Immobilization of catalase from *Aspergillus niger* in calcium alginate gel

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Introduction

The consumption of H_2O_2 in a variety of industries has been growing fast [1, 2]. The highest consumption of H_2O_2 is for pulp delignification and bleaching in the pulp and paper industry. Moreover, H_2O_2 is used for whitening natural and man-made fibers in the textile industry and as a bactericide in the pharmaceutical and food processing industries.

In a majority of cases, it is necessary to remove any residual H_2O_2. Chemical methods, which are traditionally used, often lead to the formation of undesirable byproducts. Therefore, catalase (E.C. 1.11.1.6) may be an alternative. Catalase is an enzyme from the group comprising oxydoreductases and is known to catalyse decomposition of H_2O_2 according to the following reaction:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (1)$$

Commercial uses of catalase are limited to its native form [3, 4]. Numerous attempts have been made to immobilize the enzyme [2÷7] in order to enable its repeated use in H_2O_2 decomposition processes, however, the immobilization methods used appeared to be too complicated and expensive.

The present paper relates to the immobilization of catalase from *Aspergillus niger* by entrapment in calcium alginate gel. The method is extremely simple and inexpensive and the alginate may be recovered from a deactivated biocatalyst for reuse in immobilization [8].

Materials and methods

Catalase *Aspergillus niger* C3515 and a low-viscosity sodium alginate A2158 from Sigma-Aldrich were used.

Immobilization of catalase

25 ml each of sodium alginate solutions containing 2 % w/w and 4% w/w were prepared. 30 μl catalase was added to each sodium alginate solution. The mixture was agitated for 5 min. The resulting mixtures were transferred one by one to a beaker with 0.1 mol/dm^3 $CaCl_2$ and the material in the beaker was stirred by means of a magnetic stirrer. A syringe with a ϕ 1.2-mm needle, placed in an infusion pump was used for the dropwise transferring of the viscous sodium alginate solutions. The replacement of sodium ions with calcium ions resulted in immediate gelation of the alginate drops. The resulting biocatalyst beads were separated from the $CaCl_2$ solution by means of a sieve and were kept in a refrigerator at 4°C.

Determination of the biocatalyst activity

The activity of biocatalyst was determined during decomposition of H_2O_2 at an initial concentration of 0.015 mol/dm^3. The biocatalyst beads (0.2 g) were added to 10 ml H_2O_2 solution with vigorous stirring and, after 30 min, the concentration of H_2O_2 was determined by means of manganometry. Activity was expressed in μmol of decomposed H_2O_2 per 1 g biocatalyst per 1 minute.

The effect pf pH, temperature, and storage time on the activity of biocatalyst

Measurements of the activity of biocatalyst at various pH values in the range from 5 to 8 were conducted at 30°C. Independent measurements of activity were performed at a pH of 7 at 20°C, 30°C and 40°C. Measurement of the activity of biocatalyst during storage at 4°C was carried out after 1, 7 and 14 days.

Change of activity of biocatalyst in repeated use.

Measurements were made in a similar way as in determination of activity. H_2O_2 decomposition was carried out at temperature 20°C in pH 7 for 10 minutes. Ten measurements were made for each portion of the biocatalyst (2% and 4% of calcium alginate gel), while transferring the beads consecutively to a new portion of H_2O_2 at a concentration of 0.15 mol/dm^3.

Discussion of results

The measurements have demonstrated that a maximum activity of catalase from *Aspergillus niger*, immobilized in 2% and 4% calcium alginate gel, is obtained in pH 7. Neither kind of the immobilized biocatalyst showed a loss of activity of more than 6% with a pH change of 1 compared with the neutral solution. This is consistent with the results obtained by other authors [5,9].

Table 1 shows the results of studies of the effect of temperature on the activity of the immobilized catalase. They are shown in a standardized form as a quotient of the amount of decomposed H_2O_2 at a given temperature to the maximum quantity of decomposed H_2O_2.

Table 1

Effect of temperature on activity of immobilized catalase

Temperature, °C	Biocatalyst activity [-]	
	2% calcium alginate	4% calcium alginate
20°C	0.77	0.77
30°C	1.00	0.93
40°C	0.87	1.00

The data in Table 1 show that the catalase immobilized in the 4% gel achieves a maximum activity at temperatures higher than that in the 2% gel. An increase in the maximum temperature indicates a higher enzyme stability. This is confirmed by measurements of activity fluctuations during the storage of biocatalyst (Fig. 1).

Also in that case, the biocatalyst containing 4% calcium alginate was more stable. However, the biocatalyst ought not to be stored for more than 7 days because of its gradual loss of activity in time.

Figure 2 shows changes in the activity of immobilized catalase in ten consecutive operations of H_2O_2 decomposition. The maximum rate of H_2O_2 decomposition was 30.5 μmolH_2O_2/g·min for the biocatalyst containing 2% calcium alginate and 20.8 μmolH_2O_2/g·min for the one with 4% gel. It can be observed that, initially, the activity of catalase in the 2% calcium alginate gel is higher than that in the 4% gel. This indicates lower diffusion resistances for the substrate,

which are observed in the gel with the lower concentration of alginate. A more loose structure of the gel with 2% calcium alginate is also confirmed by the gradual loss of activity of the biocatalyst in the consecutive operations, connected with the catalase being released from the gel pores. The biocatalyst with 4% calcium alginate has shown a practically constant activity. Therefore, catalase from *Aspergillus niger*, with a molecular weight of 385 kDa [10], which is much higher than for a majority if enzymes used, may be effectively entrapped in gels containing a suitable alginate content.

It is rather hard to compare the stability of the obtained biocatalyst with other preparations, given the fact that H_2O_2 decomposition was usually effected by researchers in dissimilar conditions in respect of temperatures and H_2O_2 concentrations. Much worse results were obtained in a majority of works published because the immobilized biocatalyst tended to show a clear drop in its activity after being used for 100 min [11, 12].

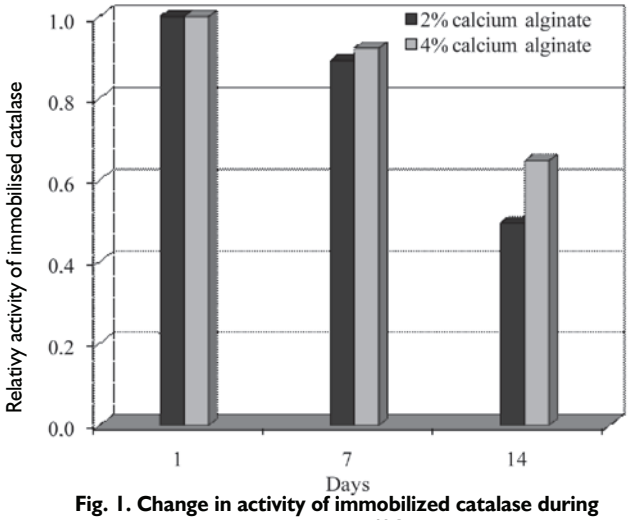

Fig. 1. Change in activity of immobilized catalase during storage at 4°C

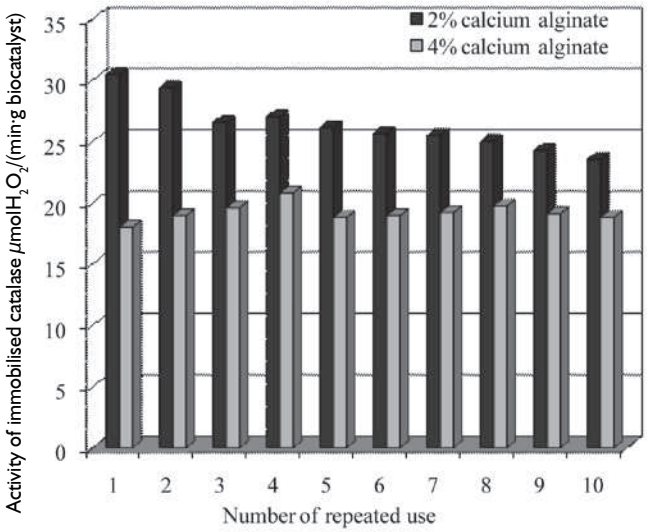

Fig. 2. Change in activity of immobilized catalase during repeated use

Conclusions

The present studies have confirmed the possibility of effective immobilization of catalase from *Aspergillus niger* by entrapment in a 4% calcium alginate gel. The biocatalyst obtained showed a maximum activity at 40°C and pH 7. The immobilized biocatalyst was found to be useful for repeated application in decomposition of H_2O_2 in a batch reactor.

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