

Encapsulation of enzymes : potentiality and applications

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Introduction

Enzymes are biocatalysts, designed mainly to act inside the biological cells or in surrounding of these cells. In many case, they interact with cell membranes or settle down on solid surface to get their full activity. While use by engineers in free solution, they often show low stability and are washed out in continuous process.

In the 60's, it was a strong interest for enzyme immobilization to get more stable and long term activity. Entrapment and encapsulation were not successful due to enzyme leaking from microcapsules. The applications were mainly based on maximum conversion speed. In microcapsules, it exists a mass transfer limitation, which reduces the overall performances.

However, strong progresses have been made in understanding how to design microcapsules for enzymes and number of applications have been proposed that didn't require maximum performances. The objective of the present contribution is to present different aspects of enzyme encapsulation through a few examples.

Enzyme versus cell encapsulation

It is important to emphasis some differences between enzyme and biological cell behaviors (even they may look obvious) to understand well the challenge.

- Cells are large biocatalysts (a few micrometers) in regards to enzymes (a few hundreds nanometers). Most cells are really immobilized while often enzymes could diffuse in hydrogel or through semi-permeable membranes.
- Cell could multiply while enzyme not. Lost of one or two log in cell concentrations during encapsulation may be acceptable as they could be recovered during fermentation. In case of enzyme, a limited lost may be acceptable if associated with a real gain in activity and stability.
- In regard to microencapsulation, cells could be regarded as quite similar while enzymes differ by their size, charge, hydrophilicity and sensibility. Generic rules are more difficult to drawn in case of enzymes.
- Most cells will profit of the immobilization to organize themselves, make exchanges with other cells and get protection. In case of enzyme, immobilization must be favorable but the interaction with the matrix may have beneficial or detrimental effect on the activity.

Artificial cells

One of the most elegant demonstrations of the interest of enzyme encapsulation are the artificial cells proposed by TMS Chang (Chang, 1964). Inside a semi-permeable membrane, several enzymes and cofactors are entrapped (Figure 1). Some enzymes degrade a substrate (catabolism) while some others regenerate the cofacteur by consuming a second substrate (anabolism). Excluding the reproduction, this could be compared to a simple model of biological cells. These types of capsules are used by injestion and allow treating different diseases.

The capsules are produced often by interfacial polymerization, i.e. reaction between two monomers at the interface of emulsion (Figure 2, Poncelet, 1990).

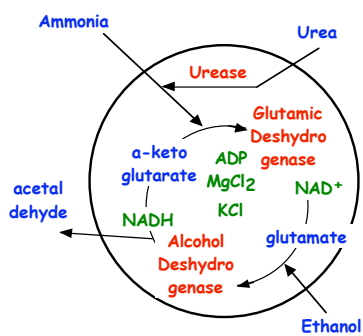


Figure 1 : Artificial cells

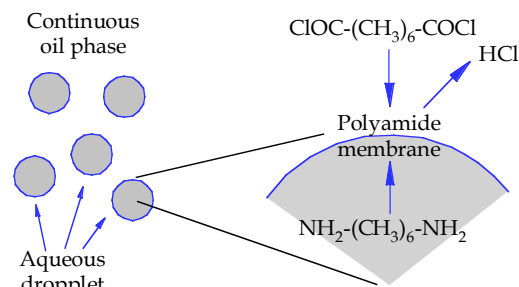


Figure 2 : Interfacial polymerization

Enzyme for detergence

Many enzymes lead to allergy and even more could attack mucus (leaps, nozze). It is then now a very strong regulation to avoid any trace of enzymes in air during manipulations. The largest applications of enzymes today are the detergence with thousands tons per year. To avoid fine enzyme particles, most enzyme producing companies (DSM, Genecor, Novozymes ..) have conducted very large research to immobilize enzymes in mechanically resistant microcapsules and avoid any dust during manipulation of enzymes. For example, Novozymes (<http://www.novozymes.com>) has developed a soft microball containing enzymes able to deform strongly without breakage (Figure 3).

The objective is to offer protection for the users during manipulations but also for the enzymes during storage. In most case, enzymes are entrapped in dry microcapsules, either in the core or in one of the layers formed around an inert core. Enzymes are generally released at a specific stage of the applications (soaking, washing). To reach it, initial particles are coated with different layers in a spray coated (Figure 4). Some layers offer mechanical protection against shock and friction, some contain protective agents against acidity or oxygen, some deal with the controlled release, some include activators or even enzymes. It is then a quite complex system. Genecor has the biggest production plan (80 spray nozzles spray coater) and production of batches could stand for 8 hours.



Figure 3 : Capsules for detergences

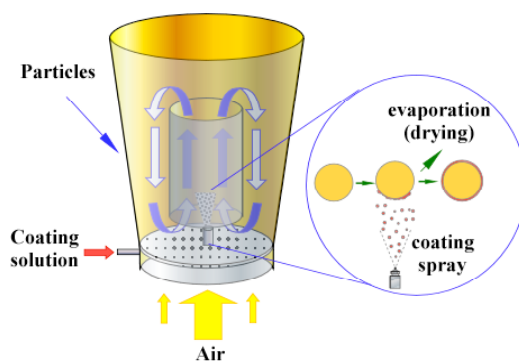


Figure 4 : Spray coating Process

One of the biggest challenges in this field is to find a technology to include enzyme in liquid detergent. Enzymes have to be protected during storage. The biggest problem is the presence of water in such liquid. Water is the smallest liquid molecule, able to “wet” and diffuse into many material. Finding a good barrier which release enzymes during applications without giving “ghost” at relatively low temperature is to day not solved.

Encapsulation of enzymes for the cosmetics

The cosmetics industry uses several enzymes including proteases for reducing skin wrinkle. However, the activity of these enzymes decreases quickly in aqueous cream due to self-digestion. One way to solve it is to immobilize the enzyme. Most of the proteases are low molecular enzymes, quite hydrophilic. In a frame of an industrial project, as a first attempt, we simply entrap the selected protease in thermogel microbeads (by emulsifying the warm hydrogel-enzyme solution in oil and dropping the temperature). While we were expecting the need for a membrane to keep the enzyme in the microbeads, it appears that 95 percents of the enzyme was kept in simple hydrogel microbeads, intrinsic activity (evaluated by degradation of small peptides) was fully maintained even during storage at room temperature.

Taking into consideration that the size of the enzyme was lower than the molecular cut-off of the thermogel, our hypothesis was that some interactions were occurring between the enzyme and the hydrogel. The binding of the enzyme on the gel network leads to a stable immobilization but also avoid self-digestion allowing stabilization of the active. The release of the size was trigger simply by a small increase of the temperature and shear during cream staggering on the skin.

Such behavior could not be generalized. Not all enzymes will form a complex with hydrogel. Opposite charge of the two components may be a criteria but a specific enzyme-hydrogel molecular interaction may also explain the complex formation. However, if such structures are too stable or if hydrogel mask the enzyme active center, activity would be lost.

Encapsulation in beer maturation

Beer is produced in two steps. In a first one, yeasts will degrade substrate to give flavors and alcohol. In the second step, flavors will be enhanced and balanced. During this maturation, unflavored components will be degraded.

This is especially the case for diacetyl, which gives a butter taste to the beer. This unflavored molecule results from a slow but spontaneous degradation of the α -acetolactate produced during first fermentation (Figure 5). One have to wait up to 3 weeks to get a complete transformation of α -acetolactate in diacetyl and degradation of this diacetyl in acetoin before to market the beer.

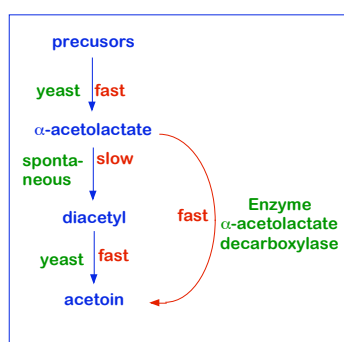


Figure 5. Diacetyl pathway

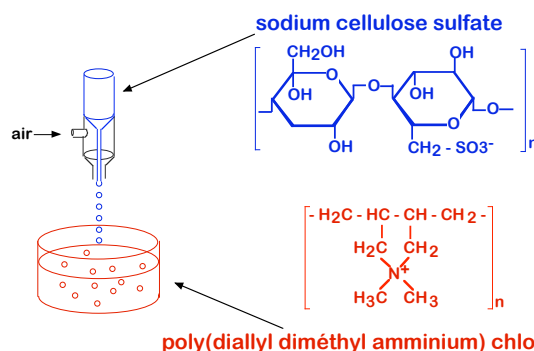


Figure 6. Interfacial coacervation method

Different solutions have been proposed to reduce the process time. One consists to use α -acetolactate decarboxylase. Due to its cost and some residual of protease in the enzyme preparation, the encapsulation and recycling of the enzyme were requested. The enzyme has low molecular weight (30 000 daltons) and is hydrophilic. Different methods of encapsulation have been tested (hydrogel beads, interfacial polymerization...). Only one seems successful to keep activity and real

immobilization : interfacial coacervation or polyelectrolyte complex membrane (membrane formed by interaction of two opposite charged polymers at droplet interface, Figure 6, Dulieu, 1990).

Figure 7a and b show that such encapsulated enzyme while added to beer allows reducing strongly the maturation period regarding the elimination of the diacetyl.

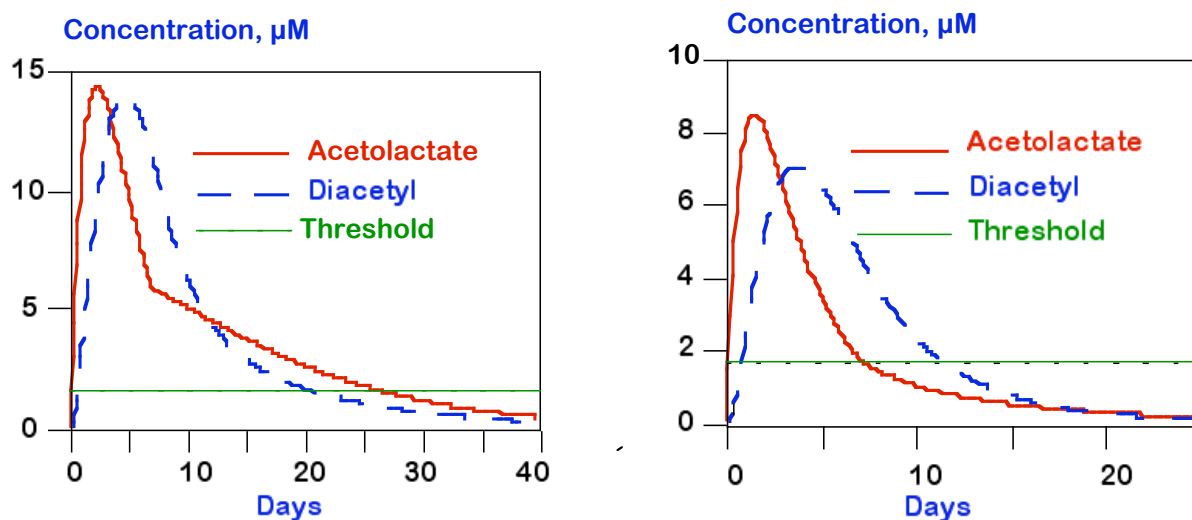


Figure 7. Second fermentation a) without b) with enzyme (α -acetolactate decarboxylase)

Enzyme encapsulation for cheese ripening

Cheese is also produced in two steps: coagulation of the milk and ripening to develop flavor. Ripening with biological cells starts slowly requesting long maturation and ends with a high activity resulting in low shelf life. Enzyme ripening starts faster and activity decreases with time, allowing short ripening time and long shelf life. However, 90 percents of the enzyme are lost in the whey. Prehydrolysis is observed during coagulation associated with yield lost and bitterness formation.

To solve it, we have tested enzyme entrapment in hydrogel beads. Enzymes release slowly after beads have been incorporated in the fresh cheese allowing getting less than 10 percents in the whey, avoiding bitterness (Reparet, 1996).

Conclusions

Enzyme encapsulation has a large potential. One could also consider it in medical applications (wound healing) or biosensors. However, the actual industrial applications remains limited, except the protection during storage and personal care, which represent already a very important business.

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