U. PRUESSE
K.–D. VORLOP

FAL–TB – Braunschweig, Germany

IMMOBILISATION TECHNOLOGIES IN LAB AND INDUSTRY

Immobilised biocatalysts play or will in future play an important role in various industries like biotechnology, medicine, agriculture, chemical, pharmaceuticals and the food industry. Thus, plenty of different immobilisation strategies and technologies exist. The choice of the right immobilisation procedure requires knowledge about the biocatalyst, the matrix material, the preferred form of the immobilisate and the technology to produce it as well as about the process and its scale. Not every immobilisation procedure which is suitable for lab–scale application in the process development can be scaled–up to industrial dimension.

IMMOBILISATION – MATERIALS, METHODS AND FORMS

In order to achieve a successful immobilisation several parameters along the process chain starting from the biocatalyst over the matrix material and the immobilisation method to the technology suitable to produce the desired immobilisate form have to be taken into consideration. This means that the different steps can not treated separately as often one influences the others.

An immobilisation process starts with the biocatalyst (cell or enzyme) which shall be immobilized. In the simplest immobilisation method – adsorption – the biocatalyst can be immobilized either by van der Waals interactions or electrostatic interactions at a wide range of matrices (Fig. 1, left). Nevertheless, depending on the process to be carried out, adsorption might not be suitable as this method generally is susceptible to contamination and loss of the desired biocatalyst. In such cases covalent bonding to a surface or crosslinking (Fig. 1, left) might be applied if the biocatalyst can tolerate such a rather severe treatment without loosing too much of its activity. As, usually, this is not the fact, other, more gentle methods like encapsulation in polymeric matrices have to be used for immobilisation (Fig. 1, left).

Author address: U. Pruesse, FAL–TB – Braunschweig, Germany

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If encapsulation methods have to be applied, a proper matrix material has to be chosen. The matrix material should not negatively affect the biocatalyst, should enable sufficient mass transfer (high porous materials, e.g. hydrogels) and should lead to chemically, biologically and mechanically stable immobilisates in the desired form (Fig. 1, right).

Plenty of different polymeric materials exist which might be divided into natural, semi–synthetic and synthetic polymers. Immobilisation inside natural polymers leading to hydrogels either by ionotropic gelation (e.g. alginate, pectinate, chitosan, carrageenan) or by temperature change (agarose, gelatine, curdlan) generally can be considered as a gentle method. Nevertheless, disadvantages result from the resulting low mechanical stability, which limits their application to reactors with low shear stress (e.g. fixed–bed reactors) and, especially, from their biodegradability and therefore the requirement of a cost–intensive sterile process environment.

In contrast, synthetic polymers (e.g. polyvinyl alcohol, polyurethanes, polyacrylates and co–polymers thereof) usually are highly elastic and mechanically stable, so that they might be applied without abrasion even in stirred tank reactors of fluidized–bed reactors. In addition, as these materials are not biodegradable, no special care must be taken about sterile conditions.

Immobilised biocatalysts can be used in different forms (Fig. 1, right). Cubes and irregular pellets, although quite easy to produce, suffer from the abrasion problem which limits their application. Foils are also easy to produce, but can only be used in special type of reactors. Special reactors have also to be used for fibers or hollow fibers. Thus, the most common form for immobilized biocatalysts is the bead. Beads can be applied in almost all type of reactor, do not suffer from abrasion and are easy to calculate as far as the mass transfer properties are concerned, which in fact is an argument for process up–scaling in industry.

A quite new form is the lens shape which has been introduced as the so–called LentiKats® (Jekel 1998). LentiKats® combine several advantages like a gentle encapsulation method in an elastic and stable polyvinyl alcohol matrix and superior mass transfer properties due

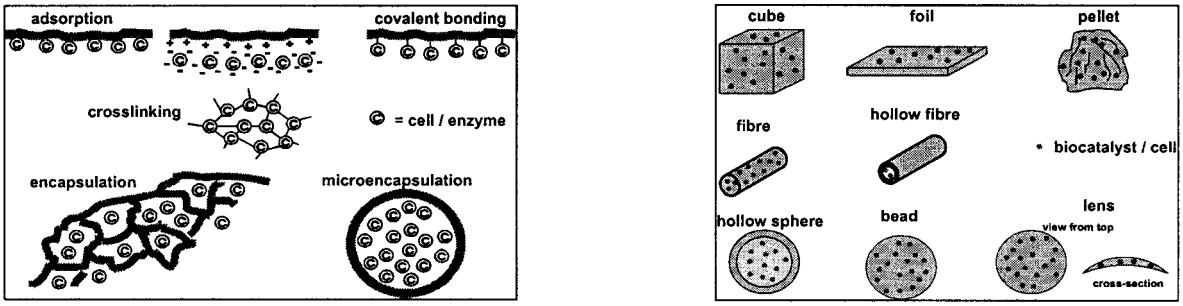

Figure 1. Immobilisation methods (left) and forms of immobilised biocatalysts (right)

to their reduced thickness (200 – 400 μm). Nevertheless, they can easily be separated by sieves due to their large diameter of a few millimeters. Thus, more and more papers deal with encapsulation in LentiKats® (Durieux 2002, Gröger 2001, Klaen 2002).

TECHNOLOGIES FOR IMMOBILISATION IN BEADS

Generally, discrete solid particles may be produced with four different approaches: i) from larger solid entities by grinding, ii) from smaller solid entities by agglomeration, granulation, pressing or tabletting – small fluid entities may also be used if in–situ drying is applied –, iii) from a larger fluid entity by dispersion in another immiscible phase (emulsions), or iiii) from fluid entities in the same size range with an immediate physical or chemical solidification step. From these four different approaches, the fourth is, in principle, best suited for the production of ideal spherical beads and quite a few different technologies for bead production exist (Fig. 2).

Dropping, electrostatic enhanced dropping and vibration technologies are well suited for the production of monodisperse beads in a wide size range from a few hundred microns up to several millimeters. Usually, the viscosity of fluids which can be processed is limited to low up to medium viscous fluids (viscosity < 1 Pa·s). The throughput per nozzle is rather low in comparison with the other technologies. For details about electrostatic dropping see (Nedović 2002), for vibration see (Heinzen 2002).

Rotating nozzle or rotating disc technologies are high throughput technologies that can be applied to bead production from low up to medium viscous fluids (viscosity < 1 Pa·s). It is disadvantageous that only a broad particle size distribution is accessible. For details about these technologies see (Prüße 2002).

The JetCutter technology enables the production of monodisperse beads in a wide size range from a few hundred microns up to several millimeters with high throughput rates per nozzle. The fluid viscosity is not limited so that low up to very high viscous fluids can be processed. For details about the JetCutter see (Prüße 2002).

TECHNOLOGIES USEFUL FOR LAB–SCALE IMMOBILISATION

All the bead production technologies named above and the LentiKat® technology can be used for lab–scale immobilisation as a low throughput can not regarded as a drawback. In fact, the easiest way to immobilise is by using a simple dropping technology or an electrostatic device which are usually home–made devices but can also be purchased from suppliers. Vibration technologies for lab–scale applications can be purchased from various supplies, e.g. Inotech, Rieter, Nisco and others (Fig. 3). Rotating disc and rotating nozzle technologies as well as emulsion techniques (Poncelet 2002) usually are not used in lab–scale. Both the JetCutter and the LentiKat® technology are available

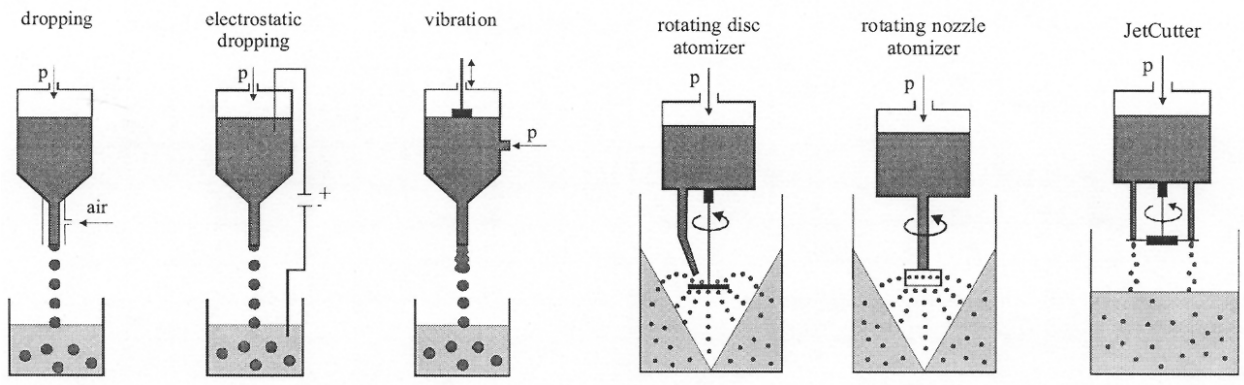

Figure 2. Technologies for the production for bead–shaped immobilised biocatalysts

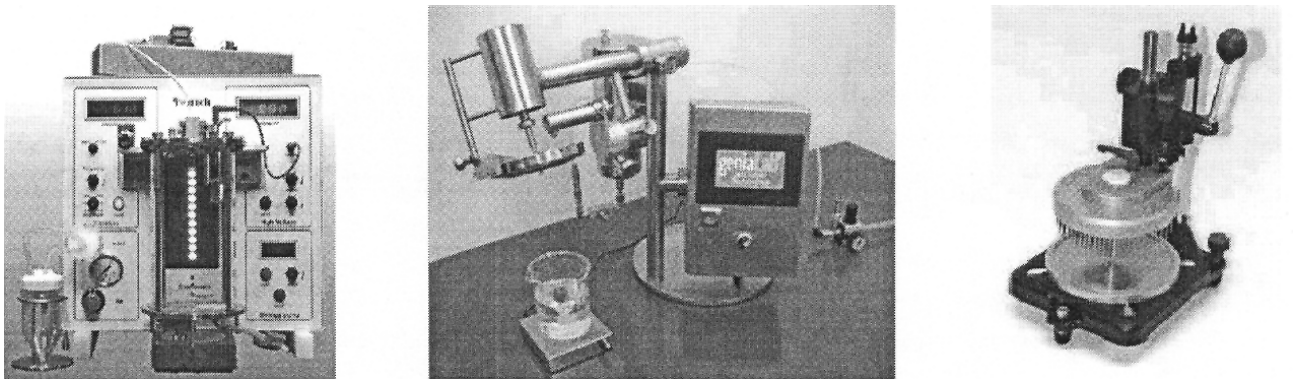

Figure 3. Lab–scale devices for the production of immobilised biocatalysts, left: Inotech Encapsualtor (Heinzen 2002), middle: geniaLab JetCutter – type JCS (Prüe 2003), right: geniaLab LentiKat® Printer (Jahnz 2001)

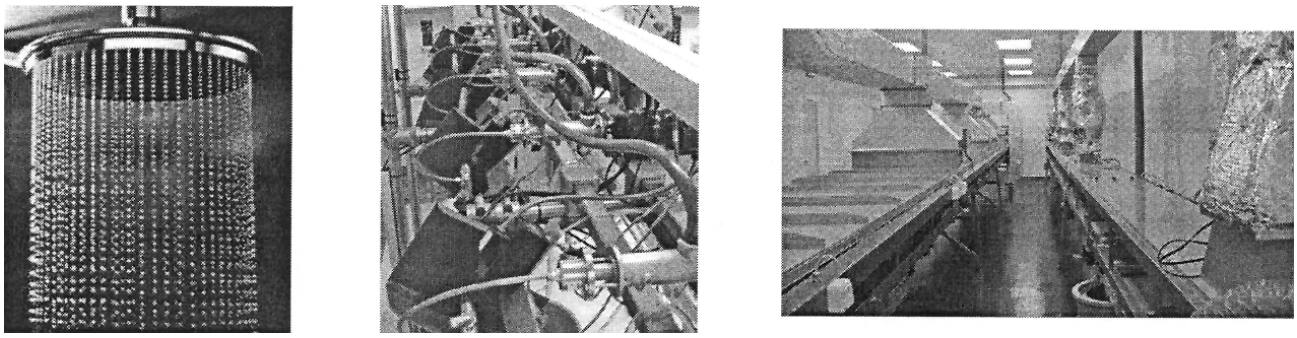

Figure 4. Industrial–scale devices for the production of immobilised biocatalysts, left: Rieter Droppo Line (Rieter 2004), middle: geniaLab JetCutter (Prüe 2003), right: Mega LentiKat® production line (Mega 2004)

for lab–scale production of biocatalysts. They are exclusively distributed by geniaLab.

Immobilisation methods other than encapsulation in beads (e.g. adsorption, covalent bonding and crosslinking) are also used in lab–scale applications.

TECHNOLOGIES USEFUL FOR INDUSTRIAL–SCALE IMMOBILISATION

As in industrial–scale immobilisation large amounts of immobilised biocatalysts have to be produced the applied technology must enable high throughput production. Depending on the process to be run sometimes simple immobilisation methods like adsorption, covalent bonding or crosslinking can be applied. These methods can easily be scaled–up. Adsorption, for example, is often used in environmental applications (biofilters or waste water treatment), whereas crosslinked glucose isomerase is used for the production of high–fructose corn syrup, which is worldwide the largest process with an immobilised biocatalyst.

If encapsulation has to be used as immobilisation method, only high throughput bead production technologies like rotating nozzle/disc technologies, emulsion technologies or the JetCutter can be used; with restrictions also vibrating systems (Fig. 4). In the latter case, multi–nozzle systems are used to achieve the desired throughput. Nevertheless, as only low and medium viscous fluid can be processed the stability of the resulting immobilisates usually is low and, thus, the applications are limited. Rotating nozzle/disc technologies as well as emulsion technology, although useful due to their high throughput, have the additional disadvantage of a broad particles size distribution which is often not acceptable as an additional sieving step is necessary and particles others than the ones in the desired size range are waste.

The JetCutter and the LentiKat® technology both have been scaled–up to industrial dimensions (Fig. 4). With these technologies high throughput production of regularly shaped beads or LentiKats®, respectively, can be achieved and both technologies are capable of processing fluids that lead to mechanical stable immobilisates. Thus, these two technologies can be regarded as the best technologies for industrial–scale encapsulation of biocatalysts.

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