Enzymatic reactor using high activity immobilized enzymes during exothermic reaction

Canh Tran Minh, V. Bougnol-Bilhou, Francis Lancelot

ENZYMATIC REACTOR USING HIGH ACTIVITY IMMOBILIZED ENZYME DURING EXOTHERMIC REACTION

C. Tran-Minh, V. Bougnol-Bilhou and F. Lancelot, Ecole des Mines de Saint-Etienne - France

The exothermic reaction used is the decomposition of hydrogen peroxide into oxygen and water, the enthalpy of which reaches -23.44 kcal.mole^{-1} at 25°C and under 1 atmosphere for the pure liquid.
An advantage of using insolubilized enzyme reactors lies in the saving of energy because enzymes allow work at ambient temperature (1-2-3-4). Nevertheless, some exothermic reactions may lead to a progressive denaturation of the enzyme. The study of the reactor using catalase for hydrogen peroxide decomposition is conducted here.

MATERIALS AND METHODS

A - Immobilization of the enzyme

The enzyme catalase employed comes from beef liver, with a molecular weight between 225,000 and 250,000 g, it is the oxydo-reductase of hydrogen peroxide. Catalase is dissolved in 17.5 % albumine (human or bovine) and placed in phosphate buffer (0.1M, pH 6.8) ; then a bifunctional agent, glutaraldehyde, is added to ensure the co-reticulation of albumin and the enzyme (5). A 2 ml solution of the enzymatic mixture contains 9,000 catalase units, 0.5 ml of 17.5 % albumine, 0.9 ml of phosphate buffer and 0.6 ml of 2.5 % glutaraldehyde. This mixture, placed in an evaporating dish, stays for several hours at a temperature below 0°C : the resulting plate has a solid foam consistency.

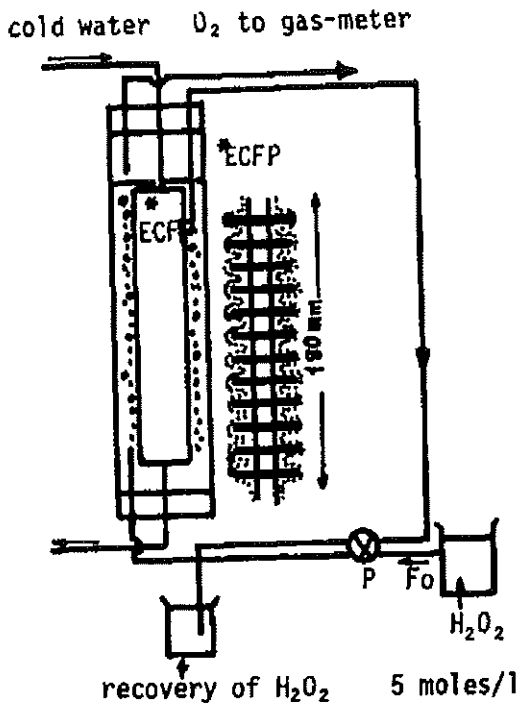
B - Detection of the heat of the reaction (bead thermistor)

The heat of the reaction is detected by measuring the temperature evolution in the bulk of the reactive layer. The calibration of the bead thermistor (Ro = 100 000 Ω ± 20 %) is made by a Wheatstone's bridge.

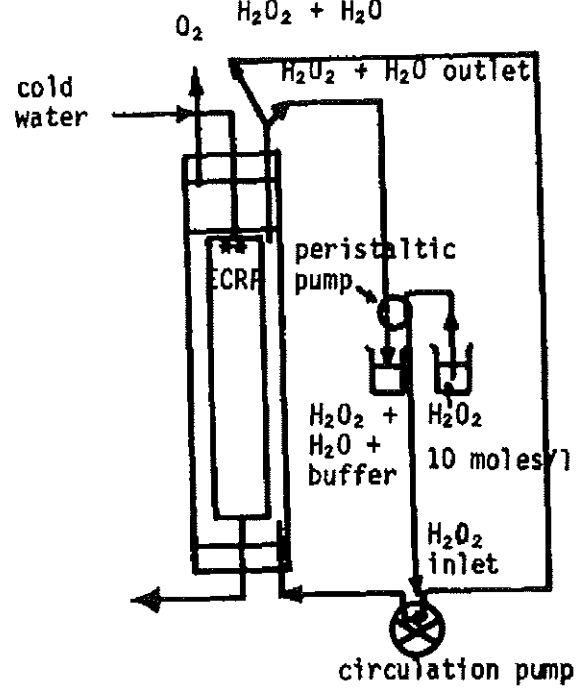
C - Enzymatic reactor (various devices)

1. Reactor I is constructed by covering rigid plates with enzyme foam. The plates are made of stainless steel and fixed on a tube, the inside of which is water-cooled (Figure 1).
Fifty ml of phosphate buffer (0.1M, pH 6.8) are first introduced into the empty reactor. Then the reactor is filled with hydrogen peroxide at the rate of 1 litre per hour. The oxygen production is measured during the course of the reaction. Samples are drawn at regular intervals, and diluted a thousand times for the analysis by U.V. spectrophotometry. The various concentrations of H_2O_2 according to the calibration curve, are determined by the values of the absorbance at 220 nm on the scale 0-2A.

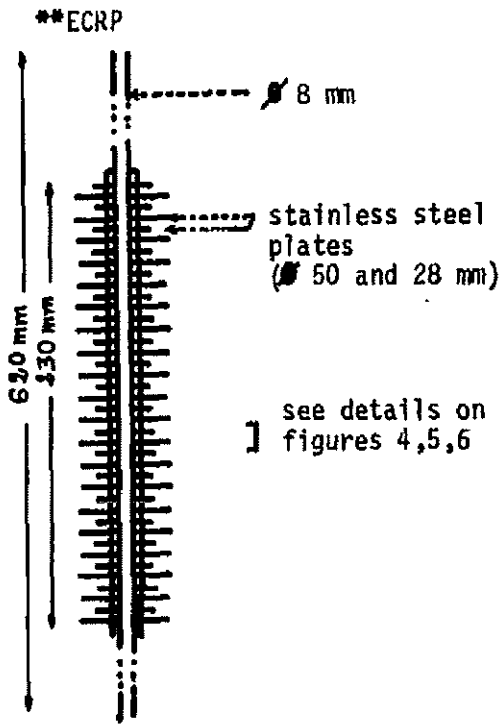
2. Reactor II is constructed with plates that can be removed (see figure 3), allowing a homogeneous distribution of the enzymatic mixture and an easier way for cleaning the plates (figure 4). The description of the different parts of the reactor is given on figure 2. The device is water-cooled by the central tube. A circulation pump of 60 litres per hour secures sufficiently quick mixing of the reactants. The initial volume of buffer in the reactor is 800 ml, the amount of catalase is 180 mg, the apparent area of reaction being 420 cm^2. Hydrogen peroxide (10 moles/l) is added at the rate of 600 ml per hour.



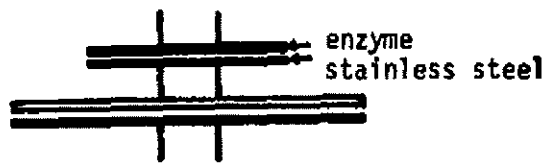
- Figure 1 -



- Figure 2 -



- Figure 3 -



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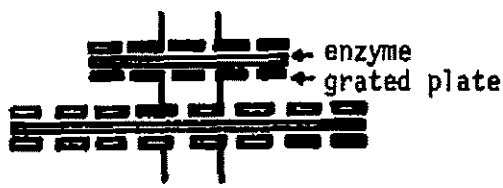
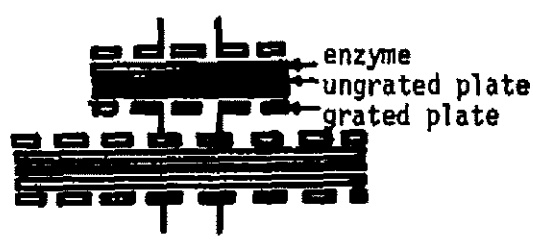


- Figure 5 -



- Figure 6 -

Figure 1 : Device using the Reactor I with the enzyme covered fixed plates (*ECFP).
Figure 2 : Device using the Reactors II, III and IV with the enzyme covered removable plates (**ECRP).
Figure 3 : Cross-section of the enzyme covered removable plates (**ECRP).
Figure 4, 5 and 6 : Detailed view of the plates for Reactors II, III and IV.

3. Reactor III uses removable plates but is improved by keeping the enzyme plates motionless (Reactor III, figure 5) between two round plates in grated stainless steel (the grate allows the transfer of heat while enabling the access of the substrate). Another way is to keep the enzyme foam between a grated plate and an ungrated one (Reactor IV, figure 6). The adjunction of the grated plate reduces by half the surface of contact of the enzyme with hydrogen peroxide : every set represented by figure 4, 5 or 6, therefore offers the same surface of contact S with the reaction mixture. The experimental conditions are the following ones :

	Reactor III	Reactor IV
amount of catalase	130 mg	260 mg
H_2O_2 10 moles/l	650 ml/h	650 ml/h
phosphate buffer pH 6.8	900 ml	900 ml
S exchange	300 cm^2	300 cm^2

RESULTS AND DISCUSSION

A - Advantages of using immobilized catalase :

The immobilized enzyme presents a higher stability and a better resistance to the attack of hydrogen peroxide. Moreover, the immobilized enzyme offers the advantage of being able to be used and reused, compared with the enzyme in solution.

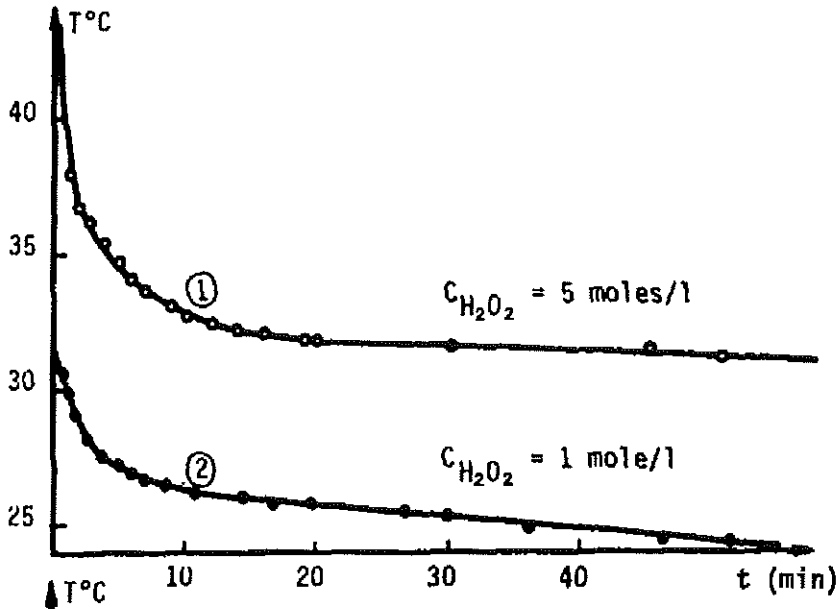
B - Effect of external temperature on the production of oxygen :

The bead thermistor, covered with a thin film of enzyme, is dipped into hydrogen peroxide solutions of different concentrations in order to measure the temperature in the bulk of the active layer.
As soon as catalase is put in contact with H_2O_2, the decomposition of H_2O_2 starts and the heat produced by the reaction raises the temperature inside the membrane. The result is a heat flow towards the outside solution.
A rapid evacuation of the heat given out enables the maintainance of the membrane at a lower temperature and the decrease of denaturation of enzyme.
At ambient temperature, the probe temperature expressing the oxygen production decreases very quickly because of destruction of the active sites of the enzyme by the heat produced (figure 7).
In order not to reach the critical temperature, an improvement consists in keeping the reaction at 0°C by placing the container in melting ice. The figure 8 gives a comparison between the results achieved for an experiment carried out at 0°C and at 21°C, the experimental conditions being the following ones :

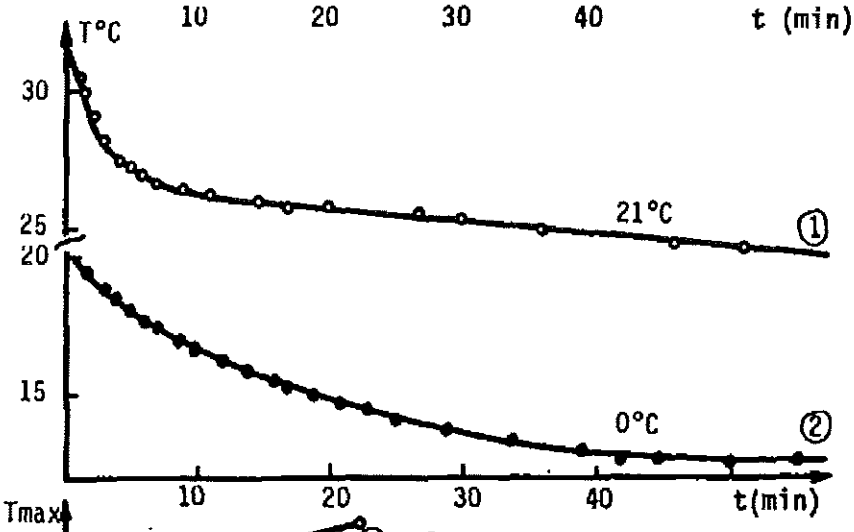
$$\begin{cases} C_{H_2O_2} = 1 \text{ mole/l} \ ; \text{ volume } H_2O_2 = 100 \text{ ml} \\ \text{thickness of the enzymatic layer} = 2e = 1{,}5 \text{ mm} \end{cases}$$

At the very beginning of the reaction, cooling produces a sensitive effect since the temperature given by the thermistance is only about 20°C instead of 32°C as before.
Moreover, the enzyme presents in these conditions an activity more regular and more stable.
That variation of about 12 degrees in temperature, due to cooling, is found again for different concentrations of hydrogen peroxide, as shown on figure 9.

The conclusion is that heat denaturation is the main cause of activity loss. Cooling is necessary to preserve the activity of the enzyme.



- Figure 7 -



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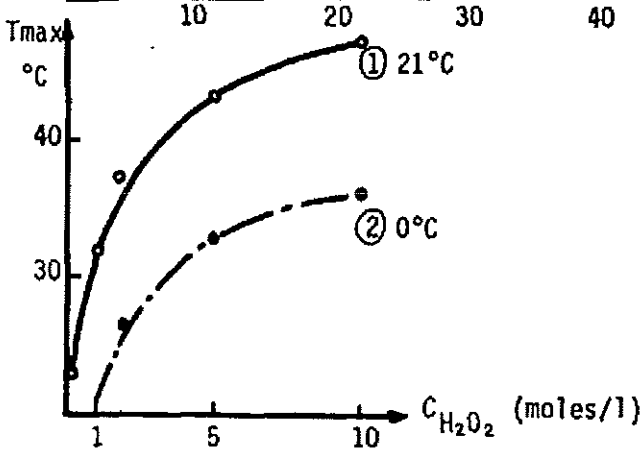


- Figure 9 -

Figure 7 : Evolution of the enzymatic reaction with $C_{H_2O_2}$ at 21°C.
Figure 8 : Evolution of the temperature during the enzymatic reaction.
Figure 9 : Effect of cooling on the maximum temperatures in the bulk of the enzyme layer - Tmax = f ($C_{H_2O_2}$).

C - Production of oxygen with the various types of reactors :

In the reactor I, the maximum production rate of oxygen reaches 33 litres per hour. The decrease in the hydrogen peroxide concentration, as well as in the oxygen production rate, is not very steady : figures 10 and 11.
In the reactor II with removable plates, the oxygen production reaches 43 litres within one hour, the momentary maximum production rate is 66 litres per hour : figures 12 and 13. In this reactor, the detached enzyme plates : figure 3 offer a larger surface of contact with the reactants, but the heat is not effectively eliminated. Reactors III and IV have been improved by the use of removable grated plates. These plates hold perfectly the enzyme foam layers while allowing the gas bubbles to rise freely.
The figure 14 shows the performance of the reactors during the first hour of the experiment, the figure 15 shows their maximum performance during the whole period of the experiment.

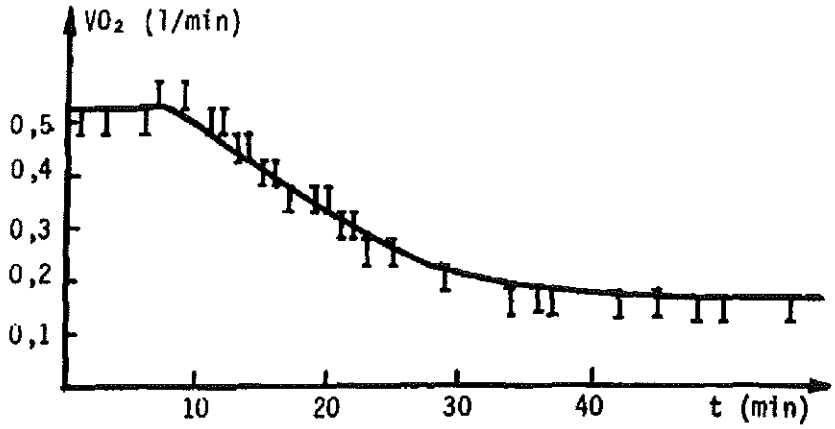
	Reference	O_2 production within one hour	VO_2 (3 h)
Reactor II	Figure 3	43 l	74 l
Reactor III	Figure 5	26 l	——
Reactor IV	Figure 6	39 l	72 l

The final presentation of the reactor we adopted (that of the figure 6) allows the supply of 39 litres of oxygen within one hour (Reactor IV), which is the equivalent of one person's consumption of oxygen per hour.
The momentary maximum production rate of oxygen is 54 litres per hour.
The enzyme denaturation, slow enough during the first hour of utilization, becomes perceptibly faster from the second hour onwards : Figures 16 and 17.
This arises from the fact that the quantity of enzyme immobilized, while diminishing, induces a lesser decomposition in H_2O_2, harmful to the enzyme.

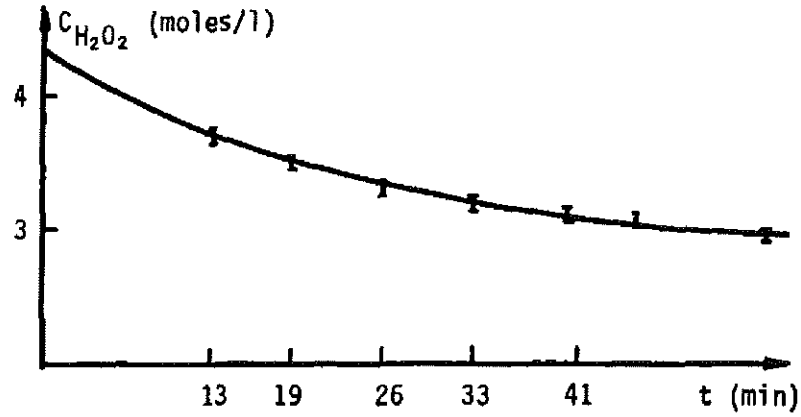
The model of Reactor IV presents several advantages compared with the previous ones : - the enzyme foam layers are kept entirely motionless by the grated stainless steel plates and the elimination of the heat is effectively carried out - the oxygen production is far more regular and the denaturation of the enzyme slower since oxygen is still emitted during several hours.
A parallel study has been made on the behaviour of the immobilized enzyme under moderate pressure. The immobilized enzyme is prepared with 4 mg of catalase, 1 ml of human albumin, 1.8 ml of phosphate buffer pH 6.8 and 1.2 ml of 2.5 % glutaraldehyde. The catalase used has an activity of 4500 units per mg ; it is presented under a foam shape of dimensions 4 cm x 2 cm x 0.5 cm. We used the apparatus given on figure 18.
The experimental conditions are the following ones :

{ amount of catalase : 4 mg ; volume H_2O_2 10 moles/l : 110 ml/h
{ volume buffer pH 6.8 : 400 ml ; p > p atm

The concentrated hydrogen peroxide is introduced regularly into the flask by means of a peristaltic pump. The magnetic stirring is strong enough to maintain the enzymatic foam constantly in motion in the reaction mixture.
The reaction flask is placed in a basin of melting ice. The working pressure is successively 1.2 ; 1.3 ; 1.4 and 1.5 atmosphere. With the temperature, the H_2O_2 flow rate, the amount of catalase and the pressure fixed, we follow the oxygen production in the course of time $VO_2 = f(t)$, the curves of which are represented on figure 19.



- Figure 10 -



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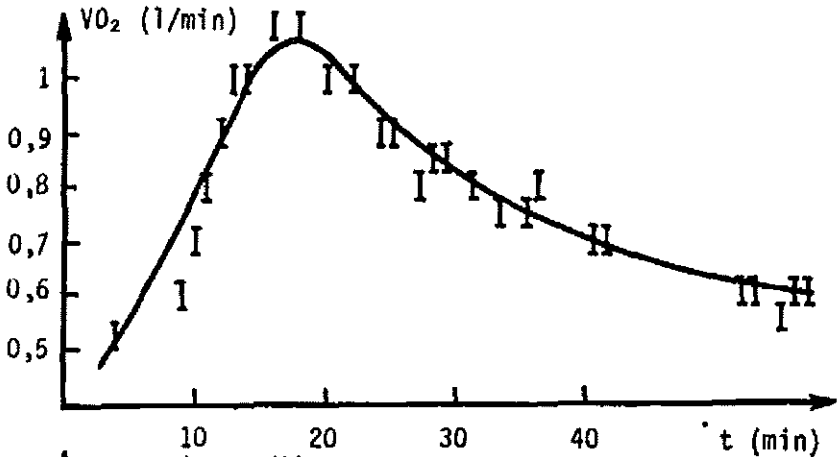
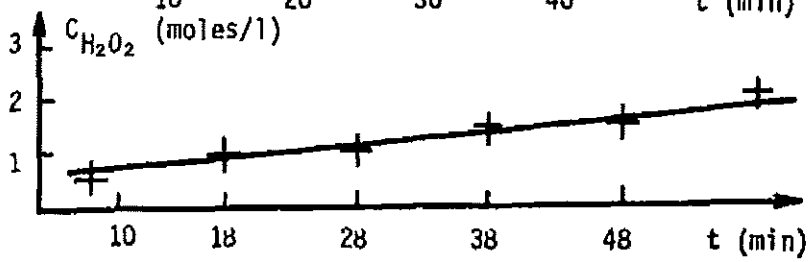


- Figure 12 -



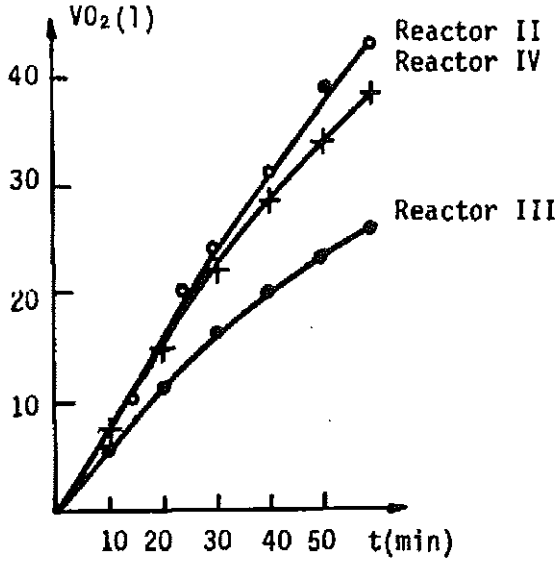
- Figure 13 -

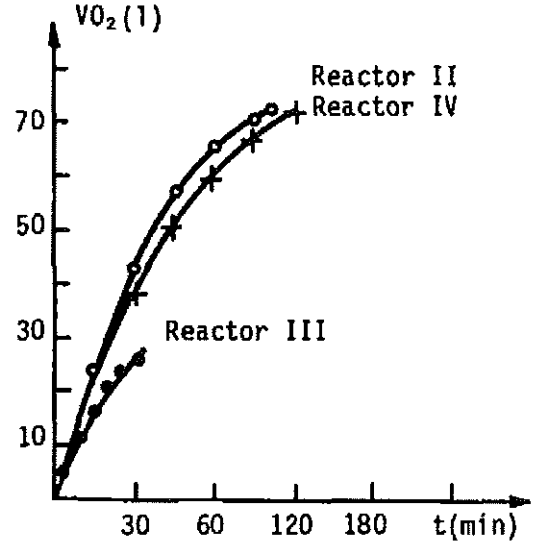
Figure 10 : Oxygen production rate during enzymatic reaction. Reactor I.

Figure 11 : H_2O_2 concentration during enzymatic reaction. Reactor I.

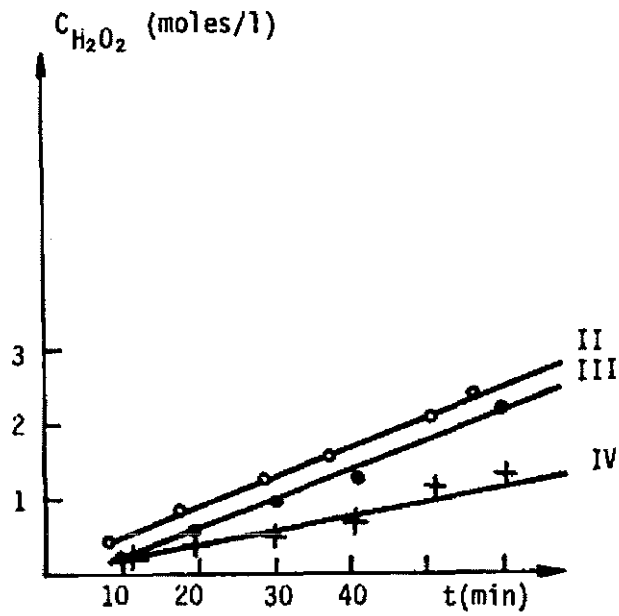
Figure 12 : Oxygen production rate during enzymatic reaction. Reactor II.

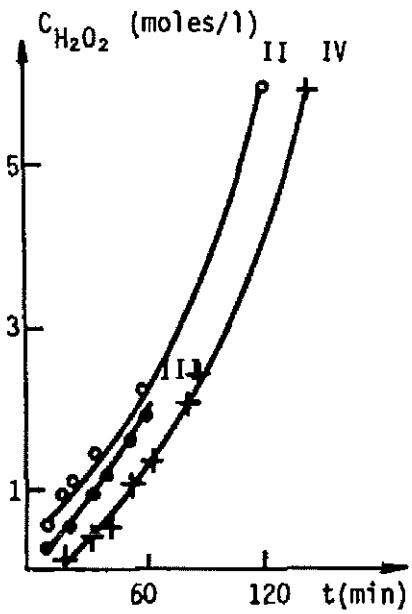
Figure 13 : H_2O_2 concentration during enzymatic reaction. Reactor II.





- Figures 14 and 15 -

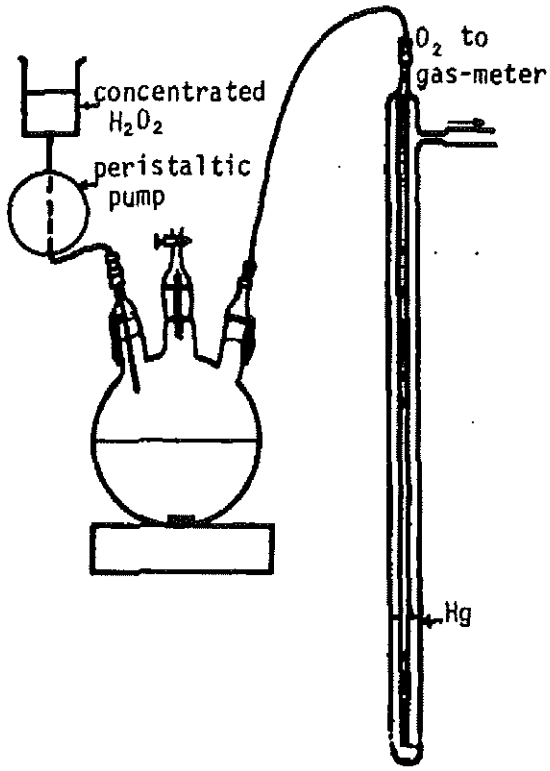




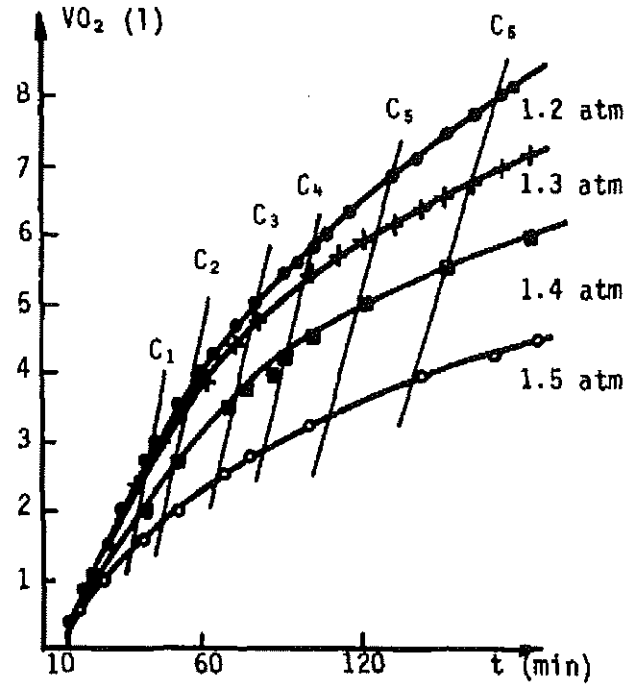
- Figures 16 and 17 -

Figures 14 and 15 : Oxygen production during enzymatic reaction.
Reactors II, III and IV.

Figures 16 and 17 : H_2O_2 concentration during enzymatic reaction.
Reactors II, III and IV.



- Figure 18 -



- Figure 19 -

Immobilized catalase under moderate pressure :

Figure 18 : Diagram of the apparatus.

Figure 19 : $VO_2 = f(t)$; C_1 = 0.919 mole/l ; C_2 = 1.252 mole/l ; C_3 = 1.691 mole/l ; C_4 = 2.053 moles/l ; C_5 = 2.536 moles/l ; C_6 = 3.042 moles/l.

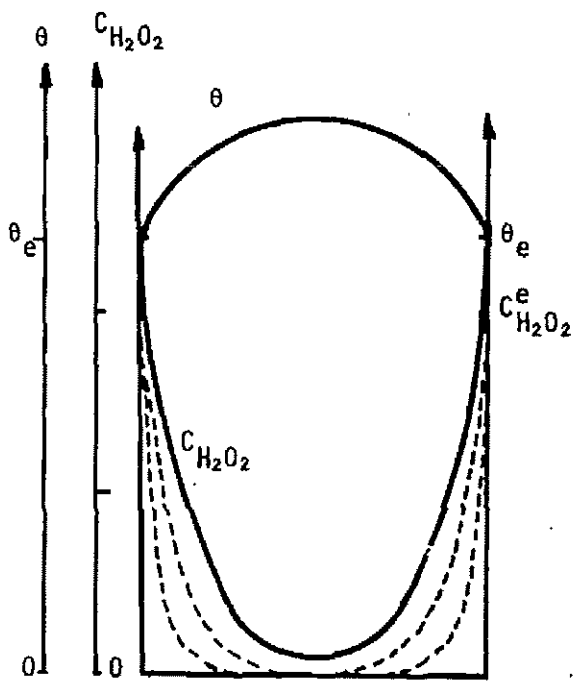


Figure 20 : Calculated profiles of temperature and concentration of H_2O_2 inside the enzyme layer for steady-state (——) and non steady-state (---).

Unit of temperature = $\frac{\Delta H \cdot D_{H_2O_2}}{\lambda} \cdot Km$

Unit of time = $\frac{e^2}{D_{H_2O_2}}$

e = thickness of the enzyme layer

Unit of concentration = Km

Some typical values are given in the following table :

P in atm	1.2	1.3	1.4	1.5
O_2 production within one hour	4.6	4.3	3.6	2.6
VO_2 (2 hr)	6.8	6.1	5.2	3.8
VO_2 (3 hr)	8.5	7.4	6.1	4.5

A small increase of pressure lowers perceptibly the emission of oxygen during the reaction. An estimation of the rate : v is graphically obtained by the slope of the tangent to the equiconcentration curve, to the factor $\frac{1}{12\ Vs}$, Vs being the solution volume.

$$v = \frac{1}{12\ Vs} \cdot \frac{dVO_2}{dt}$$

The higher the pressure, the lower the rate of the reaction : this is probably due to the reversibility of hydrogen peroxide decomposition.

D - Theoretical analysis :

The decomposition of H_2O_2 by catalase in solution can be written as follows : $H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2 + \Delta H$ where ΔH : heat of reaction. The rate of decomposition is given by the Michaelis-Menten equation :

$$v = -\frac{dC_{H_2O_2}}{dt} = \frac{1}{2} \cdot \frac{d\,C_{O_2}}{dt} = Vm \cdot \frac{C_{H_2O_2}}{Km + C_{H_2O_2}}$$

where { Km is the dissociation constant of the complex H_2O_2 - catalase
Vm is the maximum rate of the reaction.

When catalase is immobilized in a layer between two grated plates, the rate of reaction is modified by a term of diffusion given by the second Fick law :

$$\frac{\partial C_{H_2O_2}}{\partial t} = D_{H_2O_2} \cdot \frac{\partial^2 C_{H_2O_2}}{\partial x^2} - Vm \cdot \frac{C_{H_2O_2}}{Km + C_{H_2O_2}} \qquad (1)$$

where x = distance to the external plates, $D_{H_2O_2}$ = mean apparent diffusion coefficient of H_2O_2 in the active layer. Figure 20 shows the evolution of H_2O_2 concentration profiles inside the enzyme foam layer.
Similarly, the temperature θ inside the layer is given by :

$$\frac{\partial \theta}{\partial t} - \frac{\lambda}{\rho c} \cdot \frac{\partial^2 \theta}{\partial x^2} - \frac{\Delta H}{\rho c} \cdot Vm \frac{C_{H_2O_2}}{Km + C_{H_2O_2}} = 0 \qquad (2)$$

where λ = specific heat conductivity, ρ = specific gravity, c = thermal capacity.

At the steady state, we have : $\frac{\partial \theta}{\partial t} = 0$, $\frac{\partial C_{H_2O_2}}{\partial t} = 0$

By adding the equations (1) and (2), we obtain :

$$\Delta H . D_{H_2O_2} \frac{d^2 C_{H_2O_2}}{dx^2} + \lambda \frac{d^2\theta}{dx^2} = 0$$

$$- \frac{\lambda d\theta}{dx} = \Delta H . D_{H_2O_2} . \frac{dC_{H_2O_2}}{dx} + K$$

K is the integration constant. The symmetry of the system implies that at the center part of the membrane the flux of heat $(- \frac{\lambda d\theta}{dx})$ as well as the flux of oxygen $(- D. \frac{dC_{H_2O_2}}{dx})$ is equal to zero. So is the integration constant : K = 0.
This shows that the rate of transfer of heat is proportional to the rate of diffusion of H_2O_2 across unit area.

The symmetry of the system shows that the temperature θ inside the membrane depends on the concentration of the substrate H_2O_2 by the following relationship :

$$\theta - \theta_e = - \frac{\Delta H . D_{H_2O_2}}{\lambda} \left\{ C_{H_2O_2} - C^e_{H_2O_2} \right\}$$

where θ_e = temperature at the interface active layer-solution of H_2O_2 and $C^e_{H_2O_2}$ = H_2O_2 concentration at the same interface. This shows that the higher the H_2O_2 concentration used, the higher the temperature inside the membrane.

CONCLUSION

The study of the reactor described above enabled us to see that the use of enzymes for exothermic reactions needs efficient cooling to maintain the catalytic activity. The oxygen production obtained for one person may be used in first aid and possibly in skin-diving for short spells underwater. The advantage of using hydrogen peroxide as a source of oxygen lies in the fact that it is divisible and easily transportable compared to the frequently heavy and cumbersome aqualungs.

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