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

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Please cited as: CHEMIK 2011, 65, 4, 305-308

**Introduction**

The consumption of \( \text{H}_2\text{O}_2 \) in a variety of industries has been growing fast [1, 2]. The highest consumption of \( \text{H}_2\text{O}_2 \) is for pulp delignification and bleaching in the pulp and paper industry. Moreover, \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \). 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 \( \text{H}_2\text{O}_2 \) according to the following reaction:

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]  

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 \( \text{H}_2\text{O}_2 \) 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 \( \phi \) 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 \( \text{H}_2\text{O}_2 \) at an initial concentration of 0.015 mol/dm\(^3\). The biocatalyst beads (0.2 g) were added to 10 ml \( \text{H}_2\text{O}_2 \) solution with vigorous stirring and, after 30 min, the concentration of \( \text{H}_2\text{O}_2 \) was determined by means of managanometry. Activity was expressed in μmol of decomposed \( \text{H}_2\text{O}_2 \) per g biocatalyst per 1 minute.

**The effect of 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. \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 catalase 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 \( \text{H}_2\text{O}_2 \) at a given temperature to the maximum quantity of decomposed \( \text{H}_2\text{O}_2 \).

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>2% calcium alginate activity [μmol H₂O₂/g·min]</th>
<th>4% calcium alginate activity [μmol H₂O₂/g·min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>30°C</td>
<td>1.00</td>
<td>0.93</td>
</tr>
<tr>
<td>40°C</td>
<td>0.87</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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 \( \text{H}_2\text{O}_2 \) decomposition. The maximum rate of \( \text{H}_2\text{O}_2 \) decomposition was 30.5 \( \text{μmol H}_2\text{O}_2/\text{g·min} \) for the biocatalyst containing 2% calcium alginate and 20.8 \( \text{μmol H}_2\text{O}_2/\text{g·min} \) 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$_2$O$_2$ decomposition was usually effected by researchers in dissimilar conditions in respect of temperatures and H$_2$O$_2$ 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 4ºC**

**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 40ºC and pH 7. The immobilized biocatalyst was found to be useful for repeated application in decomposition of H$_2$O$_2$ in a batch reactor.

**Literature**


8. Patent nr 158020, Polska


**Translation into English by the Author**

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