Lipase immobilization in a silica gel matrix

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Introduction

There are a number of limitations to the use enzymes in their natural native form in biotransformation processes. The most significant ones are those connected with their separation from reaction products and the accompanying problem of the impossibility to reuse them. Immobilization methods, bonding or enclosing the enzymes in heterogenic systems which are water insoluble but guarantee diffusion of substrates and products, are a good solution. The present paper is focused on a method for the entrapment of lipase within a 3D, porous silica matrix, formed in a process involving the hydrolysis and polycondensation of tetramethoxysilane (TMOS), the best known representative of alkoxysilanes (organic derivatives of silicic acid). The method is known as the sol-gel process because the hydrolysis of alkoxysilanes $Si(OR)_4$ to silanols (≡Si-OH) leads to the formation of a sol which is then converted into a gel as the result of mutual condensation of silanols or their condensation with alkoxysilanes [1]. There are three methods to hydrolyze TMOS: the acid method [2, 3], the alkaline method [4], and the one induced by fluoride salt solutions such as sodium fluoride (NaF) [5÷7]. The two former ones only enable immobilization of lipase in block, in which case the enzyme solution is introduced together with the TMOS hydrolyzate into a buffer, which causes immediate gelation. The third method, which combines hydrolysis and polycondensation in a single operation, provides new ways to carry out the lipase immobilization process by the emulsion method, omitting the block crushing process. A 1 M NaF solution is a widely used catalyst in the method. At such high concentrations, immediate hydrolysis and polycondensation of TMOS leads to a gelation of the entire mixture [5, 6, 8], therefore, immobilization may also be carried out in block only. The present work is intended to slow down the gelation process by means of more diluted solutions of NaF and to check its effect on the activity of biocatalyst. Slowing down the process, if successful, would provide an opportunity to manipulate the gelling mixture and conduct the sol-gel process in emulsion.

Materials

Enzyme: lipase from Candida rugosa (Type VII, product ref. number: L1754, Sigma-Aldrich), tetramethoxysilane (TMOS, Fluka), sodium fluoride (NaF, POCh), poly(vinyl alcohol) (PVA, Fluka), tributyrin (Sigma), 0.1 M sodium hydroxide (analytical grade NaOH, POCh), calcium chloride ($CaCl_2$, POCh), potassium chloride (KCl, POCh), sodium dihydrogen phosphate (NaH_2PO_4, POCh), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, POCh), phenolphthalein (POCh).

Methods

Immobilization

A reaction vessel was filled with an aqueous lipase solution (29.4 mg/ml) and a 4 % aqueous solution of poly(vinyl alcohol) and sodium fluoride was added with concentrations of 1 mol/dm^3, 0.1 mol/dm^3 and 0.01 mol/dm^3. The system was mixed vigorously for several seconds for an effective mixing of the components. Then, tetramethoxysilane (TMOS) was added and mixing was continued. The reaction mixture was then fast transferred into plastic containers with closures and placed in a refrigerator at 4°C for 2 hours for the gel maturation. The containers were opened and left to dry for 10 days. The obtained xerogel blocks were crushed in a porcelain mortar and the resulting fine crystalline powder was subjected to a sieve analysis. Fractions with the grain size 0.1-0.2 mm and 0.325-0.5 mm were selected for activity tests.

Determination of biocatalysts' activities

The activity of the immobilized lipase was tested in a model reaction of tributyrin hydrolysis to glycerol and butyric acid:

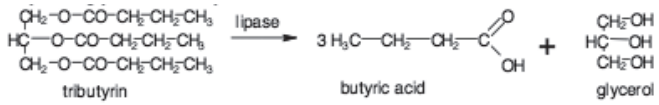

Fig. 1. Tributyrin hydrolysis reaction

Butyric acid, which was separated in the reaction, was quantified by alkalimetric titration in the presence of phenolphthalein. Aqueous sodium hydroxide (NaOH) at 0.05 mol/dm^3 was used as a titrant.

Discussion of results

The results of qualitative observations of the rate of formation of a stable gel of the biocatalyst are shown in Table 1. The results indicate that the use of a ten-fold and a hundred-fold dilution of the solution of the sodium fluoride catalyst led to longer gelation times, which increased in proportion to the increasing dilution.

Table 1

Results of qualitative observations of gelation times

NaF concentration, mol/dm^3	Gelation time, min	
	Sample 1	Sample 2
1.00	0.08	0.08
0.10	3.00	4.00
0.01	20.00	17.50

The gel volume was observed to be reduced on drying. In addition, the gel was observed to crack due to capillary stresses caused by the uneven distribution of the effect of capillary forces on pore walls with variable diameters. Unexpectedly, the observed cracking density in the xerogel depended on the concentration of sodium fluoride as the catalyst (Fig. 2).

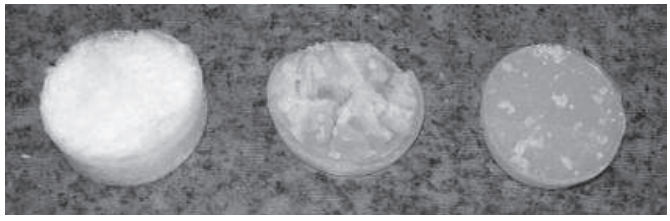

Fig. 2. A photograph of cracked biocatalyst blocks, prepared using sodium fluoride as catalyst at the following concentrations (from the left): 1 mol/dm^3, 0.1 mol/dm^3 and 0.01 mol/dm^3

The cracking density tended to be lower for lower concentrations of the sodium fluoride which was used for the hydrolysis of TMOS.

On crushing, the blocks tended to vary in terms of hardness and mechanical strength. The block obtained with the use of a NaF solution at 0.1 mol/dm^3 was the hardest block with the best mechanical strength. The poorest mechanical strength and highest fragility were shown by the block obtained with the use of the NaF solution at 1 mol/dm^3. The block obtained with the NaF solution at 0.01 mol/dm^3 showed medium hardness and mechanical strength. The information enabled the authors to plan the studies of the sol-gel process in emulsion.

Activity was expressed as the number of μmoles of butyric acid, formed by 1mg of a lipase contained in the biocatalyst during 1 hour. Typical results of the biocatalysts's activity prepared with the use of the catalyst at 3 concentrations are shown in Fig. 3. Relationships between the linear dimension of the biocatalyst grains and its activity were verified as well. Two grain sizes, having the dimensions 0.1-0.2 mm and 0.325-0.5 mm, were selected for every one of the 3 biocatalysts. Tests (Fig. 3) showed the highest activity of the biocatalyst prepared with the use of the NaF solution at a concentration of 0.1 mol/dm^3, lower for 0.01 mol/dm^3, and the lowest for 1 mol/dm^3. However, the differences were not very significant. Moreover, the biocatalyst with the higher linear dimension showed a lower activity in each case.

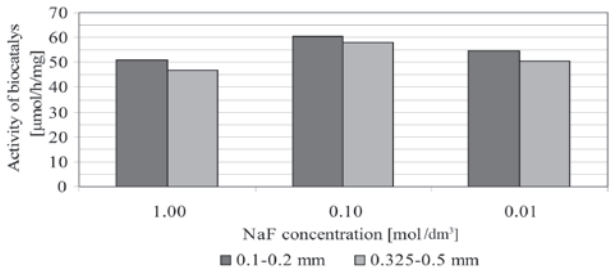

Fig. 3. Effect of concentration used during immobilization of NaF solution and of grain size of biocatalysts on their activity

This was caused by a poorer availability of the enzyme molecules, located deep in larger grains, to the substrate and the accompanying occurrence of higher diffusion resistances.

A series of activity measurements were conducted to determine the effect of the biocatalyst storage conditions on its stability. To this end, the biocatalyst was stored in two containers: at an ambient temperature (ab. 20°C) and at 4°C and its activity was measured at certain time intervals. The results are shown in Figure 4.

Changes of activity in time were comparable for all of the three biocatalysts: a higher loss of activity was observed on storage at ambient temperatures and this was probably due to the faster deactivation of the enzyme.

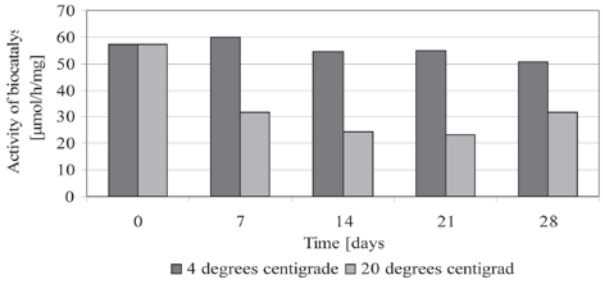

Fig. 4. Changes in the activity of biocatalyst on storage at an ambient temperature (20°C) and at 4°C

Summary and conclusions

To sum up the present studies on the process of lipase immobilization by the sol-gel method and on the activity and stability of the obtained biocatalysts, the following conclusions may be drawn:

- The use of the catalyst (NaF) at a lower concentration leads to slowing down the gelation process and has a minor effect on the activity of the obtained biocatalyst
- The grain size of biocatalyst has a minor effect on its activity
- The storage temperature of biocatalyst has a significant effect on its stability.

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Translation into English by the Author

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