of the cultures by enabling improved survival of the gastric environment as well as controlled release of the cells in the gastro-intestinal tract (Muthukumarasamy et al. 2006). Similarly, protective culture survival to antimicrobial systems used in food requires further investigation. Valuable enzymes which could have beneficial health effects, such as α- or β-D-galactosidases (for soy or milk carbohydrates, respectively), could also benefit from encapsulation in the same fashion. Thus, continued research in ICT and IET is warranted not only to improve technological processes and the safety of foods, but also to enhance the functionality of valuable bioactive ingredients in functional foods.

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