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Microfluidic reactors with immobilized enzymes—Characterization, dividing, perspectives

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ABSTRACT

It is commonly known that enzymatic transformations are considerably more specific than classical chemical reactions which usually lead to formation of byproducts. That is why the enzymes are a powerful tool in the field of analytical chemistry. The main problems occurring while working with enzymes stem from their relatively high price and sensitivity to non-physiological conditions. The above mentioned disadvantages may be overcome through enzyme immobilization which allows for reusing the biocatalyst as long as it retains its activity. When the immobilization is performed correctly the enzymes are more stable and also more resistant to denaturation. Such an approach in combination with additional benefits of miniaturization, heterogeneous catalysis and flow-mode operation contributes to the various applications of the Immobilized Enzyme Reactors (IMERs) in particular, microfluidic (μ-IMERs). In the present review various types of μ-IMERs were described. Particular attention was paid to techniques of their preparation including immobilization strategies and technical solutions connected with their applications using both capillary and chip format.

Contents

Abbreviations: AAm, acrylamide; AAO, ascorbate oxidase; ACE, angiotensin-converting enzyme; AChe, acetylcholinesterase; ADA, adenosine deaminase; ADH, alcohol dehydrogenase; ALP, alkaline phosphatase; APTES, 3-aminopropyltriethoxysilane; BuMA, butyl methacrylate; CAT, catalase; CDI, carbonyl diimidazole; ChOx, choline oxidase; CPTMS, 3-chloropropyltrimethoxysilane; CT, chymotrypsin; d-PhgATd, phenylglycine aminotransferase; EDC, *N*-ethyl-N′-(3-dimethylaminopropyl); EDMA, ethylene glycol dimethacrylate; ESI, electrospray ionization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GlDH, L-glutamate dehydrogenase; GLYMO, 3-glycidoxypropyltrimethoxysilane; GMA, glycidyl methacrylate; GOx, glucose oxidase; GPT, glutamic pyruvic transaminase; HEMA, 2-hydroxyethyl methacrylate; HDB, hexadimethrine bromide; HILIC, hydrophilic interaction liquid chromatography; K_M, Michaelis constant; LDH, lactate dehydrogenase; γ-MAPS, 3(methacryloyloxy)propyltrimethoxysilane; MBA, *N,N′*-methylenebisacrylamide; MMA, methacrylic acid; MPTMS, (3-mercaptopropyl)trimethoxysilane; MALDI, matrix assisted laser desorption/ionization; MS, mass spectrometry; MTMOS, methyltrimethoxysilane; 11-MUA, 11-mercapto-undecanoic acid thiol; NA, neuramidase; NADH, nicotinamide adenine dinucleotide; NAS, *N*-acryloxysuccinimide; NHS, *N*-hydroxysulfo succinimide; NVP, *N*-vinyl-2-pyrrolidinone; PDDA, poly(diallyldimethylammonium chloride); PEG thiol acid, *O*-(mercaptoethyl)-*O′*-(2-carboxyethyl)heptaethylene glycol; PEGDA, poly(ethylene glycol)diacrylate; PEGMA, polyethylene glycol methacrylate; PET, poly(ethylene terephthalate); PEI, polyethylenimine; PNGase F, peptide-*N*-glycosidase F; PTFE, polytetrafluoroethylene; SCX, strong cation exchange; SMA, stearyl methacrylate; TEOS, tetraethoxysilane; V_{MAX}, maximum velocity; VAL, 4-vinyl-2,2-dimethylazlactone; VTMS, vinyltrimethoxysilane; XOD, xanthine oxidase; WSC, water-soluble carbodiimide.

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1. Introduction

The enzymes are used as environmentally friendly biocatalysts in many areas of industry for large-scale production [1,2] and also as biosensors for target analytes identification or determination [3]. In the lab-scale the enzymes may be used for synthesis of some pharmaceutical ingredients e.g. production of oligogalacturidones using pectin lyase [4] or bioactive peptides employing proteases [5] but also as analytical tools. In the last case, the main task for the enzymes is a specific conversion of some particular analytes into characteristic products providing information about the sample properties and composition which may be very useful in biomedical, pharmaceutical and proteomic studies. In general, the smaller reactor dimension, the lower sample/reagent amount is needed and the yield of the reaction is higher when the products are continuously discharged and substrates introduced. That is why the microscale fluidic reactors may be particularly useful in laboratory processes. Moreover, the reduced dimension of microfluidic systems enables more precise control of the reaction time and temperature due to the better heat exchange and mass transfer [6,7].

In the miniaturized systems the enzyme may be immobilized in a microchannel/capillary to perform the heterogeneous biocatalysis (*Immobilized Enzyme Reactor* (IMER)) or injected into a chip after dissolving it in a proper solution either individually or with a sample in order to perform homogenous biocatalysis (solution-phase reaction, e.g. *Ion Exchange Based Proteomic Reactor* (IEBPR)) [8,9]. In the second case, the substrate and enzyme solutions are injected into the reaction site from the separate inlets and the reaction is carried out in a continuous flow of both reactants. For example, such a methodology was used for phenolic compounds oxidation using dissolved laccase [10]. In this case the products were separated and their concentrations were determined by HPLC. It should be noticed here that reactions with dissolved enzymes lead to contamination of the products not only with unreacted substrates but also with the biocatalysts. When proteases are used for in-solution digestion, the post-reaction mixture also contains the polypeptide fragments resulting from enzyme autolysis which additionally complicates the polypeptide mixture composition [11]. It also makes the analysis of the obtained MS spectra more difficult and often leads to decrease of the method sensitivity due to the suppression of peptide signals by autolysis peaks (this problem relates mainly to proteomic analysis) [12,13]. Moreover, in the case of using IMERs no compounds are required to stop the proteolysis while e.g. acetic acid [14] or formic acid [15] are added to post-reaction mixture to stop in-solution digestion. That is why it is more advantageous to immobilize the biocatalysts onto inner-walls of the microtubes (open-tubular microreactors) or on the surface of the porous solid support placed inside them (monolithic or packed microreactors). This approach has a lot of advantages resulting from miniaturization, flow-mode operation and immobilization procedure (heterogeneous catalysis).

The most important benefit of the immobilization is the possibility of reusing of the biocatalyst without any complicated procedure needed to its isolation from the post-reaction mixtures followed by the necessary and careful purification [16]. It minimalizes the loss of the enzymatic reagent and allows for obtaining the higher product yield per utilized enzyme amount. Additionally, the immobilization is treated as one of the methods used for increasing of the enzyme stability [17]. When the proper protocols are used the immobilized proteins are more resistant to denaturation as well as their tolerance towards the elevated temperature and high organic solvents concentration is greater when compared to their native counterpart. It directly leads to prolonged lifetime of the enzyme also due to its protection from external factors and contaminants [18].

Thanks to IMERs miniaturization, the greater reactor productivity per its volume unit and also time unit is achieved. Firstly, this is due to the high value of enzyme-to-substrate ratio which results from a large local excess of biocatalyst molecules relative to substrate concentration and secondly because of the large value of the surface-to-volume ratio. These properties have the positive effect on the reduction of diffusion-related limitations by reducing the diffusion path which, in turn, improves the ability of the substrate molecules to reach the active sites of immobilized enzymes [10,12,13,19,26]. For μ-IMERs the reaction time is precisely defined while the substrate residence time in the microreactor may be easily controlled by the flow rate [20]. It is particularly important when unstable products are formed and they should be quickly removed from the reaction system or when the equilibrium of the reaction is a factor determining the conversion yield. Additionally, this approach makes it possible to use smaller amounts of reagents which is advantageous from the point of view of sample quantity needed and the concentration of the target analytes. It makes the whole process much cheaper (limited consumption of reagents), and in the case of operation with unknown or dangerous substances also less hazardous [21]. That is why the microreactors can be used for the selection of optimal reaction conditions and evaluation of the biocatalysis economics before implementation of required processes to a larger scale. The experiments performed using μ-IMER allow for estimation of conversion cost as well as its efficiency. For example, Fu et al. immobilized threonine aldolase on three supports (Eupergit CM, silica Nanosprings and membrane) using five different methods and the three reactor designs were then subjected to cost production analysis [22]. Kundu et al. used commercially available lipase-immobilized beads for a comparison of the ε-caprolactone polymerization efficiency in a flow microreactor and conventional batch system [23]. Similar test was also performed for triglycerides hydrolysis by lipase immobilized using silica materials of different pore sizes [24].

It should also be noted that microtube-based enzymatic reactors may be easily connected with other channels or capillaries-based devices which enables coupling of the μ-IMER with separation and detection techniques (chromatography, electrophoresis and mass spectrometry) [25,26]. Such a solution allows for on-line mode working, process automatization and also prevents the reaction products from being contaminated or lost which is more risky in the case of manual handling [7,27]. μ-IMERs also facilitate the analytical process when substrates require special pretreatment before enzymatic conversion (Section 7). Additionally, due to the fact that the reaction is performed under flow and the reagents are continuously removed from the channel/capillary, the saturation of the solution by the reaction products does not affect the enzyme (at least in the entrance of the microreactor) and hence the biocatalysis is not inhibited.

The issues of immobilized enzymes are fully discussed in review articles which are focused on such aspects as general immobilization methods [28–30], selected supports (e.g Eupergit [31], chitin and chitosan [32], mesoporous silica [33]), techniques used for analysis of post-reaction mixtures (e.g. MS-based analysis [26,34]),