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An integrated process: Ester synthesis in an enzymatic membrane reactor and water sorption

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Abstract

In the case of such reactions as ester synthesis, water is produced during the reaction. Because these reactions are carried out in hydrophobic solvents an additional (water) phase in the system must not be allowed, i.e. the concentration of water saturation in the organic solvent should not be exceeded. In such a case, the reaction kinetics and product equilibrium concentration undergo undesirable changes because of the partition coefficient of the components and hampered process of product separation. Hence, removal of the water produced in the reaction determines whether the process is successful or not. For this purpose, the integrated process with water sorption in the column with molecular sieves was applied.

Integration of the process of synthesis and dehydration of a reaction phase, in which a biocatalyst is suspended and not dissolved as in water solutions, requires holding up of the catalyst in the reactor before directing the stream of reaction mixture to dehydration process. This hold-up and a possibility of multiple use of the catalyst may be accomplished by using a separating barrier, e.g. an ultrafiltration membrane or by permanent fixing of the catalyst to the matrix, e.g. a polymeric membrane.

The efficiency and activity of a biocatalyst (lipase CAL-B) immobilized on a polymer membrane by sorption and chemical binding, were determined. A subject of study was the synthesis of geranyl acetate, one of the most known aromatic compound. A hydrophobic (polypropylene) matrix was shown to be a much better carrier in the reactions performed in an organic solvent than a hydrophilic (polyamide) membrane being tested.

The reaction kinetics of geranyl acetate synthesis with the use of geraniol and acetic acid as substrates, was described by the equation defining the "*Ping-Pong Bi Bi*" mechanism that was related additionally to the inhibition of a substrate (acetic acid). The following constants of kinetic equation were obtained $k_3' = 0.344\ \text{mol g}^{-1}\ \text{h}^{-1}$, $K_{\text{mA}} = 0.257\ \text{mol l}^{-1}$, $K_{\text{mG}} = 1.629$ and $K_{\text{iA}} = 0.288$ for the native enzyme and $v_{\text{max,Gel}} = 111.579\ \text{mol l}^{-1}\ \text{h}^{-1}$, $K_{\text{mA}} = 0.255\ \text{mol l}^{-1}$, $K_{\text{mG}} = 1.91\ \text{mol l}^{-1}$, $K_{\text{iA}} = 0.238\ \text{mol l}^{-1}$ for the one immobilized by sorption on a polypropylene membrane. Half-life time of the native enzyme activity was 204 h and stability of the immobilized preparation was 70 h.

With respect to the reaction kinetics and stability of the native enzyme and immobilized preparation, from both types of membrane bioreactor more attractive appears to be the one in which the membrane is used not as a catalyst layer but only as a barrier that immobilizes the native enzyme within the bioreactor volume. When an integrated process proceeds, the method to collect water in the sorption column during the process, appeared to work very well. The reaction proceeded with a very high efficiency (after 120 h $\alpha = 98.2\%$ for native enzyme and 83.2% for immobilized enzyme) and due to low water concentration in the system (≈0.000% v/v) the second phase was not created.

Keywords: Membrane bioreactor; Organic solvent environment; Enzyme immobilization; Catalytic membrane; Geranyl acetate; Enzymatic kinetics

1. Introduction

Terpene esters are important flavoring and aromatic components widely used in food industry. Owing to numerous healing properties, they are applied also in pharmaceutical and cosmetic industry (Harborne, 1997).

Traditionally, these compounds are isolated from a vegetable material by extraction or are produced by chemical methods. The first way is usually expensive for commercial production, while the other one is a non-specific process that requires further purification. Hence, an enzymatic method with the use of a natural catalyst gains importance (Claon and Akoh, 1994a; Yee et al., 1995; Lee et al., 1998). A high cost of the biocatalyst requires a technology that will enable its multiple use.

Therefore, it becomes vital to obtain such an enzymatic preparation that will be characterized by high activity and stability,

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Nomenclature

A	membrane surface (m^2)
c_A	concentration of acetic acid ($mol\,l^{-1}$)
c_{A0}	initial concentration of acetic acid ($mol\,l^{-1}$)
c_E	concentration of enzyme ($g\,l^{-1}$)
c_G	concentration of geraniol ($mol\,l^{-1}$)
c_{G0}	initial concentration of geraniol ($mol\,l^{-1}$)
c_{Gel}	enzyme concentration in gel layer ($g\,l^{-1}$)
$c_{Prod.}$	concentration of product (geranyl acetate) ($mol\,l^{-1}$)
$k_{'}$	inactivation constant (h^{-1})
$k_3^{'}$	bisubstrate reaction constant ($mol_{Prod.}\,g_E^{-1}\,h^{-1}$)
K_{iA}	inhibition constant for acetic acid ($mol\,l^{-1}$)
K_{mA}	Michaelis constant for acetic acid ($mol\,l^{-1}$)
K_{mG}	Michaelis constant for geraniol ($mol\,l^{-1}$)
$N_{Prod.}$	amount of product (geranyl acetate) (mol)
Q_r	retentate stream ($m^3\,h^{-1}$)
$v_{'}$	rate of bisubstrate reaction ($mol_{Prod.}\,l^{-1}\,h^{-1}$)
$v_{max,Gel}^{'}$	maximal rate of bisubstrate reaction in catalytic layer ($mol_{Prod.}\,l^{-1}\,h^{-1}$)
V_b	volume of substrate solution (l)
V_{Gel}	volume of gel layer (reactor) (l)

Greek symbols

α	limiting substrate conversion degree (%)

and at the same time will enable the process to be carried out with a simultaneous separation of by-products, especially when they might have a negative impact on the process. This is feasible in the systems with an immobilized catalyst (Paiva and Malcata, 1997; Ivanov and Schneider, 1997; Trusek-Holownia, 2005a).

Due to esterifying and hydrolyzing properties of lipases which are the enzymes catalyzing the synthesis of terpene esters, and owing to the equilibrium of such a reaction, the reactions are carried out in an organic solvent which contains a minimum amount of water necessary to induce the enzyme activity (Claon and Akoh, 1994b; Shieh et al., 1996) or almost in an anhydrous system (Anderson et al., 1998; Kontogianni et al., 2003). Holding up a proper amount of water in this type of catalysis is a key parameter. On the other hand, the control of water amount in the system causes many problems, specially in the reactions in which water is formed as a by-product, i.e. in the esterification reactions.

Depending on the enzyme type, the required minimum amount of water in the reaction system is different (in the catalysis it is often expressed as water activity) (Zaks and Klibanov, 1988; Matsue and Miyawaki, 2000). Water content in the system depends also on the presence of additional system components, e.g. a matrix for immobilization (Chulalaksananukul et al., 1992).

In the case of reactions carried out in hydrophobic solvents, water concentration in the reaction system changes in the range of dehydrated solvent-saturated solvent. For obvious reasons, an additional (aqueous) phase in the system must not be allowed, i.e. the concentration of water saturation in the organic solvent should not be exceeded. In such a case, the reaction kinetics and product equilibrium concentration undergo undesirable changes because of the partition coefficient of the system components and hampered process of product separation. Hence, the removal of water produced in the reaction determines whether the process is successful or not. For this purpose, the process of sorption (de Castro et al., 1992; Wehtje et al., 1997), and pervaporation (Van der Padt et al., 1993), distillation (Gubicza et al., 2000) or sparging of dry inert gas (Jeong and Lee, 1997) can be used.

Integration of the process of synthesis and dehydration of the reaction phase, in which a biocatalyst is suspended and not dissolved as in water solutions, usually requires holding up of the catalyst in the reactor before directing the reaction stream to dehydration process. This hold-up and a possibility of multiple use of the catalyst may be accomplished by using a separating barrier, e.g. an ultrafiltration membrane or by permanent fixing of the catalyst to the matrix.

In this study, efficiency and activity of a biocatalyst immobilized on polymer membranes by sorption and chemical binding, are determined. Properties of the immobilized preparations are compared with the native enzyme properties and a method of an integrated process: reaction with water sorption will be proposed. A subject of study is the synthesis of geranyl acetate, one of the most known aromatic compound, which is carried out in the presence of lipase from *Candida antarctica*.

2. Materials and methods

The enzyme, lipase B from *C. antarctica* (CAL-B) recombined in *Aspergillus oryzae* was obtained from Fluka (USA). The reaction substrates geraniol (>96% pure) and acetate acid (99.8%) were purchased from Fluka (USA) and Aldrich (USA), respectively. The standard of product (geranyl acetate) was obtained from Aldrich (USA).

A flat polyamide (PA) membrane 25 mm in diameter with pore diameter 0.2 μm, was purchased from Millipore (USA), a flat polypropylene (PP) membrane 25 mm in diameter with pore diameter 0.2 μm, was obtained from Euro-Sep (Poland).

Molecular sieves 3 Å were obtained from Fluka (USA).

In concentration analyses a Dani gas chromatograph (Italy) and a Shimadzu spectrophotometer (Japan) were used. Water concentration in the organic solvent was measured by a Karl–Fisher apparatus (Mettler-Toledo, Switzerland).

A membrane modules (with perpendicularly flow and cross-flow) were prepared in a laboratory, a gear, pressure pump was supplied by Cole-Parmer (USA) and thermostated shaken baths by Merck (Germany).

2.1. Selection of an organic solvent

The following organic solvents were used in the experiments: isooctane ($\log P = 4.5$), hexane ($\log P = 3.5$), pentane ($\log P = 3.0$), toluene ($\log P = 2.5$), 1-butanol ($\log P = 0.8$) and ethyl acetate ($\log P = 0.7$). Before the process, the solvents were saturated with 0.05 M Tris–HCl buffer, pH 7.6 (selected for this enzyme).