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Immobilised Enzymes in Industrial Biocatalysis: Supports, Methods and Operational Stability

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ABSTRACT

Enzymes offer selectivity and mild reaction conditions that are hard to match with conventional catalysts, yet most are used dissolved, once, and discarded. Immobilisation, in which the enzyme is attached to or confined within a solid phase, is the usual route to reuse, continuous operation and cleaner products. This narrative review asks which choices of support and attachment method are most likely to give an immobilised enzyme that stays useful in a working process, and what the literature actually shows about operational stability. It draws on published reviews and research papers on carriers, binding chemistry, the effects of immobilisation on activity and selectivity, and industrial practice, and it reports no new data. Adsorption, covalent binding, entrapment and cross-linking each trade simplicity against stability, and the better supports are those that match the enzyme, the solvent and the reactor rather than those that look best in a laboratory test. Stability figures are rarely comparable between studies because conditions, endpoints and activity measures differ. The review concludes that reuse over a few cycles is weak evidence of industrial value, and it sets out the information that a stability claim should carry.

Keywords: Immobilised enzymes; Biocatalysis; Enzyme supports; Covalent attachment; Operational stability; Cross-linked enzyme aggregates; Narrative review

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Article Class: Review Article

1. Introduction

Enzymes catalyse reactions with a selectivity that is hard to reproduce with conventional chemistry, and they do so in water, near ambient temperature and at modest pressure (1, 2). These qualities explain why biocatalysis has moved from a

curiosity into the manufacture of fine chemicals, pharmaceutical intermediates, foods and fuels. The same enzymes, however, are usually soluble proteins that were never designed to sit in a stirred tank for weeks. Dissolved in the reaction mixture they are difficult to recover, they



contaminate the product, and many lose activity quickly under process conditions (3, 4).

Immobilisation is the standard answer. The enzyme is bound to a solid carrier, enclosed in a porous network or joined to other enzyme molecules, so that it can be filtered off, packed in a column and used again (5, 6). The idea is old, and the literature on it is very large. What the literature rewards, though, is novelty of carrier and ease of preparation, whereas an industrial user is concerned with something plainer: how long the preparation keeps working, and at what cost per kilogram of product (7, 8).

That second concern is what we mean by operational stability, and it is the thread of this review. A new immobilised preparation is often reported with a handful of reuse cycles and a bar chart of retained activity. Such a result is a start, not a verdict. The questions that matter to a process, namely whether the enzyme stays attached, whether the carrier survives, whether mass transfer limits the rate and whether impurities in a real feed poison the catalyst, are answered less often than they are asked (9, 10). The article is a narrative review of published work, and no local or experimental data are reported. The aim is a balanced account of what is known and what is simply repeated.

2. Literature Review

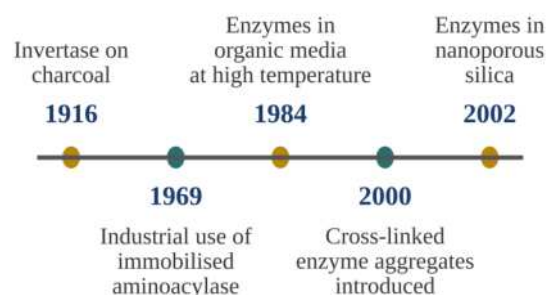
2.1 Why Immobilise, and What It Costs

The case for immobilisation rests on a short list of benefits. Reuse spreads the cost of an expensive enzyme over many batches. Continuous operation in packed or fluidised beds becomes possible, and the product stream is free of protein, which simplifies downstream work (11, 12). In favourable cases the enzyme is also more stable than its soluble form towards heat, solvents and extremes of pH, because

attachment restricts the unfolding of the protein (13, 14).

Against these benefits stand real costs. The carrier and the chemistry used to join the enzyme to it add to the price of the catalyst. Some of the enzyme is inevitably lost or inactivated during preparation, and what remains is often less active per unit of protein than the free enzyme, partly because of diffusion limits inside the particle and partly because attachment can distort the active site (15, 16). An immobilised preparation is therefore worth having only when the gain in lifetime and reuse outweighs these losses, and that balance depends on the enzyme, the reaction and the scale, not on the elegance of the method.

The history of the field is a useful corrective to enthusiasm (Figure 3). Early industrial successes rested on a few robust enzymes used in large volumes, and a preparation that works in the laboratory but cannot be handled, regenerated or afforded at scale does not become a process (5). A process chemist reading a paper needs one answer: would this survive a month in my reactor?



Schematic timeline; spacing is not to scale

Figure 3. Selected milestones in the development of immobilised enzymes (schematic timeline, not data).

2.2 Supports and Carriers

Carriers fall into a few broad families (Table 2). Natural polymers such as



agarose, alginate and chitosan are hydrophilic and gentle towards proteins, and activated agarose in particular has long served as a platform for stable covalent attachment (17, 18). Their weakness is mechanical: soft beads compress under flow and may swell or shrink with changes in the medium. Synthetic polymer resins, including acrylic and styrene-based beads with engineered pores, are rigid and widely used at scale, and a considerable body of work describes how carrier chemistry, pore size and particle size should be matched to the enzyme and the reactor (19).

Inorganic carriers, above all silica, offer mechanical strength and tunable pore structure. Ordered mesoporous silicas allow the pore diameter to be chosen to suit a particular protein, which has made them popular in research (20, 21). They are less forgiving in practice: silica dissolves slowly at alkaline pH, and fragile particles generate fines that block filters. Nanomaterials, including particles, tubes and magnetic cores, give a large surface area and easy recovery, but questions of cost, aggregation and scale-up are still largely open (22, 23).

The choice of carrier is also a choice of microenvironment. A hydrophobic surface can pull a protein into an unfolded state, whereas a hydrophilic one tends to leave it hydrated and active, and charged groups on the surface shift the local pH and the concentration of ions around the enzyme (6, 24). Because the microenvironment is invisible in a bulk measurement, two preparations that look identical on paper can behave differently in the same medium. Particle size is a similar compromise: small particles shorten diffusion paths but raise the pressure drop across a packed column (19). A support that has been equilibrated with the medium behaves differently from one used straight from the bottle.

Table 2. Families of support materials and what to ask of each.

Family	Examples	Strength	Concern
Natural polymers	Agarose, alginate, chitosan (17, 18)	Gentle	Soft; swelling
Synthetic resins	Acrylic and styrene-based beads (19)	Rigid; set pore size	Apolar surface
Inorganic oxides	Silica, mesoporous silicas (20, 21)	Robust; tunable pores	Alkaline attack; fines
Nano-particles	Particles, tubes, magnetic cores (22)	High area	Costly; clumping

Note: Qualitative; the suitability of any carrier depends on the enzyme, the medium and the reactor.

2.3 Methods of Attachment

Four routes are used most often (Table 1). Adsorption relies on weak interactions between enzyme and carrier, is easy to carry out and is mild towards the protein, but the enzyme can desorb when pH, ionic strength or solvent change (25). Covalent binding forms stable bonds with reactive groups on the carrier, such as epoxide, aldehyde or glyoxyl groups, and gives preparations that rarely leach; its risk is that the chemistry modifies residues the enzyme needs (26, 27).

Entrapment encloses the enzyme in a gel or a porous matrix. It leaves the protein largely unmodified, but substrates and products must diffuse through the network, and the pore must be small enough to hold the enzyme and large enough to let reactants pass (28). Cross-linking without a carrier forms aggregates of enzyme molecules joined by a bifunctional reagent, the best known being cross-linked enzyme aggregates (29, 30). These offer a high concentration of catalyst and avoid the cost of a carrier, though the particles are often soft and difficult to handle (31). The



workhorse cross-linker, glutaraldehyde, is itself chemically complex, and its behaviour in solution is not as simple as a textbook scheme suggests (32, 33).

Table 1. Principal immobilisation methods compared qualitatively.

Method	Principle	Strength	Weakness
Adsorption	Weak forces hold the enzyme on a carrier (25)	Simple, gentle, carrier often reusable	Enzyme can leak with changes in pH, ionic strength or solvent
Covalent binding	Stable bonds to reactive groups on the carrier (26)	Little leaching; room for stabilisation	Chemistry can distort or deactivate the enzyme
Entrapment	Enzyme confined in a gel or porous network (28)	Mild; protects the enzyme	Diffusion limits; slow release of enzyme possible
Cross-linking (CLEAs)	Enzyme aggregates joined without a carrier (29, 31)	High enzyme loading; no carrier cost	Soft particles; poor flow and handling

Note: General tendencies drawn from the cited reviews; individual enzymes and carriers can behave differently.

2.4 What Immobilisation Does to the Enzyme

It is tempting to treat immobilisation as a neutral act of fixing the enzyme in place. It is not. Attachment can raise or lower activity, alter selectivity towards a substrate and shift the pH optimum, and the effects depend on the orientation of the protein and the number of points at which it is bound (13, 34). Binding through several residues on one face of the protein tends to rigidify the structure and can sharply raise its stability to heat and solvents; binding through a single point

leaves most of the flexibility and most of the fragility (35, 36).

Multimeric enzymes bring a particular hazard: if only some subunits are attached, the rest can dissociate in use and be lost (37). A preparation that shows no leaching of protein may therefore still lose activity, which is why how the enzyme was bound matters as much as whether it was. Such differences look small in a single run but compound over many cycles.

2.5 Industrial Practice

Industrial reviews describe a technology that is widely used but concentrated in relatively few enzyme classes (7, 8). Lipases are prominent, notably the lipase B of *Candida antarctica*, which is usually supplied adsorbed on a macroporous acrylic resin and is used in a large variety of organic syntheses (38, 39). Work on enzymes in organic solvents opened synthesis in low-water media, where immobilised preparations are the norm (40, 41). Immobilised enzymes also serve in the conversion of renewable feedstocks, where the economics are set by the very large volumes and the low value of the product (42, 43). In pharmaceutical manufacture the picture is mixed; some of the most celebrated enzymatic processes rely on engineered enzymes used in solution, which shows that immobilisation is a tool and not a requirement (2, 44).

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts. Which combinations of carrier and attachment method are most often associated with an immobilised enzyme that stays active in a working process, and how well does the published evidence on operational stability support that association? To answer it we divided the question into the points a stability claim would need to settle: what the enzyme and



carrier were, how they were joined, how activity was measured, in what medium and reactor the preparation was used, and for how long. None of these steps is difficult, but each is easy to skip.

3.2 Literature Assembly

The review draws on peer-reviewed reviews and research papers in four areas: carriers and attachment chemistry; the effect of immobilisation on activity, selectivity and stability; industrial use of immobilised enzymes; and the characterisation of heterogeneous biocatalysts. Sources were chosen for relevance, citation in the field and the clarity of their methods.

We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. Its practical consequence is that the review is a better guide to the questions that should be asked of a stability claim than to the exact lifetime of any particular preparation, and it avoids quoting numerical results from individual studies that could not be checked against the primary papers for this article.

3.3 Appraisal Framework

To judge how far a report could be trusted we used four questions. Is the immobilised preparation described well enough to be reproduced, including the loading and the activity measure? Is stability measured under conditions close to use, rather than under a convenient test condition? Are leaching and carrier damage checked directly, and is a real or realistic feed used (7, 9)? And is the lifetime expressed in a way that allows comparison, for example as productivity per amount of enzyme and not only as a number of cycles? Table 3 lists the causes of lost activity we looked for.

Table 3. Common causes of lost activity in a working immobilised biocatalyst.

Cause	What happens	What to check
Thermal or chemical inactivation	Enzyme unfolds in use (9, 13)	Activity retained over time at process conditions
Leaching	Enzyme detaches from the carrier (25)	Protein and activity in the product stream
Subunit dissociation	Multimeric enzyme falls apart (37)	Whether every subunit is attached
Carrier failure	Beads crush, swell or dissolve (7)	Particle size and fines after long runs
Fouling and poisoning	Substrate impurities coat or block sites (8)	Feed quality; cleaning cycles

Note: Causes are those discussed in the cited literature; relative importance differs from process to process.

4. Results

4.1 Matching the Support to the Enzyme and the Medium

Across the sources, the strongest recurring message is that no carrier is best in general. The better preparations in the literature are those in which the carrier, the attachment chemistry and the medium were chosen together (Figure 1). Hydrophilic carriers with covalent attachment are favoured where the reaction is in water and leaching must be avoided, whereas adsorption on a hydrophobic resin works well for lipases in organic media, where the enzyme has little reason to leave the surface (19, 38). A carrier is rarely judged on its own: the same resin can suit one enzyme in one solvent and fail with another enzyme in water, which is why published rankings of supports transfer poorly (11). Whatever the choice, the preparation should be tested in the medium in which it will actually be used, not only in the buffer in which it was made (9).

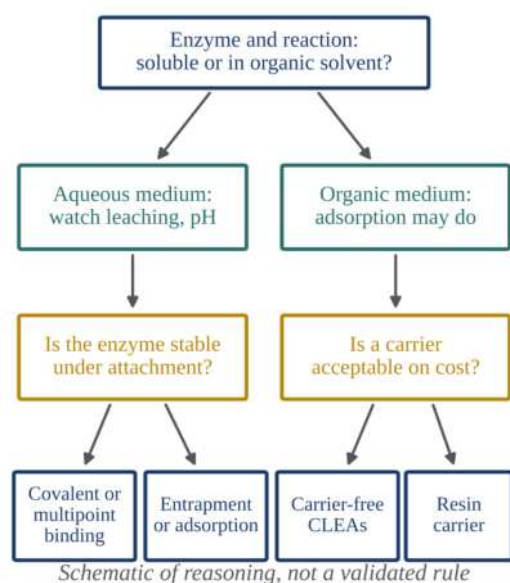


Figure 1. Questions that lead from an enzyme to a choice of method (schematic, not data).

Preparing the carrier matters as well. Activated supports must be washed and equilibrated, and residual reactive groups are usually blocked, since groups left unreacted can go on binding the enzyme or the substrate in unintended ways (17, 27).

4.2 Choosing and Controlling the Attachment Chemistry

The sources agree that the chemistry should be treated as a variable to be optimised and not a fixed recipe. Immobilisation through several covalent links on the same face of the protein generally gives the largest gains in stability, while a single link gives the mildest effect on activity (26, 35). Heterofunctional carriers, which combine a group that adsorbs the enzyme with a group that then binds it covalently, allow the two steps to be separated and the orientation to be steered (26, 36).

Methods differ in how they trade simplicity, leaching, retained activity and loading (Figure 2). Adsorption is simplest but least secure; covalent attachment is more secure but more demanding; entrapment protects the enzyme but slows

the reaction; carrier-free aggregates give high loading at the cost of handling (11, 14). These are tendencies, and exceptions are common enough that the figure should be read as a prompt for questions and not as a ranking. That is a modest claim, and it is the one the evidence will currently bear.

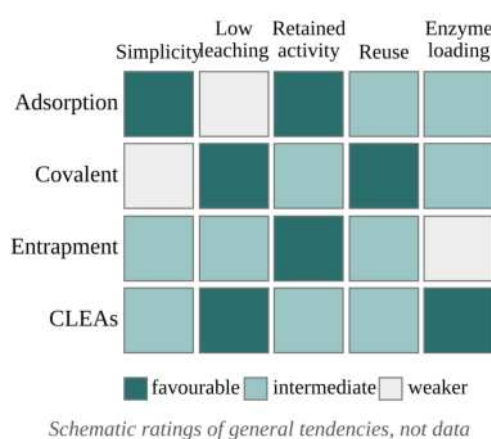


Figure 2. Qualitative matrix of methods against desirable properties (schematic, not data).

4.3 What Operational Stability Actually Means

Operational stability is the capacity of a preparation to keep delivering product while it is in use, and it is shaped by several processes that run together. The enzyme can unfold or be inactivated by heat, solvent or the reaction itself; it can detach from the carrier; the subunits of a multimeric enzyme can separate; the carrier can crush, swell or dissolve; and impurities in the feed can coat or poison the particle (9, 13, 37). Each of these has a different remedy, and a preparation can fail through any one of them.

Mass transfer deserves particular attention. When the reaction is faster than the diffusion of substrate into the particle, the interior of the bead is starved and the apparent activity falls, which can be mistaken for inactivation (7, 9). The reverse also happens: a sharp fall in measured activity after cycles of use may reflect fouling of the pores rather than



damage to the enzyme. Read this way, a loss of activity in a short test is not proof that a method is unsuitable.

The simple count of reuse cycles is the weakest way to express stability, because the cycle length, the substrate concentration and the conversion reached differ from paper to paper. A preparation that is reused ten times in short tests may have processed far less substrate than one that is used three times in long ones. Measures that tie the output to the amount of enzyme, such as the product obtained per unit of enzyme over the life of the catalyst, are more informative and are better matched to the economic question (7, 8). This is why the same enzyme can look excellent in one report and unremarkable in the next.

4.4 Quality and Comparability of the Evidence

The reviews we examined converge on a set of recurring weaknesses in the primary literature. Characterisation is often thin: loading, particle size, pore size and the activity of the free enzyme under the same conditions are not always reported (9, 24). Leaching is inferred from the absence of protein in the supernatant rather than from the retained activity, and the reuse of a preparation is tested over a few cycles with fresh buffer and pure substrate, a situation that rarely resembles a plant. The point is not that a study is poor, only that its result travels less far than its abstract suggests.

Comparisons between studies are therefore hard to make, because enzymes, carriers, media and endpoints, whether half-life, retained activity or number of cycles, are combined in every possible way. A claim that one carrier is better than another is secure only when both were tested with the same enzyme under the same conditions (4, 10). Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5. Discussion

5.1 Interpretation

The central message is that an immobilised enzyme is a system, and its lifetime belongs to the system and not to any single component. A well-chosen carrier cannot rescue an enzyme that is inactivated by the reaction medium, and a stabilising attachment chemistry cannot protect a carrier that dissolves. For a practitioner this suggests starting from the process conditions and working backwards: the solvent, pH and temperature of use define which carriers are possible, the enzyme's sensitivities define which attachment chemistries are tolerable, and the reactor type decides how much mechanical strength is needed (7, 19).

It follows that the most useful early experiments are those that expose how a preparation fails: a long run at process temperature, a test with a realistic feed, and a check for activity in the product stream.

5.2 Limitations

The review has several limits that readers should keep in mind. It is a narrative synthesis, so the selection of sources reflects the authors' judgement, and a systematic search might have surfaced studies we did not consider. It relies chiefly on reviews and on well-known papers and so is likely to over-represent the enzymes and carriers that have been studied most, notably lipases and a handful of silica and polymer supports. It gives no figures for the lifetime of specific preparations, because those depend on conditions we cannot reproduce here. It is a habit worth keeping even when the outcome seems obvious.

5.3 Directions for Future Work

Three kinds of work would make the field more useful. The first is routine reporting of a minimum set of details, namely



loading, activity of the free enzyme, medium, reactor, endpoint and a direct check for leaching (9, 24). The second is comparative studies in which several carriers and attachment methods are tested with the same enzyme under the same conditions. The third is longer tests in real or realistic feeds, expressed as productivity per amount of enzyme, so that the result can be set against the cost of the catalyst.

Combining immobilisation with protein engineering is also promising, since enzymes can be designed to attach in a chosen orientation (2, 36). Whether such combinations beat simple preparations in real processes remains to be shown. Nothing in this reading argues against immobilisation; it argues for measuring what happens after it.

6. Conclusion

Immobilisation can make an enzyme reusable, stable and easy to separate from a product, and the technology is established in several industrial processes. The literature, however, does not allow a general ranking of carriers or methods, and many reports of stability are not strong enough to carry the conclusions drawn from them. What it does show is that the carrier, the attachment chemistry, the medium and the reactor must be chosen together, and that stability is a property of the whole preparation under use.

For the practitioner the message is practical. Define the process conditions first, characterise the preparation, test it in the way it will be used and report the lifetime in terms that link to output. For the researcher, the opportunity lies in comparative and long-term studies in realistic feeds, which would turn a large collection of promising preparations into evidence on which a process can be built. These points apply with equal force to any heterogeneous catalyst, not only to

enzymes.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

M.V. conceived the review and wrote the sections on supports and attachment methods. M.B. wrote the sections on industrial practice and process considerations. D.P. wrote the sections on enzyme stability and the appraisal of evidence. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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