

Enzyme Immobilisation in Printed Photopolymer Structures: Activity Retention, Mass Transfer, Leaching and Reuse

Qualification of immobilised biocatalysts in photopolymer supports and structured flow reactors

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Abstract

Printed photopolymer structures can immobilise enzymes by adsorption, covalent attachment, entrapment or post-print functionalisation. The engineering value arises only when retained catalytic activity, substrate and product transport, enzyme leakage and reusability are measured together. This review separates loading from active loading, intrinsic enzyme stability from reactor transport, and one-cycle conversion from long-term operational stability. It defines a qualification framework for printed immobilised-enzyme structures without assuming that conditions optimised on conventional carriers transfer to a printed reactor.

Keywords: enzyme immobilisation; photopolymer; 3D printed reactor; biocatalysis; mass transfer; leaching; reuse; continuous flow

1. Significance and scope

Additive manufacturing can place catalytic surface area and fluid pathways in geometries that are difficult to machine, but a printed reactor is useful only if immobilisation chemistry and transport are compatible. Marchetti and co-workers demonstrated a screening-to-reactor route using the same photopolymeric formulation and showed that immobilisation conditions identified in a screening format could be translated into a honeycomb flow reactor [1]. This makes the support chemistry, not merely the CAD geometry, part of the reactor identity.

Immobilisation route must be declared because adsorption, covalent tethering, physical entrapment and affinity capture expose the enzyme to different environments and impose different transport penalties. A support with a high total protein loading can still have poor catalytic utilisation if active sites are sterically blocked or buried. The relevant engineering comparison is therefore activity retained per unit enzyme and per unit reactor volume or area, not loading alone.

2. Loading is not active loading

Protein mass on a printed surface does not establish catalytic capacity. Immobilisation can block active sites, alter conformation or place enzyme in poorly accessible regions. Qualification should report total loading, retained specific activity and activity per printed volume or geometric area.

Comparing free and immobilised enzyme on an equivalent enzyme basis helps distinguish immobilisation loss from transport limitation. Active loading should be measured after all post-print operations, including washing, functionalisation and storage, because each step can remove protein or alter conformation. If activity is inferred only from the initial solution depletion, irreversible adsorption of inactive protein will be counted as successful immobilisation.

A direct activity assay on the final support closes that ambiguity.

3. Photopolymer chemistry can affect protein state

Residual monomer, photoinitiator fragments, surface hydrophobicity and post-cure conditions can alter enzyme activity. Post-print functionalisation can decouple bulk printability from biochemical surface chemistry. Iakimova and co-workers showed that a photopolymer resin could be chemically functionalised to support stable protein immobilisation in printed microfluidic devices, illustrating that activation chemistry and print fidelity can be engineered separately [2].

Photopolymer surfaces can continue to evolve after cure through extraction, hydrolysis, oxidation or adsorption of reaction components. A functionalisation protocol should state whether it is performed on a freshly cured or aged surface and how conversion is verified. When coupling agents are used, an unfunctionalised printed control helps separate the effect of surface chemistry from changes caused by print geometry or roughness.

4. Mass transfer can dominate apparent kinetics

Structured supports introduce boundary layers, pore diffusion and residence-time distributions. A lower observed rate may therefore arise from transport rather than enzyme deactivation. Flow rate, channel geometry, substrate concentration and temperature should be reported together with conversion.

Where possible, apparent kinetics should be compared across geometries or agitation conditions to detect external transfer limitation. Apparent kinetic parameters in a structured reactor are effective device parameters unless internal and external transport limitations have been excluded. Dimensionless analysis, residence-time variation or particle/strut-size variation can help identify transfer control.

For flow reactors, pressure drop belongs beside conversion because a geometry that improves mass transfer at an unacceptable hydraulic cost is not an engineering improvement.

5. Leaching and reuse define operational value

An immobilised catalyst intended for reuse should be challenged over multiple cycles or extended continuous operation. Activity loss should be accompanied by protein-leaching measurements where feasible, because declining conversion may reflect enzyme loss, deactivation, fouling or support damage. Nakahara and co-workers showed that immobilised aldehyde dehydrogenase on a 3D-printed reactor could be recycled while retaining broad substrate specificity, providing a direct example of reuse as a distinct performance metric [3].

Reuse should be reported as a trajectory rather than a final retained percentage. Cycle-to-cycle conversion, selectivity, protein loss and pressure drop can reveal different degradation modes. A stable conversion accompanied by increasing residence time or declining flow is not equivalent to stable catalyst performance, just as retained protein without retained activity is not evidence of enzymatic stability.

Axis	Measurement	Failure mode
Immobilisation	total loading and retained specific activity	high protein loading with inactive enzyme
Surface chemistry	activation/post-cure record	residual chemistry deactivates catalyst
Transport	flow, geometry, substrate concentration	mass-transfer-limited apparent kinetics
Leaching	protein/activity in effluent	conversion decline from enzyme loss
Reuse	cycles or hours on stream	single-cycle performance presented as durability

Table 1. Domain-specific qualification matrix for enzyme immobilisation in printed photopolymer structures.

6. Device-level verification

The qualification unit is the printed reactor under its intended hydrodynamic condition. Pressure drop, residence time, conversion, selectivity, enzyme release and structural integrity should be tracked together. Cleaning or storage between runs can change both enzyme state and polymer surface, so reuse protocols belong in the test definition.

ER-437 owns deliberate enzyme-programmed degradation of the polymer network. ER-434 instead treats the enzyme as the retained catalyst whose loss from the support is undesirable. The same enzyme can therefore appear in both reviews under opposite design objectives, and the intended role must be explicit.

Reactor qualification should include a blank printed support processed identically but without active enzyme. That control captures substrate adsorption, uncatalysed conversion and any catalytic contribution from the support chemistry itself. For reactions sensitive to trace metals or acidic/basic sites, this distinction is important because the photopolymer or functionalisation chemistry can produce apparent conversion that is not enzymatic.

7. Minimum qualification framework

Report printer and material, post-cure and surface activation, immobilisation chemistry, enzyme identity, loading, retained activity, flow or mixing condition, conversion/selectivity, leaching and reuse duration. A claim of improved thermostability should distinguish enzyme stabilization from reduced apparent deactivation caused by transport limitation. A claim of continuous operation should state the on-stream duration and acceptance threshold.

A minimum data package should also report enzyme basis consistently. Activity per gram of support, per printed volume and per amount of immobilised protein answer different questions and should not be interchanged. For scale-up, geometric similarity is insufficient if Reynolds number, residence-time distribution or surface-to-volume ratio changes; the reactor should be requalified under the scaled hydrodynamic condition rather than credited with small-device performance.

Operational qualification should include the transitions between runs. Cleaning, rinsing, solvent exposure, drying or cold storage can alter both the enzyme and the printed support, so a reuse claim should state the complete inter-cycle protocol. If regeneration or re-loading of enzyme is allowed, the restored state is a new operational state and should be distinguished from true retention of the original catalyst.

For scale-up, the acceptance criteria should also include hydraulic reproducibility across nominally identical prints: variation in channel dimensions or partial blockage can shift residence time and conversion even when the biochemical preparation is unchanged. A robust process therefore couples print metrology, pressure-drop control and catalytic performance across batches.

8. Engineering conclusions

Printed immobilised-enzyme systems are coupled catalytic-transport devices. Their advantage is not established by enzyme attachment alone, but by retaining accessible activity in a geometry that delivers substrate and removes product while limiting enzyme loss. The most

informative qualification therefore combines biochemical activity with hydrodynamic and lifetime measurements on the final printed structure.

References

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Competing interest

Juan Segurola is founder, CEO and CTO of 3Dresyns by Resyner Technologies S.L., which develops and commercialises photopolymer materials and related additive-manufacturing technologies. This review is an engineering analysis and does not constitute a product-performance claim or a device qualification.