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PARALLEL REACTOR SYSTEM FOR SCREENING AND CHARACTERISATION OF BIOCATALYSTS

UKŁAD RÓWNOLEGŁYCH APARATÓW REAKCYJNYCH W KONTROLI I CHARAKTERYSTYCE BIOKATALIZATORÓW

Abstract

Screening, characterisation and estimation of reliable kinetic parameters of biocatalysts is a complex and time consuming task in bioprocess development. The commercially available screening and characterisation systems are mostly operated in (fed-)batch mode, so the data obtained from such systems do not offer the possibility to describe/design a continuous process in e.g. a membrane reactor. Thus, the primary goal of this project is the development of a membrane-based screening and characterisation system that enables physical immobilisation of the dissolved catalyst in the reactor volume while at the same time, products can be removed and so the system can be operated continuously.

Keywords: screening system, miniaturized membrane reactor, process development

Streszczenie

Kontrolowanie, charakteryzowanie oraz szacowanie wiarygodnych parametrów kinetycznych biokatalizatorów to złożone i czasochłonne zadanie w ramach rozwoju bioprocesowego. Dostępne na rynku systemy kontroli i charakterystyki działają zazwyczaj w trybie pakietowym, co powoduje, że uzyskane dane nie dają możliwości opisania czy zaprojektowania procesu ciągłego, np. w membranowym aparacie reakcyjnym. Tak więc zasadniczym celem niniejszego projektu jest opracowanie systemu kontroli membranowej i charakterystyki, który umożliwi fizyczne unieruchomienie rozpuszczonego katalizatora w pojemności aparatu reakcyjnego przy jednoczesnym usuwaniu produktów, co zapewni ciągłe działanie systemu.

Słowa kluczowe: system kontroli, zminiaturyzowany membranowy aparat reakcyjny, rozwój procesów

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1. Introduction

Industrial biocatalysis is often carried out continuously e.g. in membrane reactors. In comparison to batch or fed-batch reactors, these offer versatile advantages: Membranes with a MWCO from 5−10 kDa easily separate homogeneously distributed enzymes from the product. Thus, there is no need neither for an immobilization procedure which would cause additional costs and − what is even more important − development time, nor for the enzyme to be substituted after each batch which would also cause additional costs as well an additional procedure for enzyme deactivation, since not all products tolerate the residual enzymes. Wöltinger et al. [1] summarize: "The use of soluble enzymes in biotransformations presents significant advantages over immobilized enzymes in terms of productivity, selectivity and economics". However, to our knowledge, none of the commercial available screening and characterization systems can operate in continuous mode with homogeneously distributed catalysts without catalysts losses. So, the data collected from such systems helps one to design a fed-batch pilot-scale reactor, but cannot help to estimate the potential of the continuous operation in a membrane reactor. The exceedingly important data, such as enzyme stability in continuous operation mode, enzyme leaching through the membrane, enzyme adsorption on the membrane surface or membrane fouling effects and membrane long-term performance stay completely obscured.

Recently we proposed [2−4] a new concept of a membrane reactor based screening and characterization system. The system contains initially 2 parallel membrane reactors of small-scale (approx. 90 mL) and standard geometry (h/D = 1.8) and enables continuous operational mode. Temperature and hydraulic retention time (HRT) are monitored and closed-loop controlled. For precise dosing of small values of additional components (e.g. for enzyme activity or pH control) a simple low-cost dosing system based on a switching mechanism of parallel micro solenoid valves was designed and built into the system. In this contribution we validate the potential of the system for precise HRT control, dosing of small liquid values as well as for long-term operation.

2. Materials and methods

2.1. Set-up

The main components of the developed screening and characterisation system (Fig. 1A) are: membrane reactor (1, made in the departmental workshop of TU Berlin, Germany), pressure regulator (2, MPPE-3, Festo AG, Germany), mixing device (3, MIX1, 2MAG, Germany), thermostat (4, Thermo Haake GmbH, Germany), precision balance (5, ALT 310, Kern & Sohn GmbH, Germany), pH-sensor (6, QP930X, ProSense, Netherlands) as well as a safety valve (7, EC-218.12, Riegler GmbH, Germany). All components are united over Visual Designer™ (Ver. 4.0) interface for data collection, processing and closed-loop process control.

The membrane reactor was designed based on a commercial dead-end test cell from Millipore Corp. (XFUF-047) with a working volume of approx. 90 mL and a net membrane surface area of 14 cm^2. The main components of the constructed membrane reactor (figure 1B) are: membrane (1), magnetic stirrer (2), connectors for pH and temperature sensors (3 and 4, respectively) as well as connectors for inlet and outlet pipes (5 and 6).

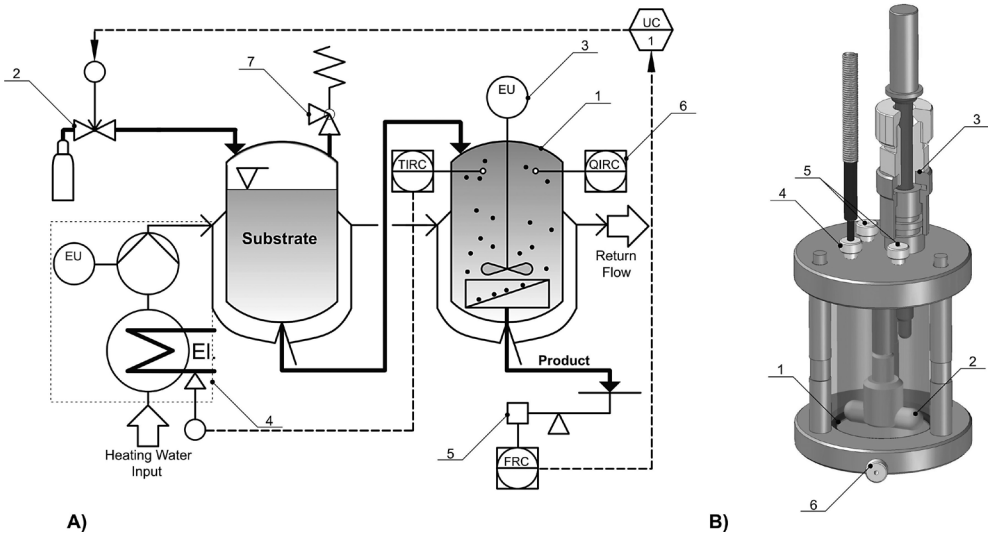

Fig. 1A. Simplified flow-sheet of the screening and characterisation system (for clarity, only one of the parallel reactors is shown); 1B. Design of the membrane reactor (from [4])

Rys. 1A. Uproszczony blokowy schemat działania systemu kontroli i charakterystyki (dla przejrzystości pokazano tylko jeden z równoległych aparatów reakcyjnych); 1B. Projekt membranowego aparatu reakcyjnego ([4])

2.2. Chemicals and membranes

The enzymatic hydrolysis of N acetyl L-methionine (NAM, 22003320, Molekula GmbH, Germany), represents a well-known and industrially important reaction (Wöltinger et al., 2001). This model reaction with the acylase I from Aspergillus melleus (534862, Sigma-Aldrich Corp., USA) was used to validate the continuous operation with typical problems encountered at full-scale and during long-term operation. The reaction was buffered by means of tris buffer. Polyethersulfon (UP005, 5 kDa, Microdyn Nadir GmbH, Germany) and regenerated cellulose (Hydrosart 14429, 5 kDa, Sartorius AG, Germany) membranes were used. D-glucose (101174Y, VWR International, USA) was used as a tracer in dosing experiments.

2.3. Enzyme activity test

Enzyme activity for acylase I was measured at T = 37 °C, pH = 8.0, $C_{NAM,0}$ = 20 mM, and C_{BUFFER} = 50 mM where 1 unit of acylase I liberates 1 μmol/h of L-Methionine (MET) from NAM.

2.4. Analytical methods

The concentration of the produced MET was measured by means of spectrometry (Specord 200, Analytic Jena AG, Germany) according to [6]. The concentration of the D-glucose was measured by means of refractometry (DD-7, ATAGO Co, Ltd., Japan).

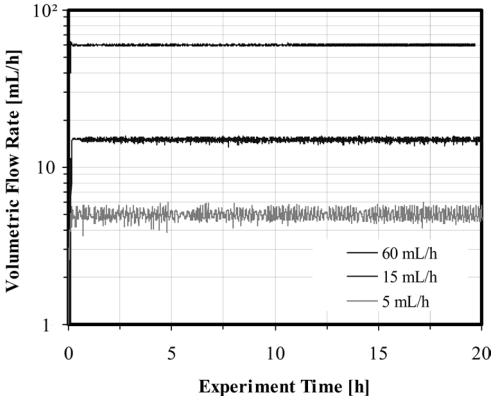

Fig. 2. Flow control from 5-60 mL/h−over experiment time, T = 30°C, UP005, ultrapure water

Rys. 2. Kontrola przepływu z 5-60 mL/h w czasie przeprowadzania eksperymentu, T = 30°C, UP005, kryształowa woda

3. Results and discussion

3.1. Flow control

The maintenance of a desired HRT (i.e. a desired flow rate) is one of the key process characteristics. As we previously reported in [2, 3] it was possible to keep it at any desired value within the range of 7.5−30 mL/h with an accuracy of ±1 %. Recently we extended this range to 5−60 mL/h (flux of approx. 3.5−45 L/(m²h)) and to 1.5−18 h HRT, accordingly (Fig. 2). The extended range gives an additional opportunity not only for new reactions with long or short residence times but also for precise flux behaviour investigations (e.g. measurement of critical flux). From Fig. 2 it can be seen, that even at very low flow rates of 5 mL/h, the system allows a precise control. Although the system is incapable of maintaining a precise feed pressure, caused by the maximal pressure regulator precision of ±20 mbar, the integrated PI/PID flow controller works very well, so that the standard deviation was less than 7% even for the low flow rate of 5 mL/h, and the standard deviation of the averaged flow (over 1 h) was less than 0.3%.

3.2. Dosing system

Figure 3 represents the proof of the dosing concept and shows dosing tests from trials with 0.25−2 mL dosing volume. Small liquids volumes could be dosed with a precision of more than 97% (2σ-deviations are less than 3%). This gives as a reliable basis for further automation, i.e. for integration of pH control, dosing of additional components or enzymes for an activity control.

3.3. Continuous NAM hydrolysis

Figure 4 shows the comparison between three continuous NAM-hydrolyses, with error bars representing the expected analytical errors. Two hydrolyses were carried out under the

same conditions, to prove the reproducibility. In the third hydrolysis the enzyme activity as well as temperature were increased.

Figure 4 proves good reproducibility of the results. Both experiments which were carried out under the same conditions show an identical reaction course: all measurement points lie within the analytical error range. The increase of the enzyme activity as well as temperature leads as expected to higher conversion, but the deactivation rate achieves after 140 h of operation nearly 20%. This represents a typical optimisation task, which can be investigated in the developed system.

Considering the proven reproducibility also for another reaction − the hydrolysis of cellulose (reported in [2, 3]) − it can be stated that we developed a reliable instrument for process characterisation.

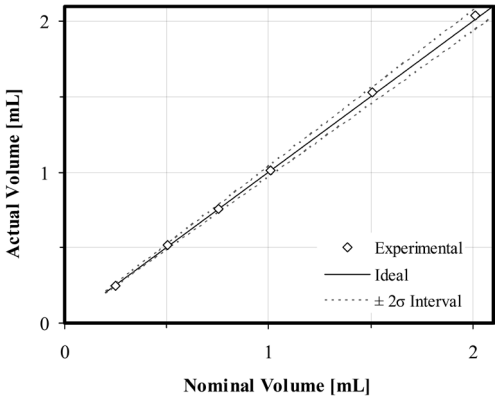

Fig. 3. Proof of the dosing concept, T = 30°C, tracer: D-glucose solution with $C_{D\text{-}g}$ = 100 g/L

Rys. 3. Próba koncepcji dozowania, T = 30°C, wskaźnik izotopowy: roztwór glukozy D przy $C_{D\text{-}g}$ = 100

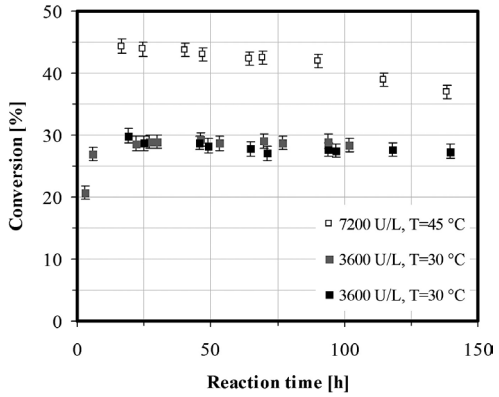

Fig. 4. Hydrolysis of NAM during continuous operation, at HRT = 6 h, $C_{NAM,0}$ = 20 mM, $C_{ENZYME,0}$ = 3600−7200 U/L, T =30−45°C, pH = 8.0

Rys. 4. Hydroliza NAM podczas operacji ciągłej przy HRT = 6 h, $C_{NAM,0}$ = 20 mM, $C_{ENZYME,0}$ = 3600–7200 U/L, T = 30–45°C, pH = 8,0

4. Conclusions

A concept of a novel screening and characterisation system, based on 2 parallel miniaturised membrane reactors was developed and presented. The system allows continuous operation mode with soluble enzymes without enzyme depletion. Variable hydraulic retention times from 1.5−18 h can be easily adjusted with precision of typically more than 99%. Two industrially important reactions were investigated in the presented system. The reactions have shown excellent reproducibility, proving the usability of the system for reaction and process description. A simple low-cost dosing concept was implemented and evaluated, showing a great potential for system extension. The successful integration of the dosing concept into the whole system will allow pH control, dosing of additional media e.g. for enzyme activation, additional substrates or co-factors. The system will be further developed to include new features and reactions.

Symbols

C	–	Concentration [mol/m³] or [kg/m³] or U/L
D	–	Diameter [m]
HRT	–	Hydraulic retention time [h]
h	–	height [m]
MWCO	–	Molecular weight cut off [Da]

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