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A multicommutated tester of bioreactors for flow analysis

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ABSTRACT

Enzymes are often used in the modern analytical procedures allowing selective recognition and conversion of target analytes into easily detected products. In flow analysis systems, enzymes are predominantly applied in the immobilized forms as flow-through bioreactors. In this research the multicommutated flow analysis (MCFA) system for evaluation and comparison of analytical parameters of bioreactors has been developed. The MCFA manifold allows simultaneous testing up to four bioreactors, but if necessary their number can be easily increased. The system allows comparison of several parameters of tested bioreactors including activity, repeatability, reproducibility, operational and storage stability. The performance of developed bioreactor tester is presented using urea-urease model system based on plastic open-tubular bioreactor with covalently immobilized enzyme. Product of enzymatic reaction is detected using two different chemical methods and by dedicated optoelectronic ammonium detectors. Moreover, the utility of developed MCFA manifold for evaluation of other enzyme bioreactors is demonstrated.

1. Introduction

The application of enzymes in analytical chemistry seems to be very attractive, especially because it can ensure significant expansion of the range of available analytes for conventional analytical methods and significantly improves of selectivity of determination due to the high specificity of biocatalytic recognition. Analytical uses of enzymes are predominantly connected with the detection of respective substrate of biocatalytic process. These applications require high activity of biocatalytic system to provide highest conversion of an analyte into a detected product and this is typically involved with high enzyme consumption. Hence from economical and practical point of view, enzymes are mainly applied in the immobilized forms such as biosensors and bioreactors.

Both bioreactors and biosensors operation is based on kinetic character of enzymatic reaction. The kinetics of biocatalytic conversion is a function of enzyme activity, time, reaction condition, reagent concentration, etc. Evidently for the accurate determination of a substrate concentration all these parameters should be strictly controlled and this could be readily available under flow analysis conditions. The reaction time and transport are well defined by the configuration of flow manifold. The chemical condition of the process is controlled by the composition of carriers used. And here comes the main drawback of biosensors. These biodevices required compromise between optimal condition for enzymatic reaction and detection process and they can be used only with sensor detectors. In case of bioreactor application, each single step can be independently optimized and flow system can be coupled with various electrochemical and optical detection systems operating under different conditions. Additionally different bioreactors could be placed in parallel, giving the opportunity for simultaneous determination of two or even more analytes with the same detector. Thanks to this advantages bioreactors find vide application in flow analysis.

A lot of analytical bioreactor types are applied in flow analysis systems. There are various construction designs, from simple capillary [1,2] and open tubular [3,4] bioreactors to mechanized rotating bioreactor [5–7] and most popular packed bioreactors [8–10]. There are a lot of described enzyme immobilization supports: aminopropyl glass [9,11] as well as controlled porous glass beads (CPG) [12–16], silica [17,18], resins [19], nanoparticles [20,21], porous amalgams [22], plastics such as polyvinyl chloride (PVC) [3,4,23,24] and poly (methyl methacrylate (PMMA)) [25,26], nylon fibers [27], ceramics [28,29], paper [30] or even biological matrix such as eggshell membrane [31], and they all can provide base for such biological material as enzymes [12,19,32], whole cells [33], DNA [34] or antibodies [35], immobilized with a various routines

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[32,36]. As can be expected all version differ in activity, stability, repeatability of obtained bioreactors [37].

Not only various types of bioreactor differ each other. Unfortunately the result of the same immobilization process can be quite random. Protein molecules can be immobilized in random orientation via different moieties which can cause different arrangement of active site and different accessibility of substrate molecule [38]. Also, even small matrix surface defects could effect in a different amount of bounded protein. In effect bioreactors of the same type obtained using the same immobilization protocol can vary in individual properties. Therefore preparation procedure as well as analytical utility should be evaluated on a base of several of bioreactors tests.

In this paper multicommutated flow analysis system (MCFA) for evaluation and comparison of analytical parameters of bioreactors is reported. The performance of presented multicommutated bioreactor tester is presented using a model urea/urease system based on plastic open-tubular bioreactor [3,4] with covalently immobilized enzyme and dedicated optoelectronic PEDD (paired emitter- detector diodes) detector [39,40]. Utility of developed manifold for other substrate-bioreactor-detector systems are also demonstrated. Presented manifold allows simultaneous testing up to four bioreactors but if necessary their numbers can be easily increased up to eight or sixteen. In the effect the presented MCFA system enable simultaneous, repeatable testing of multiple bioreactors.

2. Experimental

2.1. Reagents and materials

All applied enzymes: urease (lyophilized powder isolated from Jack bean, 100 U/mg, EC 3.5.1.5), alkaline phosphatase (AlP, lyophilized powder, isolated from bovine intestinal mucosa, 24 U/mg, EC 3.1.3.1) and glucose oxidase (GOx, lyophilized powder isolated from *Aspergillus Niger*, 130 U/mg, EC 1.1.3.4), immobilization components: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and high-molecular-weight carboxylated polyvinylchloride (PVC-COOH), as well as p-nitrophenyl phosphate disodium salt (NPP), Nessler reagent and Berthelot reaction components: sodium salicylate and sodium hypochlorite, were purchased from Sigma (USA). All other reagents including urea, β-D-glucose and ascorbic acid, buffer components and solvents were obtained from Avantor (Poland). Water used for experiments was distilled and passed through a Milli-Q purification system.

Bioreactors tubes and optical flow through cells was made of PEEK (Poly-Ether Ether Ketone). The body of these devices was micromachined using manually operated milling machine and lathe 630 nm red, 470 nm blue and 405 nm violet light emitting diodes for construction of PEDD detectors were purchased from Optosupply (China): 375 nm UV LED was obtained from Amecam (Poland). For supplying LED emitter lab-made circuit was prepared with typical electronic components (TME Poland). For recording the voltaic signal [40] generated by a PEDD detectors a voltmeter model U1231A from Keysight Technologies (USA) connected with PC via Bluetooth®-IR interface was applied.

2.2. Bioreactors preparation

For each experiment new set of bioreactors was prepared. First step of preparation was fabrication of plastic reactor. The 8 mm (OD) PEEK rod was micromachined to obtain tubes with adequate dimension: 30 mm length, 5.8 mm outside diameter and 2.4 mm of inside diameter. Next the internal surface of tube was chemically modified by deposition of PVC-COOH. 5% solution of PVC-COOH dissolved in tetrahydrofuran (THF) was injected into PEEK tube and left for 30 s. Then solution was removed and tubes left for THF evaporation in room temperature. After THF evaporation freshly prepared, unbuffered solution of enzyme with activating reagent (EDAC) was prepared. Enzyme solution was placed inside tubes and left for water evaporation. The EDAC and enzyme content were variable in consecutive experiments. Finally, the bioreactors were several times vigorously rinsed alternately with distilled water and working buffer to remove excess of non-covalently bonded enzyme.

2.3. Optoelectronic detectors and sensors

In this study four photometric analytical methods were applied. To determine the content of ammonium ions, generated in the course of urea hydrolysis in urease bioreactors, Nessler's [41] and Berthelot's [24] reactions were applied. For evaluation of ALP bioreactor the hydrolysis of NPP to intensively yellow p-nitrophenol was photometrical detected [42]. For determination of hydrogen peroxide generated by GOx bioreactor photometric method with Prussian Blue/Prussian White optosensing film was applied [43,44].

Photometric flow cells used in Nessler, Berthelot and p-nitrophenol assays were lab-made as described elsewhere [45] and have 10 mm optical path length and 3 mm aperture. PEDD-optosensor has 1.7 μL internal volume and active sensing area approx. 0.07 mm^2.

2.4. Multicommutated flow system

The developed MCFA manifold was arranged with microsolenoid pumps (stroke volume of 10 μL, product no. 120SP1210-4TE), three-way microsolenoid valves (product no. 100T3MP12-62-5), three way pinch valves (product no. 100PD3MPAL-02S), six-way pinch valves (product no. 100P3MPAL-02S), check valve (product no. CF5C) purchased from Bio Chem Fluidics (USA) and PTFE Microbore tubing (ID 0.8 mm) obtained from Cole-Palmer (USA).

Solenoid devices were controlled by microcontroller prototype board Arduino™ Mega (Arduino, Italy) with ULN2803A (Texas Instruments, USA) integrated circuit and external AC adapter (12 V, 4 A, LED-POL, Poland). Connection scheme of simple solenoid driver is given in details in the Supplementary material Additionally, an exemplary program (as a zip.file) is there attached (Supplementary material 2).

The modular flow system presented on Fig. 1 consists of several components. Single standard calibration module (SSC, red) which details have been discussed in detail elsewhere [46], bioreactor selector module (BSM, green), injection module (IM, purple) for substrate of enzyme reaction and detection module (DM, orange) with vary construction dependently on applied analytical procedure.

Modules are operated in parallel by programmed microcontroller to perform desired tasks. The protocol of operation begin from “Rinsing”. During this state devices V1, PV2, PV3 are in NO (normally open) position, P1 actuated 2 Hz 500 times. Carrier buffer is directed through each bioreactor respectively and switching BSM valves PV1 and SPV1 states result in selection of desired bioreactor flow-through.

Next step of protocol is “Aspiration” when devices PV2, PV3 are in NO position, P1 actuated 2 Hz 300 times, and V1 actuated to obtain chosen calibrant which is directed to desired bioreactor. Then the segment is stopped inside bioreactor for selected incubation time. After this step some time with no actuation was applied to equalize the time of incubation of consecutive bioreactor, if needed.

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