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Soluble and Immobilized Catalase

Effect of Pressure and Inhibition on Kinetics and Deactivation

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ABSTRACT

This article examines the effect of pressure on the steady-state kinetics and long-term deactivation of the enzyme catalase supported on porous alumina. The reaction studied is the decomposition of hydrogen peroxide. The results of studies carried out in a continuous stirred-tank reactor under isothermal conditions are presented and compared with results obtained for soluble catalase. For soluble catalase, it is found that in the range of pressures studied, the oxygen flow rate increases with increase in pressure up to a certain value and then decreases. Hydrogen peroxide concentration appears to have a strong influence on pressure effects. With immobilized catalase, the pressure effects are not as prominent. Fluorescent microscopy studies of the immobilized enzyme suggest that this is probably because of pore diffusional limitations.

Index Entries: Immobilized catalase; inhibition; pressure effects.

Nomenclature: a, activity of the enzyme; A, dimensionless substrate concentration; E, free enzyme concentration; E_0, total enzyme concentration; ES, primary catalase peroxide complex; ES^*, secondary catalase peroxide complex; ESS, diperoxy catalase complex; H, nondimensional space velocity; I, inactive form of the enzyme; k_1, rate constant in the association of free enzyme and substrate; k_{-1}, rate constant in the dissociation of the primary complex; k_2, rate constant

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in the association of ES and substrate; k_{-2}, rate constant in the dissociation of the diperoxy complex; k_3, reaction rate constant, s^{-1}; k_4, rate constant in the association of ES^* and S; k_d, deactivation constant, s^{-1}; K_m, Michaelis constant, M; K_i, inhibition constant, M; q, volumetric flow rate, mL/ min; S, substrate concentration, M; S_o, initial substrate concentration, M; t, time, s; v, reaction rate, mol/L s; V, volume of the reactor, mL; v_{max}, maximum reaction rate, mol/L s; ΔV_b^*, activation volume accounting for the effect of pressure on K_m; ΔV_c^*, activation volume accounting for the effect of pressure on v_{max}; ΔV_i^*, activation volume of inhibition; ΔV^*, total activation volume; Greek letters: β, (K_m / S_o); γ, (K_i / S_o).

INTRODUCTION

Pressure affects a wide variety of processes in biological systems, and it is therefore important to investigate the effect of pressure on enzymatic reactions. Pressure studies give information on molecular substitution effects, and this has practical consequences in genetic engineering. Studies related to aerobic respiration in biological systems have focused interest on the study of reactive oxygen metabolites. One aspect that demands attention is the effect of pressure on enzyme deactivation, and very little research has been done in this area. It is important to study the effect of pressure on the decomposition of reactive oxygen metabolites, such as hydrogen peroxide. Little information about the effect of pressure on heterogenous enzyme catalysis in solution is available in the literature.

An important aspect associated with the use of enzymes in vitro is their susceptibility to poisoning or deactivation. Pressure may have a positive or a negative effect on the reaction rate, and may contribute to deactivation of the enzyme as well. Numerous papers reporting the pressure effects on chemical reactions with soluble enzymes have been published (*1–3*), to cite a few, but are focused on the effect of pressure on initial rates only. There has been progress in the field of high-pressure biology. "Conformational drift hypothesis" of Weber et al. (*4–7*) and Bautista et al. (*8*), and conformational interconversions by Quieroz et al. (*9*) are a few examples. The effect of pressure on long-term deactivation of soluble catalase was first investigated by Haddock (*10*). The study was experimental in nature.

In conjunction with studying the physiological effects of pressure on deep sea divers, experimentation to determine the effect of pressure on catalytic activity of catalase was carried out by Morild and Olmheim (*11*). Data were gathered for bovine liver catalase at reaction pressures up to 100 MPa. The duration of each experimental run was 3–5 min, with the enzyme still in the α-phase of activity (an initial burst of activity characteristic of bovine liver catalase), and conducted in a batch mode.

The authors designed the experiments so as to minimize the effect of both substrate inhibition of the enzyme and long-term enzyme deactivation. This was achieved by keeping both the enzyme concentration and substrate concentration fairly low. Morild and Olmheim observed an increase in catalytic activity with pressure with the activity peaking around 50 MPa, when the substrate concentration was about 0.02*M*. On doubling the substrate concentration, they observed little change in the activity in the range 0–30 MPa. Above 30 MPa, activity was found to decrease, which tended to flatten out above 100 MPa. Measurements of reaction rate were done by both polarography and spectrophotometry. Morild and Olmheim observed that the spectrophotometric measurements tended to flatten out above a certain pressure, whereas the polarographic measurements exhibited a continuous steep slope. Based on their studies, Morild and Olmheim concluded that the activation volumes were concentration-dependent.

The experimental model used by the above authors to interpret their results is straightforward, and does not take into consideration the steady-state (β-phase) activity of catalase, or the effect of substrate inhibition or long-term parallel poisoning by hydrogen peroxide (*12*). This article examines the effect of substrate inhibition (achieved by using a high feed concentration) and long-term deactivation of the enzyme at different pressures ranging from 0.15–0.41 MPa during the steady-phase of enzyme activity.

MATERIALS AND METHODS

Materials

In all the experimental runs, bovine liver catalase (product number C-10 from Sigma Chemical Company, St. Louis, MO) was used. It had an activity of about 2500 IU/mg protein, activity being defined as the amount of peroxide decomposed in μmol/min at pH 7.0, temperature 25°C, and an initial peroxide concentration of 0.01*M*. The buffer used was a sodium phosphate-citric acid buffer of pH 7.0. Reagent-grade hydrogen peroxide stabilized with sodium stannate (30% wt) from Fisher Scientific was used in all the experiments. This was diluted to the required concentration with buffer. Alumina support was obtained from Davison.

The alumina pellets were silanized by immersing them in a 2.5% (v/v) γ-aminopropyltriethoxy silane in acetone solution at 45°C for 24 h. The silanized pellets were thoroughly washed with distilled deionized water. The pellets were then immersed in a 2% (v/v) aqueous glutaraldehyde solution for 2 h at room temperature, washed with distilled deionized water, and then immersed in an enzyme solution of known concentration.

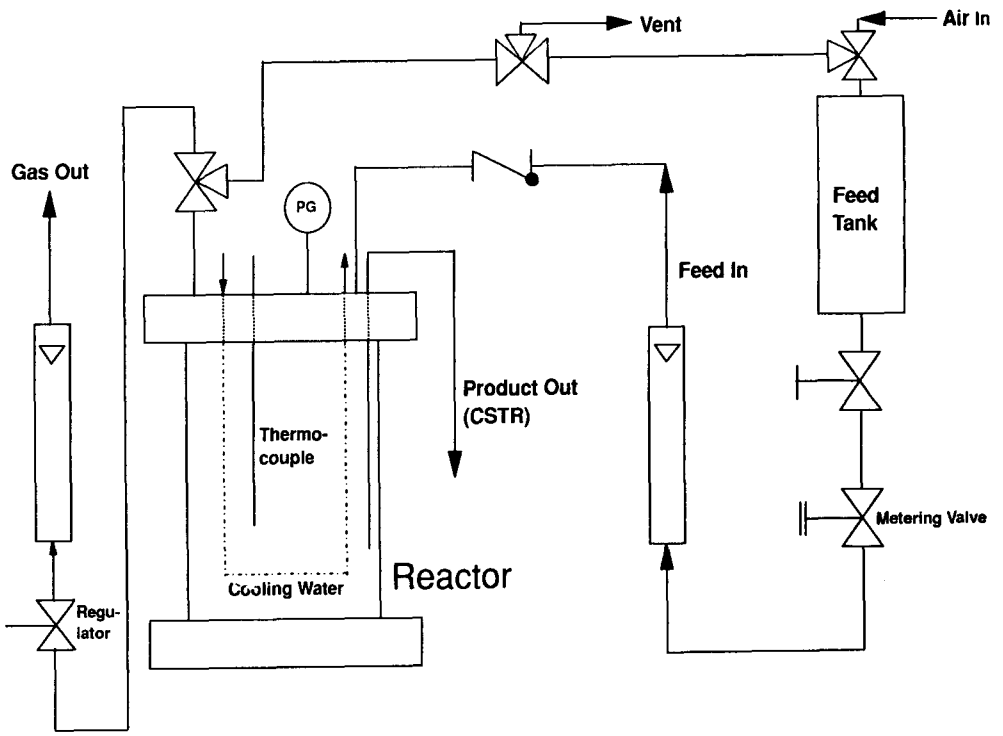

Fig. 1. Schematic of experimental setup.

Ten grams of the treated support were immersed in an enzyme solution (containing 150,000 IU/mL) for 5 h at room temperature. The immobilized enzyme pellets were thoroughly washed with distilled deionized water and kept refrigerated in pH 7 buffer until further use.

Experimental Setup

A 1500-mL reactor was constructed out of a PVC pipe with a PVC bottom flange and a thick clear acrylic top flange (schematic is shown in Fig. 1). The reactor was constructed to make it pressure-proof. There were seven ports on the top of the flange. A pressure gage (0–0.51 MPa) was threaded into the tapped port. One of the ports was used to feed hydrogen peroxide solution to the reactor, and one for the outflow of product oxygen. A thermowell was inserted into another port, and a thermocouple was inserted for temperature indication of the reaction mixture. The remaining two ports were used for the inlet and outlet of refrigerated water from a temperature-controlled bath, in order to maintain the reaction mixture at a desired temperature. A cooling coil made of stainless steel was connected to these ports. A 200-mesh screen was fixed to a steel pipe at one end with the help of Teflon™ tube piece that held the mesh tightly to the steel pipe. This pipe was attached to the last port. A tygon tube was connected to the other end of this pipe, and a pinch clamp was used as a valve. This arrange-

ment gave a dip tube to withdraw product continuously from the reactor. A magnetic stir bar and a magnetic stirrer/hot plate provided the agitation.

Hydrogen peroxide was fed from a pressurized feed tank and regulated by a micrometering valve. This flow rate was monitored by a rotameter that was placed at the exit to the metering valve. The product gas exited the reactor via the exit port and then passed through a relief valve. The relief valve could be adjusted from 0.135 to 0.44 MPa, and controlled the pressure in the reactor. The relief valve dropped the pressure to near atmospheric, and then the product gas was directed to a rotameter.

Experimental Method

A feed solution of strength 4.9*M* was made using 30% hydrogen peroxide and pH 7.0 sodium phosphate-citric acid buffer. A high feed concentration was chosen in order to be able to measure oxygen flow rates as accurately as possible. Five hundred milliliters of the buffer solution containing an antifoaming agent were transferred to the reactor through the thermowell port. Ten grams of the immobilized enzyme were used in each run. Agitation was started, and the cooling system was initiated simultaneously. The temperature was maintained at 24°C in all the runs. The shut-off valve was opened, and the reactor was pressurized by air flowing through the empty feed tank. The desired reaction pressure was set at this time by adjusting the relief valve. The shut-off valve was then closed, and sufficient time was allowed for the relief valve to reseat, as indicated by a zero reading in the product flow rotameter.

Pressure was bled from the feed tank via a three-way valve connected after the pressure regulator. The top of the tank was unscrewed, and 100 mL of the feed solution were added to the tank and resealed. The feed tank was pressurized to about 0.2 MPa over the reactor pressure with air from the regulator for control. The metering valve was closed 90%, and the shut-off valve was opened. The line to the reactor was filled slowly allowing for adjustment of the flow rate using the metering valve. The feed flow rate was maintained constant at 0.65 mL/min in all the experiments. The feed flow rate, reactor pressure, reactor temperature, and gas evolution rate were monitored periodically. The gas evolution rate was measured by the product gas rotameter.

RESULTS AND DISCUSSION

Effect of Pressure on Enzyme Kinetics

All the runs using immobilized enzyme were started with an initial buffer volume of 500 mL in the reactor. The lowest pressure studied was 0.149 MPa. It was found that at pressures below this, the oxygen flow rate was not stable. The relief valve had a range of 0.135–0.44 MPa, and 0.149 MPa was the lowest pressure attainable.

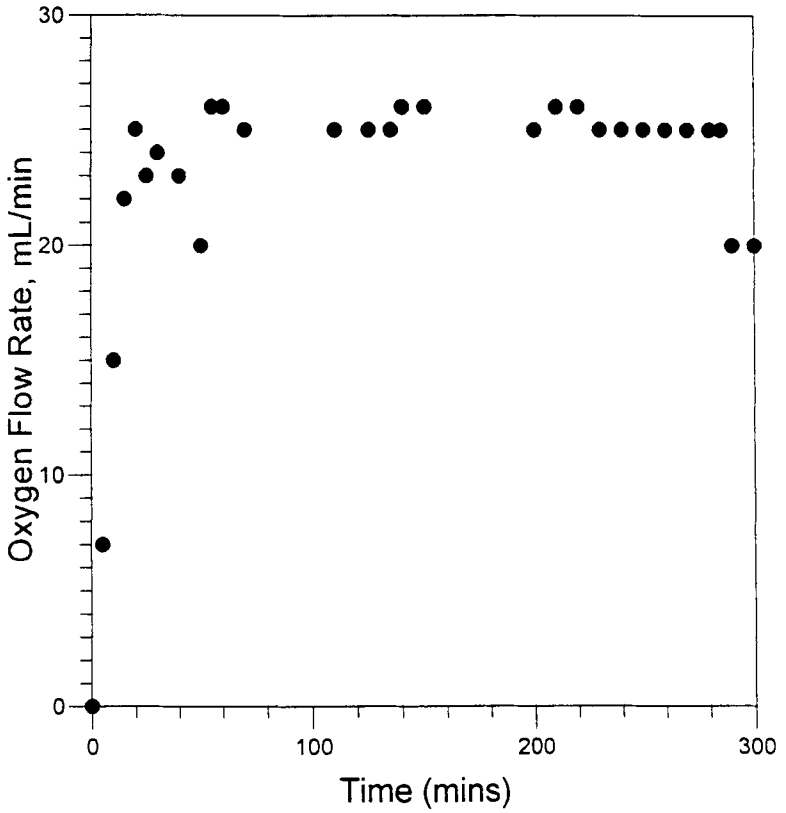

Fig. 2. Oxygen production vs time. Pressure = 0.149 MPa, temperature = 24°C, pH = 7.0, S_0 = 4.9*M*, 10 g of immobilized enzyme.

Previous studies on the effect of pressure on soluble enzyme activity in a fed-batch reactor (*10*) indicated that the oxygen flow rate went through a maximum between the pressure range of 0.21 and 0.31 MPa. However, the temperature was not maintained constant, since there was no arrangement for cooling water circulation. Studies conducted by Thakur with soluble enzyme in an isothermal fed-batch reactor in which the temperature was maintained at 24°C in all the runs showed that the peak flow rate occurred around 0.31 MPa. The duration of these experiments was between 90 and 140 min at the end of which the feed to the reactor was stopped since it was operated in a fed-batch manner.

Representative results for immobilized catalase are presented in Figs. 2 and 3. Figure 2 is a plot of oxygen production rate in mL/min vs time in minutes at a pressure of 0.149 MPa, temperature 24°C, pH 7.0, and a feed concentration of 4.9*M*. Figure 3 is a plot of oxygen production rate vs time at 0.33 MPa; all other conditions remain the same. The average production rate of oxygen at steady state for each pressure is presented in Table 1. The average values are reported as a range and represent 95% confidence limits.

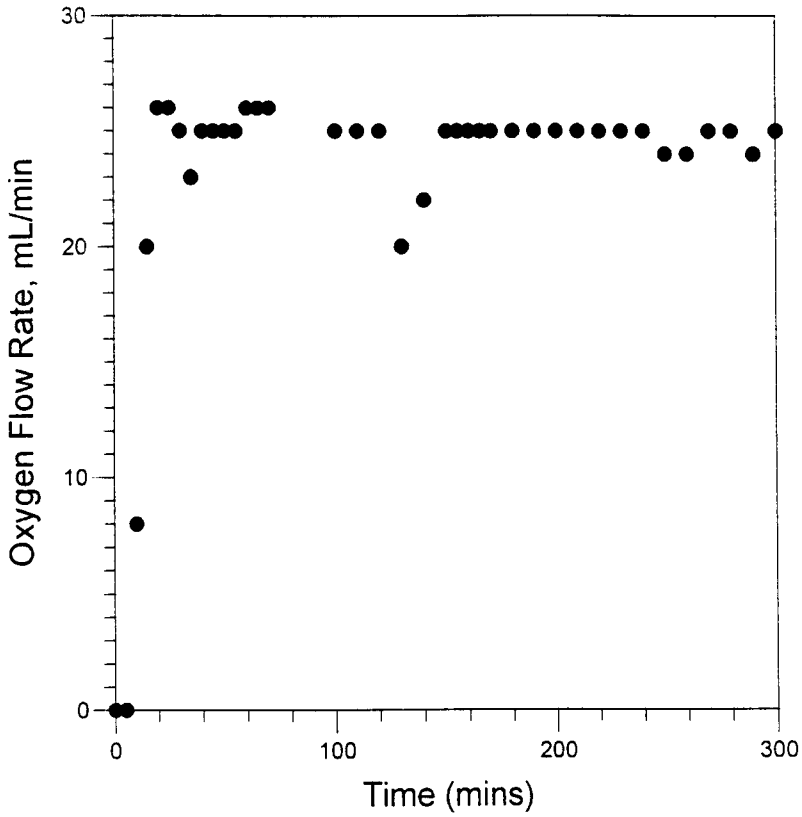

Fig. 3. Oxygen production vs time. Pressure = 0.33 MPa, temperature = 24°C, pH = 7.0, S_0 = 4.9*M*, 10 g of immobilized enzyme.

Table 1
Steady-State Average Values
of the Rate of Oxygen Production
at Different Pressures for Immobilized Enzyme

Pressure, MPa	Average oxygen flow, mL/min
0.149	24.2 ± 0.5
0.294	24.7 ± 0.6
0.301	28.2 ± 0.6
0.311	30.5 ± 0.7
0.325	25.5 ± 0.6
0.332	24.5 ± 0.4

Representative results for soluble catalase are presented in Figs. 4 and 5. Figure 4 is a plot of oxygen production rate in mL/min vs time in minutes at a pressure of 0.28 MPa, temperature 24°C, pH 7.0, and a feed concentration of 4.9*M*. Figure 5 is a plot of oxygen production rate vs time at 0.38 MPa; all other conditions remain the same. The average oxygen

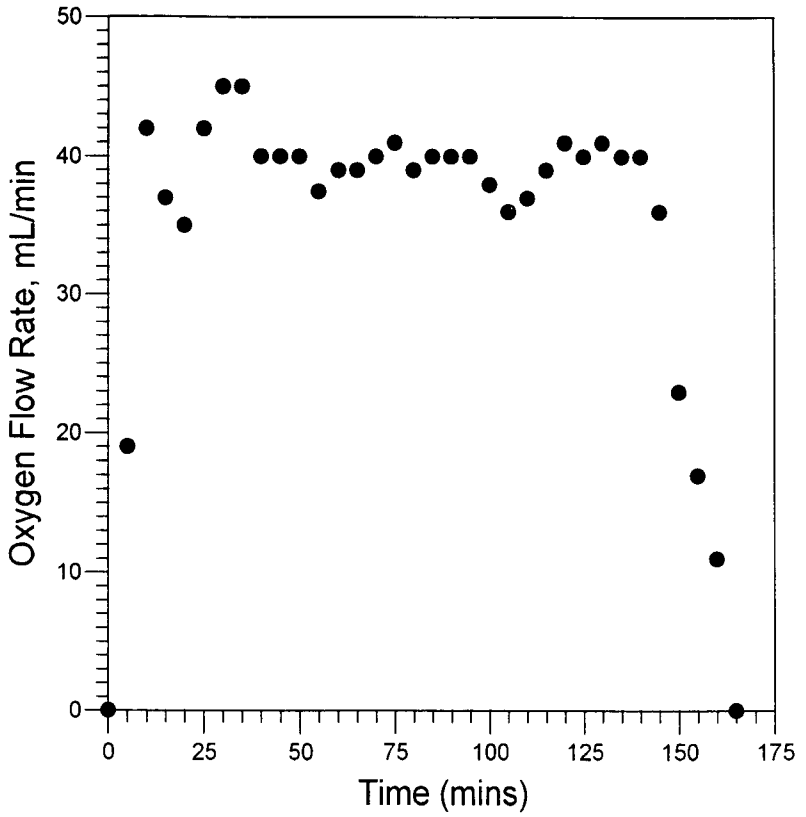

Fig. 4. Oxygen production vs time. Pressure = 0.28 MPa, temperature = 24°C, pH = 7.0, S_0 = 4.9*M*. Soluble enzyme.

production rates for soluble catalase are presented in Table 2 for the sake of comparison. Runs with soluble catalase were conducted in a fed-batch reactor; 21.3 mg of catalase (also Sigma C-10) were used in each run. Since the studies were conducted in a fed-batch reactor, the duration of the experiments with soluble catalase was much less. The sudden decline in the oxygen production rate observed around 120 min or so is as a result of stoppage of feed to the reactor.

Immobilized Catalase

The reaction using immobilized bovine liver catalase was carried out in a continuous stirred-tank reactor. It was ensured that external diffusional limitations were absent. Internal pore diffusion was a problem, since the alumina pellets were fairly large (average diameter of 3.2 mm). Photomicroscopy studies during different stages of immobilization of the enzyme confirmed that most of the enzyme was confined to an outer shell of the alumina support. It is important to examine this, since it sheds light on the differences observed in the oxygen flow behavior between soluble and immobilized catalase, as will be explained in a subsequent paragraph.

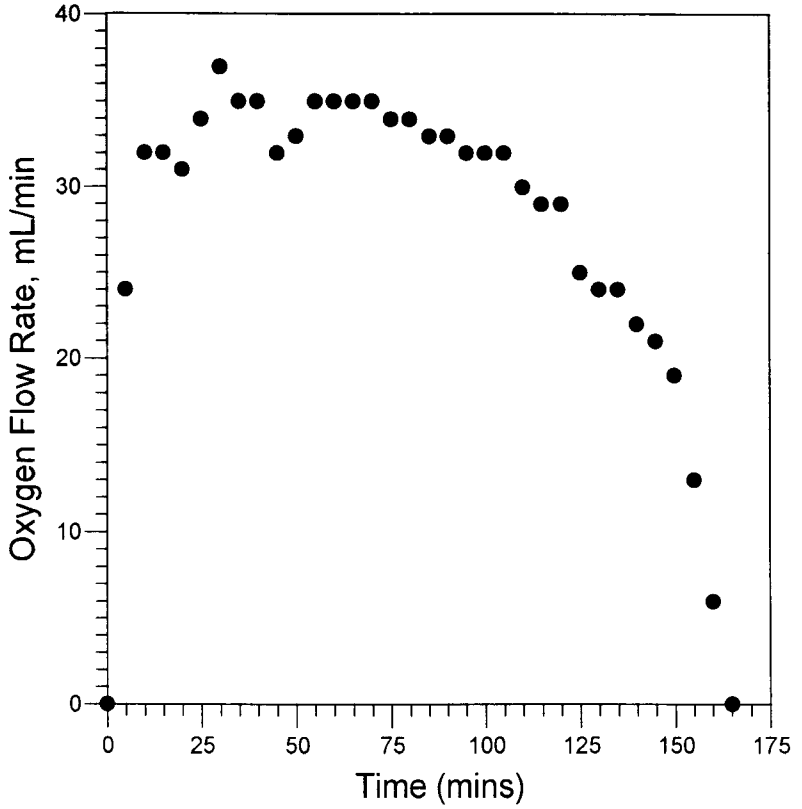

Fig. 5. Oxygen production vs time. Pressure = 0.38 MPa, temperature = 24°C, pH = 7.0, S_0 = 4.9*M*. Soluble enzyme.

Table 2
Steady-State Average Values
of the Rate of Oxygen Production
at Different Pressures for Soluble Catalase

Pressure, MPa	Average oxygen flow, mL/min
0.149	25.3 ± 1.8
0.177	27.7 ± 1.9
0.210	28.5 ± 1.8
0.256	34.5 ± 1.6
0.287	38.4 ± 2.3
0.294	43.5 ± 2.2
0.310	45.9 ± 2.3
0.320	41.6 ± 2.0
0.342	35.5 ± 2.0
0.377	31.8 ± 1.9
0.404	25.9 ± 1.6

Enzyme Distribution in Immobilized Alumina

Alumina used in this study had an average surface area of 230 m^2/g and an average diameter of 3.2 mm. In order to determine if pore diffusion is a problem, photomicroscopic studies were carried out. A very small amount ($\approx$0.005 g) of Fluorescein isothiocyanate hydrochloride (FITC, 4022F, Research Organics) was added to the enzyme. The immobilization was carried out according to the procedure outlined under Materials and Methods. Fluorescent microscopy studies of the enzyme on plain alumina, silanized alumina, and glutaraldehyde-treated alumina were carried out. A separate set of runs was also conducted in which the FITC was used instead of glutaraldehyde.

The pellets were sliced using a microtome blade, and the following sections were chosen to give an indication of the enzyme distribution in the pellet. Only photographs of the supported enzyme relevant to this article will be discussed.

- Outer segment;
- Segment (not through the geometric center); and
- Geometric center of the pellet.

A BH2-RFL Olympus microscope attached with a reflected light fluorescent attachment was used to study the distribution. The unit was also equipped with a supplementary exciter filter slider and a barrier filter in addition to the exciter filter that aided this study. A 100-W high-pressure mercury burner and a halogen lamp were the sources of light used to illuminate the specimen. A PM-6 Olympus 35mm camera was used for photomicroscopy. A shutter release cable was used to take the pictures, and the exposure time ranged from 2–50 s. The exposure time was determined by an EMM-7 Olympus exposure meter. Kodak Ektachrome 1000 and Kodak 125-X pan film were used to take the pictures. Two barrier filters, one 530 nm and the other 570 nm, were used in the study.

Plain alumina scattered light from external sources, which made it visible under a fluorescent microscope. One of the ways to get around this was to use planar slices of the specimen to prevent structural imperfections in the specimen from scattering light. Care was taken to ensure that all the specimens were reasonably planar. However, in total darkness, plain alumina did not scatter light even if the specimen was not cut properly.

As expected, catalase immobilized on plain alumina showed no fluorescence. Silanized alumina had a definite fluorescence, and so did silanized alumina immobilized with enzyme (without the fluorescent label) and silanized alumina treated with glutaraldehyde. A combination of exciter filter and a 570-nm barrier filter was used to eliminate this.

Figure 6 is a photomicrograph of alumina subjected to silanization followed by treatment with glutaraldehyde and enzyme immobilization.

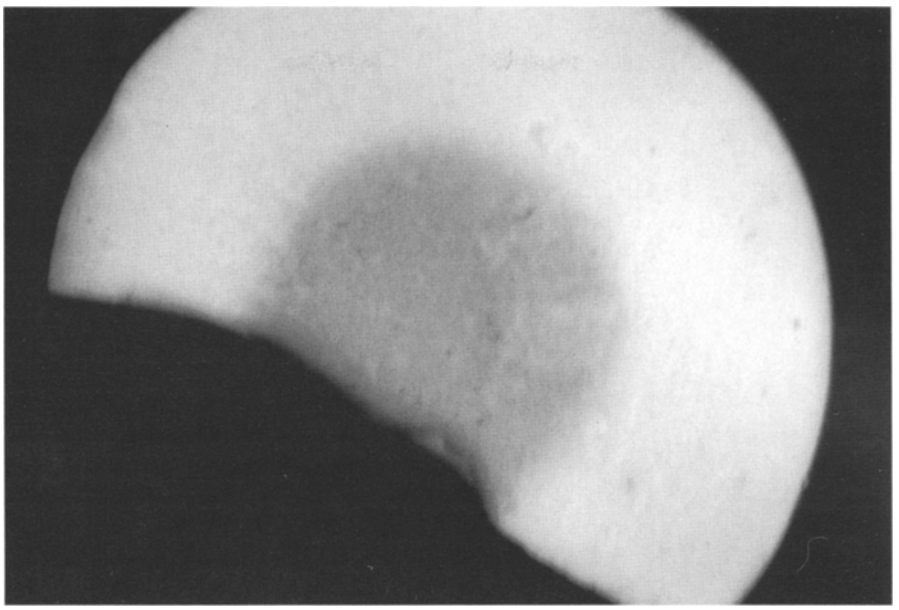

Fig. 6. Enzyme distribution in sectioned alumina pretreated with silane coupling agent and glutaraldehyde, and immobilized with FITC-marked enzyme, near geometric center.

It is clear from this picture that no enzyme is present at the core and that the enzyme is confined to an outer shell. Figure 7 is a photograph of an alumina pellet in which FITC was used as the crosslinking agent instead of glutaraldehyde. Again, it can be seen that the enzyme is confined to a very thin outer shell.

Experiments were also conducted in which the silanized support was exposed to glutaraldehyde or FITC for a prolonged length of time (10 h). This was followed by immobilization with FITC-marked enzyme or plain enzyme. However, it was noticed that in spite of using longer times, there was no change in the dimension of the dark core. Also, higher concentrations of enzyme in the immobilization solution appeared to have no effect on the dimension of the vacant core (not immobilized enzyme activity).

The photomicroscopy studies suggest that a concentration gradient exists inside the alumina pellet. If the concentration of hydrogen peroxide inside the pellet is lower than the bulk concentration, then inhibition of the enzyme inside the pellet would be considerably less. The experimental data for oxygen production show a flat profile (even at long times), indicative of the fact that inhibition of the enzyme by peroxide and/or long-term deactivation of the enzyme is probably minimal because of the low concentration of peroxide inside the pellet. This might explain the differences observed in deactivation rates between soluble and immobilized

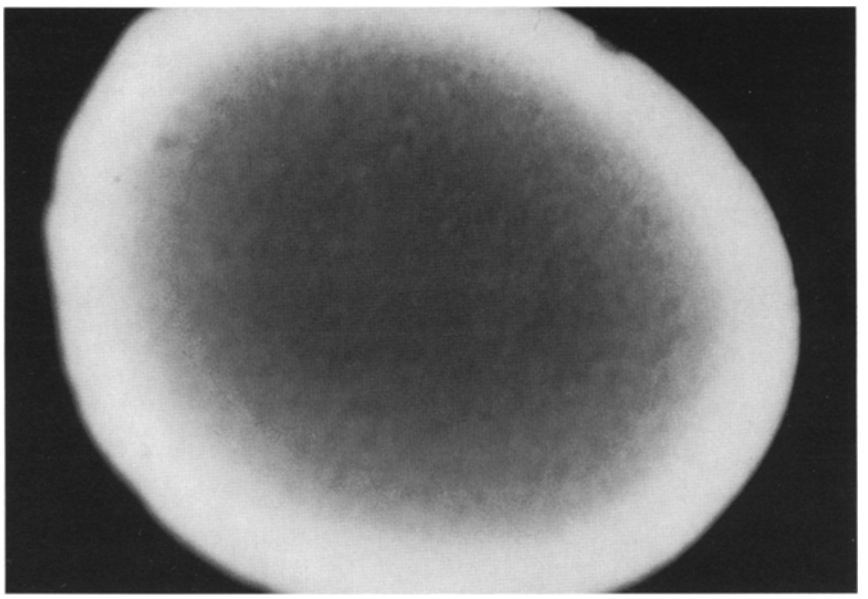

Fig. 7. Enzyme distribution in sectioned alumina pretreated with silane coupling agent and and FITC, no glutaraldehyde, and immobilized with catalase, geometric center.

catalase (even though the feed concentration to the reactor was the same in both cases).

Discussion

The main differences between the experiments with soluble enzyme and immobilized enzyme are as follows. First, a steadier rate of production of oxygen was observed with immobilized catalase compared to soluble catalase. Second, the soluble enzyme runs were conducted in a fed-batch reactor, whereas the experiments with the immobilized enzyme were conducted in a CSTR. The most striking observation with the immobilized enzyme runs was the steady oxygen flow rate even at long times (up to 300 min), unlike soluble catalase, where inhibition and deactivation of the enzyme had a noticeable effect on the oxygen flow rate. In the case of soluble catalase, Table 2 clearly shows that the maximum in oxygen production rate is around 0.31 MPa. For immobilized catalase, the presssure peak is not as prominent.

Theoretical Analysis

For catalase, the mechanism proposed by Jones and Wynne-Jones (*13*) is as follows:

$$E + S \underset{k-1}{\overset{k_1}{\rightleftharpoons}} ES \tag{1}$$

$$ES + S \underset{k-2}{\overset{k_2}{\rightleftharpoons}} ESS \tag{2}$$

$$ES \xrightarrow{k_3} ES^* \tag{3}$$

$$ES^* \xrightarrow{k_4} E + P \tag{4}$$

A steady-state analysis yields the rate equation:

$$v = [v_{max}S / K_m + S + (S^2 / K_i)] \tag{5}$$

where

$$v_{max} = 2k_3E_o \tag{6}$$

$$(1 / K_i) = (k_2 / k_{-2}) \tag{7}$$

and

$$K_m = [(k_{-1} + k_3) / k_1] + (k_3 / k_4) \tag{8}$$

The above mechanism is typical of an enzyme-catalyzed reaction in which the enzyme is inhibited by the substrate. For a fed-batch reactor (soluble catalase experiments), mole balance gives:

$$(dV / dt) = q \tag{9}$$

Balance on the substrate gives:

$$[d(V S) / dt] = qS_0 - [v_{max}Sa / (K_m + S + S^2 / K_i)] \tag{10}$$

The above equation can be expanded and combined with Eq. (9) to give:

$$(dS / dt) = [q(S_0 - S) / V] - [v_{max}Sa / (K_m + S + S^2 / K_i)] \tag{11}$$

The above equation contains the enzyme activity, a, which is included to account for the deactivation of the enzyme. Since the feed flow rate, q, was kept constant, Eq. (9) can be integrated and combined with Eq. (11). The combined equation is:

$$(dS / dt) = [q(S_0 - S) / (qt + 0.5)] - [v_{max}Sa / (K_m + S + S^2 / K_i)] \tag{12}$$

Vasudevan and Weiland (*12*) have studied the long-term deactivation of bovine liver catalase immobilized on nonporous glass. The deactivation model proposed in their study for catalase is:

$$(da / dt) = [-k_dSa / (K_m + S + S^2 / K_i)] \tag{13}$$

Equations (12) and (13) were solved numerically using an IMSL routine, IVPRK. The values of K_m, K_i, and k_d were taken from the work of Vasudevan and Weiland (*12*). These values are intrinsic constants obtained

for the same system and using the same enzyme (Sigma C-10, bovine liver catalase). Vasudevan and Weiland also corroborated George's (*14*) observation that rate decreased above a substrate concentration of about 0.08*M*, clearly indicative of a substrate-inhibited reaction. The results of the above simulation and comparison with experimental data are presented later.

Effect of Pressure on Reaction Parameters

The steady-state rate equation for a nondeactivating catalyst (*5*) can be written as:

$$v = v_{max} / [1 + (K_m / S) + (S / K_i)] \tag{14}$$

Taking logarithm on both sides and differentiating partially with respect to pressure keeping temperature constant:

$$[(\partial \ln v / \partial p)]_T = [(\partial \ln v_{\max} / \partial p)]_T - \{1 / [1 + (K_m / S) + (S / K_i)]\}$$
$$\{(K_m / S)\,[(\partial \ln K_m / \partial p)]_T - (S / K_i)\,[(\partial \ln K_i / \partial p)]_T\} \tag{15}$$

Substitution for the partial derivatives in terms of activation volumes gives:

$$-(\Delta V^* / RT) = (\Delta V_c^* / RT) + \{1 / [1 + (K_m / S) + (S / K_i)]\}$$
$$[(K_m / S)\,\Delta V_b^* - (S / K_i)\,\Delta V_i^*] \tag{16}$$

As reported earlier, Morild and Olmheim observed that the rate of hydrogen peroxide decomposition goes through a maximum with pressure. This suggests that the activation volume, ΔV_c^*, goes through a maximum, starting with a negative value at low pressures, reaching zero at the maximum, and then becoming positive. This is possible if there is a pressure-induced change in the rate-determining step from ES isomerization (low pressure) to product formation (high pressure). Stated otherwise, the rate-determining step has shifted from reaction 3 to reaction 4. Although this interpretation is plausible and would explain why a maximum is observed in the activity in general, it is clear that in the present study, this would imply a large change in the activation volume (for the range of pressures studied), which is highly improbable.

The experimental studies have shown that oxygen flow rate increases with an increase in pressure up to a certain value and then decreases. This phenomenon is not as prominent for immobilized catalase as it is for soluble catalase. In the case of soluble catalase, the activity peaks at about 0.31 MPa. Comparison with the data of Morild and Olmheim suggests that there is a shift in the activity peak in the direction of decreasing pressure as peroxide concentration is increased.

It is well known that catalase from bovine liver exhibits two phases of activity (*14*). The α-phase is characterized by a burst in activity lasting only a few minutes followed by an irreversible transition to the steady-state (β-phase) of activity. The kinetic parameters for the α-phase alone would be very different. For instance, during the transition from the α to

the β phase, v_{max} changes from a large value to a smaller value (this ratio can be as high as 3) (*12–14*). It is not well understood why this transition is peculiar to bovine catalase. It is possible that application of pressure affects this transition in activity (and the substrate-induced binding) in a manner not clearly understood, but resulting in an altered steady-state activity of the enzyme.

The studies conducted by Morild and Olmheim (*11*) investigated the effect of pressure on enzyme activity in the α-phase alone. As mentioned earlier, these studies were conducted at very low enzyme and fairly low substrate concentrations. In this study, although the range of pressures studied is fairly low, the observation that the oxygen flow rate starts to decrease beyond 0.31 MPa is striking. Morild and Olmheim observed that the rate goes through a maximum with pressure. Since the substrate concentration in vivo is small, they concluded that the catalase activity will increase with pressure up to about 30–40 MPa. They also concluded that the "position of the maximum seemed to be a complicated function of the substrate concentration" and noted that "a drastic change in pressure effects" occurred with the addition of a salt (KCl). The addition of a salt led to a monotonous decrease in the rate of hydrogen peroxide reduction with pressure.

The substrate concentration used in our study is much larger, and hydrogen peroxide concentration appears to have a strong influence on pressure effects. This may explain the shift in the activity peak (that is a shift in the maximum value compared to the results of Morild and Olmheim, who used lower concentrations of hydrogen peroxide) in the direction of decreasing pressure, as peroxide concentration is increased. With immobilized catalase, the shift is not so prominent probably because of the lower concentration of peroxide inside the catalyst compared to the bulk substrate concentration. Comparison of Tables 1 and 2 clearly shows that the range of values for oxygen flow rate is much narrower for immobilized catalase compared to soluble catalase. Also, the effect of the buffer and antifoaming agent on the rate, if any, is not known. It is also possible that the fugacity of the dissolved oxygen tends to inhibit the rate. For example, inhibition of catalase may be the result of superoxide ($O_2^{\cdot}$). It is known that catalase is inhibited by O_2 through formation of an oxidized ferroxycatalase, which is an inactive species (*15*). It is possible that superoxide production is increased as the pressure is increased. Clearly, the fundamental question arises as to the cause of the decline in activity beyond a certain pressure. We do not have a concise and unambiguous rationale for this observation.

Deactivation

For soluble enzyme, Eqs. (12) and (13) were solved numerically using an IMSL routine, IVPRK. The values of K_m, K_i, and k_d were taken from the work of Vasudevan and Weiland (*12*). These values are intrinsic constants

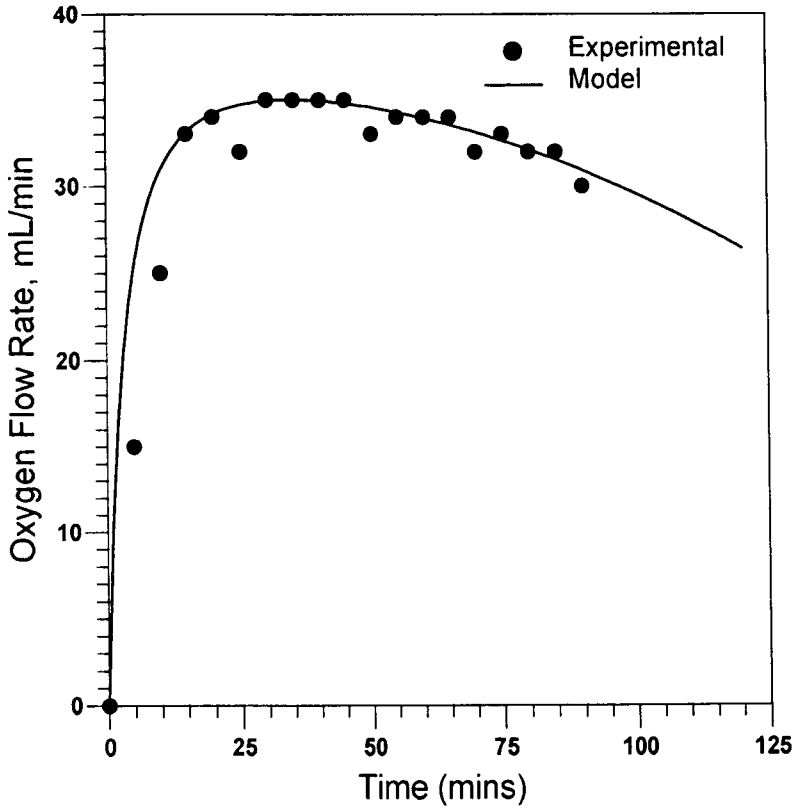

Fig. 8. Model vs experimental data. P = 0.23 MPa, v_{max} = 2.1 × 10^{-4} mol/L s, K_m = 0.035M, K_i = 0.23M. Soluble enzyme.

obtained for the same system and using the same enzyme (Sigma C-10, bovine liver catalase). For example, Fig. 8 compares the results predicted by the model at a pressure of 0.23 MPa with experimental data at the same pressure. v_{max} was found to be 2.1 × 10^{-4} mol/L s and is the only fitted parameter. The solid line represents the model, and the •s indicate the experimental data. There appears to be reasonable agreement between the two. The important observation is that the values of the kinetic parameters determined in a previous study for catalase immobilized on nonporous glass (*12*) appear to be valid for soluble catalase also. The effect of pressure (in the range studied) on long-term deactivation appears to be minimal. For soluble enzyme, the experiments typically lasted about 2 h, and in this time, no sudden loss of activity was observed as a result of increase in pressure.

In the case of catalase immobilized on porous alumina, deactivation appears to be minimal probably because of a lower substrate concentration inside the pellet. Theoretical analysis of the data verified this observation since a much lower value of the deactivation constant (almost two orders of magnitude less) resulted in a fair agreement between theory and experimental data. However, the problem is complicated by pore dif-

fusion, and such an analysis for catalase immobilized on porous alumina is simplistic at best. As with soluble catalase, no sudden loss of activity was observed as a result of increase in pressure.

CONCLUSIONS

The effect of pressure on enzyme kinetics and on long-term deactivation of bovine liver catalase was investigated. It was found that the oxygen flow rate increased somewhat with an increase in pressure up to a certain value and then decreased. This phenomenon is not as prominent for immobilized catalase as it is for soluble catalase. For immobilized catalase, the enzyme appears to be distributed in an outer shell, and the effect of pressure on enzyme kinetics is complicated by pore diffusion. The effect of pressure on long-term enzyme deactivation appears to be minimal in the range of pressures studied.

REFERENCES

1. Hamann, S. D. (1957), in *Physico-Chemical Effects of Pressure.* Butterworths, London.
2. Ludwig, H. and Greulich, K. O. (1978), *Biophys. Chem.* **8,** 163–170.
3. Weale, K. E. (1987), E & F. N. SPN. London.
4. Ruan, K. and Weber, G. (1989), *Biochemistry* **28,** 2144–2153.
5. Weber, G. (1989), *J. Mol. Liquids* **42,** 255–268.
6. Ruan, K. and Weber, G. (1988), *Biochemistry* **27,** 3295–3301.
7. King, L. and Weber, G. (1986), *Biochemistry* **25,** 3632–3637.
8. Bautista, J. M., Fuentes, J. M., Diez, A., Gutierrez, M. C., and Soler, G. (1992), *Biochimica et Biophysica Acta* **1122,** 99–106.
9. Quieroz, C. C., Valon, C., and Quieroz, O. (1988), *Chronobiology International* **5,** 301–310.
10. Haddock, G. (1991), M.S. Thesis, University of New Hampshire.
11. Morild, E. and Olmheim, J. E. (1981), *Physio. Chem. Phys.* **13,** 419–428.
12. Vasudevan, P. T. and Weiland, R. H. (1990), *Biotechnol. Bioeng.* **36,** 783–789.
13. Jones, P. and Wynne-Jones, W. F. K. (1962), *Transactions of Faraday Society* **58,** 1148–1158.
14. George, P. (1947), *Nature* **160,** 41–43.
15. Halliwell, B. and Gutteridge, J. M. C. (1989), *Free Radicals in Biology and Medicine.* Clarendon, New York.