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One-step isolation and immobilization of His-tagged amine transaminase from cell lysate in a microreactor with functionalized nanofiber mat

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PURPOSE OF THE ABSTRACT
One-step isolation and immobilization of His-tagged amine transaminase from cell lysate in a microreactor with functionalized nanofiber mat

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Microreactors are powerful tools for rapid development and intensification of biocatalytic processes. Their main advantages derive from their small dimensions, which allow very efficient mass and heat transport, controlled flow regimes and continuous process operation [1], To achieve long-term use of enzymes in the flow, biocatalysts are usually immobilized inside the microreactors, which also improves their operational stability [2,3]. Since enzyme purification significantly contributes to biocatalysts costs and process time, a one-step isolation and immobilization of the target proteins constructed with different types of affinity tags offers an excellent alternative [4].
In this work, His-tagged wild-type amine transaminase (ATA -wt) from the c-LEcta metagenome library was expressed as a soluble protein in Escherichia coli BL21(DE3). The cell lysate was centrifuged and used to immobilize the enzymes on functionalized nonwoven nanofiber membranes in a microreactor. The microreactor consisted of two poly(methyl methacrylate) plates and two polytetrafluoroethylene gaskets forming a hexagonal channel in which the nonwoven nanofiber mat was embedded between the two gaskets. The commercial polymeric nanofiber mat functionalized with Cu2+ ions (Tiss-IMAC-Cu from NanoMyP) was selected based on preliminary experiments with purified enzyme and offers very large surface for enzyme bonding. The retained enzyme activity, volumetric productivity (space-time yield, STY) of the microreactor, and stability were monitored in operando based on a model reaction using (S)-α-methylbenzylamine as amine donor, pyruvate as amine acceptor, and pyridoxal-5'-phosphate (PLP) as a cofactor that was present only in the immobilization step [5].
Immobilization of unpurified amine transaminase from the cell lysate in a microreactor yielded enzyme load of over 1000 U mL-1. By skipping the purification step, the amount of waste produced, and the time required for enzyme isolation, purification and immobilization were significantly reduced. A STY of over 20 U mL-1 was achieved at tested conditions. Over 90% of enzyme activity was retained after 4 days of continuous operation without exogenously added PLP.

Keywords: amine transaminase, microreactor, immobilization, biotransformation

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

amine transaminase | microreactor | immobilization | biotransformation

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