KINETICS AND STABILITY OF IMMOBILIZED GLUCOSE OXIDASE AND CATALASE

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Glucose oxidase (GOD) has been immobilized for both technical and analytical purposes. Since it is deactivated by its reaction product $\text{H}_2\text{O}_2$ (1), its activity may be stabilized by immobilized catalase (2). The catalase decomposes $\text{H}_2\text{O}_2$, but it is itself also deactivated by $\text{H}_2\text{O}_2$ (3). It therefore becomes necessary to analyze the production and decomposition rates of $\text{H}_2\text{O}_2$ and the deactivation rates for both enzymes in the presence of this compound.

GOD and CAT have been immobilized in a polyacrylamide matrix by a procedure presented elsewhere (2). The granular material had a diameter of approximately 0.4 mm.

EXPERIMENTAL

Experiments have been carried out in thermostated stirred vessels of 200 to 400 ml volume, provided with oxygen and pH electrodes. The latter was connected to an autotitrator providing the neutralization by NaOH solution of the gluconic acid produced. In some experiments, the reaction was followed in a sealed stirred vessel by the oxygen consumption rate with catalase being inhibited.

In continuous experiments, the stirred vessel was aerated, glucose solution was pumped into the vessel and product solution

Nomenclature: CAT, catalase; G, glucose; $G_a$, gluconic acid; $G_l$, glucono-lactone; GOD, glucose oxidase; GOD$_r$, reduced glucose oxidase (FADH$_2$-enzyme); $k_i$, kinetic constants; r, reaction rate (mole/sec. enzyme); $\eta$, effectiveness factor (ratio of overall reaction rate in the heterogeneous system to that in a homogeneous system under similar conditions).

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removed through a filter. The latter was analyzed for glucose by an autoanalyser and for H$_2$O$_2$ by a peroxidase catalyzed indicator reaction. The material balances gave reaction rates for glucose oxidation, H$_2$O$_2$ production and decomposition. 100 to 200 mg of immobilized GOD/CAT were used in each run. The stirrer speed was above the range where it influenced the reaction rate.

RESULTS

We used a simplified model for the glucose oxidase catalyzed reaction

\[
\text{GOD} + G \xrightarrow{k_1} G_1 \xrightarrow{k_2} G_a + \text{GOD} \quad \text{r} \quad + \quad \text{H}_2\text{O}_2 \xrightarrow{k_4} \text{CAT} \quad \text{O}_2 + \text{H}_2\text{O} 
\]

The reaction rate, r, for the glucose oxidation is expressed by the following approximation:

\[
\frac{1}{r} = \frac{1}{k_1 \cdot G} + \frac{1}{k_3 \cdot O_2} \quad \text{mol/sec . g}^{-1} \quad (1)
\]

The equation gives the exact rates for rate limiting glucose or oxygen concentration levels respectively. The overall reaction rate is first order in respect to the glucose concentration for \( G \ll 5 \times 10^{-4} \text{ M} \), \( O_2 \gg 2 \times 10^{-4} \text{ M} \), and first order with respect to the oxygen concentration for \( G \gg 3 \times 10^{-2} \text{ M} \) and \( O_2 \ll 2 \times 10^{-4} \text{ M} \). The H$_2$O$_2$ decomposition is first order with respect to H$_2$O$_2$. Apparent kinetic constants are calculated from Eq. 1 and the mass balances for the quasi-stationary state. The values for the kinetic constants are: \( k_1 = 5.5 \times 10^{-4} \), \( k_3 = 10^{-2} \), \( k_4 = 5.5 \times 10^{-3} \) (all 1/g.sec for pH 4.9, 25°, limit or error ± 20%). \( k_1 \) refers to total glucose concentration. In our experiments, mutarotation of α-glucose is much faster than the oxidation reaction.

The GOD deactivation has been observed by the slow rise of the quasi-stationary glucose concentration, which is reached after 5 to 10 h of operation.