

Immobilized enzymes for the chemical and pharmaceutical industry

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Introduction

Enzymes meet the increasing demand by manufacturers of chemicals and pharmaceuticals for enantiomerically pure compounds, because of their greater selectivity and specificity and because of their mild reaction conditions and lower energy consumption. Immobilization of enzymes offers easier separation and reuse of enzymes thereby making production processes more cost effective (Schoevaart 2007). Additionally process conditions can be chosen with increased flexibility.

In 1916 Nelson and Griffin discovered that invertase “exhibited the same activity when absorbed on a solid (charcoal or aluminium hydroxide) at the bottom of the reaction vessel as when uniformly distributed throughout the solution”. This discovery was the first of various enzyme immobilization techniques currently available. Besides absorption, different covalent methods of enzyme immobilization were developed in the 1950s and 1960s. Up to now, more than 5000 publications and patents have been published on enzyme immobilization techniques. Several hundred enzymes have been immobilized in different forms and approximately a dozen immobilized enzymes, for example penicillin G acylase, lipases, proteases, invertase, etc. have been used as catalysts in various large scale processes.

In the last two decades there has been an important transition in the development of immobilized enzymes. Approaches used for their design have become increasingly more rational: this is reflected in the use of more integrated and sophisticated immobilization techniques to solve problems that cannot be easily solved by previously developed single immobilization approaches. For this reason development of more robust immobilized enzymes which can work under hostile conditions, especially in non-aqueous media came to the forefront of many research interest.

Designing an immobilized enzyme

Numerous efforts have been devoted to the development of insoluble immobilized enzymes for a variety of applications. These applications can benefit from the use of immobilized enzymes rather than the soluble counterparts, for instance as reusable heterogeneous biocatalysts. Benefits are e.g. reduction of production costs by efficient recycling and control of the process. Two functions are associated with immobilized enzymes: catalytic functions and non-catalytic functions. The first comprises activity and stability of the enzyme and the second the shape, mechanical and chemical stability of the carrier. In practice, catalytic functions are designed in line with the desired activity, selectivity, etc. The selection criteria for non-catalytic functions, especially geometric properties are largely dependent on the design of reactor configurations (e.g. batch or column), the type of reaction medium (aqueous, organic solvent or two-phase systems), the reaction system (slurry, liquid-to-liquid, liquid-to-solid or solid-to-solid) and the process conditions (pH, temperature, pressure).

The objectives when designing the non-catalytic properties are mainly to achieve easy separation of the immobilized enzymes from the reaction mixture, broad applicability in different reaction media and reaction systems and general control over the process. Easy and fast filtration is a major issue:

depending on the application filtration must be completed in up to minutes. An enzymatic resolution where the reaction has to be stopped at a certain conversion to preserve the high ee (enantiomeric excess) of the product calls for swift separation of the catalysts from the medium. This can only be afforded with beads with the size of at least a few hundred micrometers in combination with a suitable sieve.

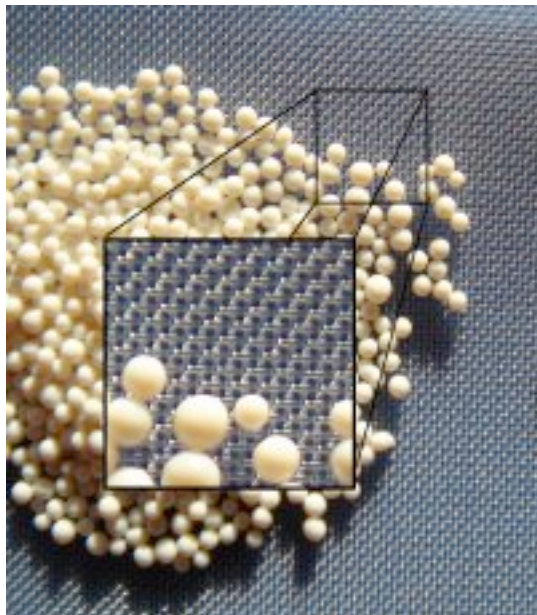


Fig 1. CaLB covalently bound to polymeric beads (150 – 300 μm in size) filters very easy on a 50 micrometer sieve.



Figure 2: Immobilized enzymes are easy to handle.

Since only a dozen immobilized enzymes are used as catalysts in diverse industrial processes, only a few enzymes are commercially available in immobilized form. The most well-known one used in diverse chemical applications is NZ 435 produced by Novozymes which is *Candida antarctica* lipase B absorbed on macroporous beads. This product has become an industrial standard over the years. Covalent immobilized forms of CaLB have also been produced (Figure 1 and 2) For the production of antibiotics covalent immobilized penicillin G acylase has been used for over decades. Few others are available, most of them are exclusively used in food processing like immobilized glucose isomerase for the continuous production of fructose syrup. This has forced some chemical producing companies to immobilize enzymes themselves: although both the enzyme and the carrier are available on the market, no immobilized enzyme “to-go” can be obtained.

Growing markets

With the dawning of the “White Biotechnology” came an increasing demand for enzyme technology. Many new companies concentrated on the development of new and improved enzymes which contributed to the enzyme’s catalytic properties turning biocatalysis into a mature technology. Large chemical producers now have their own biocatalysis groups (e.g. BASF, Dowpharma, Degussa, DSM) with a strong product based focal point. Relative low production volumes keep new enzymes rather expensive, however. Another strategy is concentrating on improving the chemical processes in which cheap bulk enzymes can be applied. By integrating them in a smartly designed chemical route very low enzyme costs per kilogram product are feasible -nothing comes as “dirt” cheap as a laundry enzyme. Not only for pharmaceutical manufacturers

this is important, especially intermediate producers facing tough competition need affordable technology since enzymes are still considered to be expensive. In most cases the implementation of a commonly used enzyme like a lipase or a protease leads to new intellectual property. Recycling of the enzyme by immobilization and associated lower operating costs contribute to further growth of the biocatalysis market.

Resolution with immobilized enzymes on industrial scale

Chiral intermediates and fine chemicals are in high demand from the pharmaceutical and agrochemical industries for the preparation of bulk drug substances and agricultural products. A number of multi-ton industrial processes use enzymatic resolution, often with lipases that tolerate different substrates (Table 1). BASF, for example, makes a range of chiral amines by acylating racemic amines with proprietary esters. Only one enantiomer is acylated to an amide, which can be readily separated from the unreacted amine, the unreacted amine can be racemised off-line and fed back into the process to increase the final yield. This process is considered to be the largest application of a single immobilized enzyme. Other resolutions use nitrilases where only one isomer is converted in the corresponding acid. DSM uses enzymatic resolution for racemic 2-pentanol and 2-heptanol with lipase B from *Candida antarctica*.

Manufacturer	compound	enzyme	scale (tons / y)
BASF	chiral amines	<i>C. antarctica</i> lipase B	1000
DSM	chiral alcohol	lipase	multi ton
Kaneka	D-amino acids	hydantoinase* / carbamoylase	1-5000
various	6-aminopenicillanic acid	penicillin G acylase	20,000

Table 1 : Some examples of enzymatic resolution used in production (immobilised enzyme)

New developments

In addition to tailor fitted route design and designer enzymes, more companies including enzyme producers are now offering an increasing range of immobilized enzymes with a wide choice of enzymes and carriers with particle sizes from micrometers up to millimeters suiting both batch and continuous processes. Most of the enzyme carriers used are readily available on the market and guarantee minimal costs and bulk availability. Custom immobilization services provides the specific knowledge now to the market and makes the technique also accessible for custom enzymes. With these new developments the choice for an immobilized formulation might soon not only be based on an inevitable technical or economical decision: with prices coming down it may well be just a matter of convenience.

A practical approach

Enzymes can be immobilised in several ways including absorption and covalent attachment. Absorbed lipases of ChiralVision are on the market under product type T-1. Also covalent attached enzymes under the name T-2 have been introduced. These T-2 Immozymes are formulated as a dry powder. Problems arise during scaling up and production of T-2 Immozymes. Recently new insights gave rise to the development of the next generation (T-3) of covalently bound Immozymes formulated as wet beads. Preliminary experiments showed a decreased loss of enzymatic activity.

Moreover, the production process is much easier. The storage stability, as well as the operational stability, the effect of additives, and substitutable carrier materials were investigated, for a wide range of lipases.

References

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