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Structured Hydrogel Reactors for Continuous Enzymatic Production of Allicin

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Allicin, a broad-spectrum antimicrobial compound derived from garlic, is produced by enzymatic conversion of alliin catalyzed by alliinase. Despite its promising biological activity, this system is limited by allicin's high reactivity, which can lead to gradual enzyme inactivation via thiol modification, interaction with Pyridoxal-5′-phosphate (PLP) cofactor, and physical hindrance of the active site, reducing the efficiency of conventional batch processes.

This work investigates the continuous production of allicin in a plug-flow reactor using immobilized alliinase entrapped in 3D-printed hydrogel structures, which provide a high surface-to-volume ratio. Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting is employed to fabricate constructs with defined internal architecture. Different geometries, including gyroid and hexagonal infill, are used to study the effect of structure on transport and catalytic performance.

Compared to conventional immobilization approaches such as randomly packed hydrogel beads, structured architectures offer improved control over flow distribution and mass transport. Interconnected channel networks reduce diffusion distances, minimize stagnant zones, and provide more uniform exposure of the enzyme to the flowing substrate. At the same time, the absence of random packing should lead to lower pressure drop and more predictable hydrodynamic behavior. The printed hydrogel modules are assembled into a modular packed-bed configuration and operated under continuous flow. The system is evaluated for substrate conversion, enzyme stability, and operational cycles, with direct comparison to batch conditions. Particular attention is given to the extent of enzyme immobilization and its physical retention within the hydrogel matrix during operation.

The combination of continuous flow and controlled internal architecture is expected to mitigate enzyme deactivation by limiting local accumulation of reactive products and by-products, reducing oxidative stress and pH shifts, and improving transport conditions. This approach provides a platform for studying the relationship between reactor design and biocatalytic performance and may contribute to more efficient utilization of reactive enzymatic systems by reducing enzyme consumption, improving recovery, and increasing product yield.