around 2.5. These values are within the range reported for tightly compacted materials.

## NOTATION

$A$ = bulk cross section of sample
$D_i$ = controlling pore entry diameter
$D_j$ = pore diameter
$D_l$ = diameter of large pore segment
$D_s$ = diameter of small pore segment
$D_{12}$ = binary diffusion coefficient
$D_{12,eff}$ = effective diffusion coefficient
$E$ = electric potential
$F$ = quantity defined by Equation (3)
$I$ = current
$k_m$ = permeability predicted by Dullien's model
$l_{ij}$ = length of a pore segment associated with the volume $V_{ij}$
$l_l$ = length of a large pore segment
$l_s$ = length of a small pore segment
$L$ = length of sample
$L_e$ = effective path length in sample
$m$ = quantity defined in Equation (12)
$n_{ij}$ = number of pore segments associated with the volume $V_{ij}$
$S'$ = quantity defined by Equation (5)
$T$ = tortuosity
$V_{ij}$ = bivariate pore volume distribution
$x$ = $D_l/D_s$
$X$ = quantity defined by Equations (2) and (3)
$y$ = $l_l/l_s$

### Greek Letters

$\alpha$ = angle defining orientation of sample with respect to direction of macroscopic current
$\beta$ = angle defining orientation of sample with respect to direction of macroscopic current
$\epsilon$ = porosity
$\rho_0$ = specific resistivity of porous medium saturated with electrolyte
$\rho_w$ = specific resistivity of electrolyte

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# A Solution for the Oxygen Mass Transfer Problem in Immobilized Enzyme Systems


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The kinetic limitations due to oxygen transfer problems were widely described in chemical and biochemical engineering (Ryu et al., 1973, Topiwala and Hamer, 1973). This subject was especially studied in the field of industrial fermentors. In the growing field of immobilized enzymes, not only has the problem not been solved, but also it has never been clearly studied. Numerous papers (Weetall, 1974) have been devoted to immobilized enzymes and recently reviewed (Gryszkiewicz, 1971; Goldman, 1971).

The aim of this note is to show that in immobilized enzyme systems the oxygen can be generated inside the support itself through another enzyme reaction. The basic principle of the described solution is the existence of substrate and product concentration profiles inside the support (Thomas et al., 1974; Barbotin and Thomas, 1974). In the experimental system glucose-oxidase (E.C. 1.1.3.4.—Sigma) and catalase (E.C. 1.1.1.1.6.—Sigma) are immobilized inside proteic membranes with a co-cross-linking method previously described (Broun et al., 1973). The glucose-oxidase catalyses the transformation of glucose into gluconic acid and $H_2O_2$ in presence of oxygen. Its kinetics were monitored at $p$H 8, the optimum $p$H for the immobilized enzyme, in a phosphate solution $5.10^{-3}$M by injection of NaOH in with a $p$H-stat (Metrohm). Due to the $p$H value and the presence of lactonase, the lactone decomposition is not the limiting stop of the overall reaction. Under steady state conditions, the enzyme activity is given by the measurement of the $H^+$ production.

The catalase transforms $H_2O_2$ into water and oxygen. The reaction rate was followed spectrophotometrically at 240 nm. In both experiments performed with free and immobilized catalase and glucose oxidase, activities were respectively 7500 I.U. (with $H_2O_2$ $10^{-2}$M) and 250 I.U. (with a glucose solution of 20 g/l) 100-ml bath solution. The Thiele modulus values for the catalase are 186 and 5.8 for the glucose-oxidase. The diffusion coefficients were


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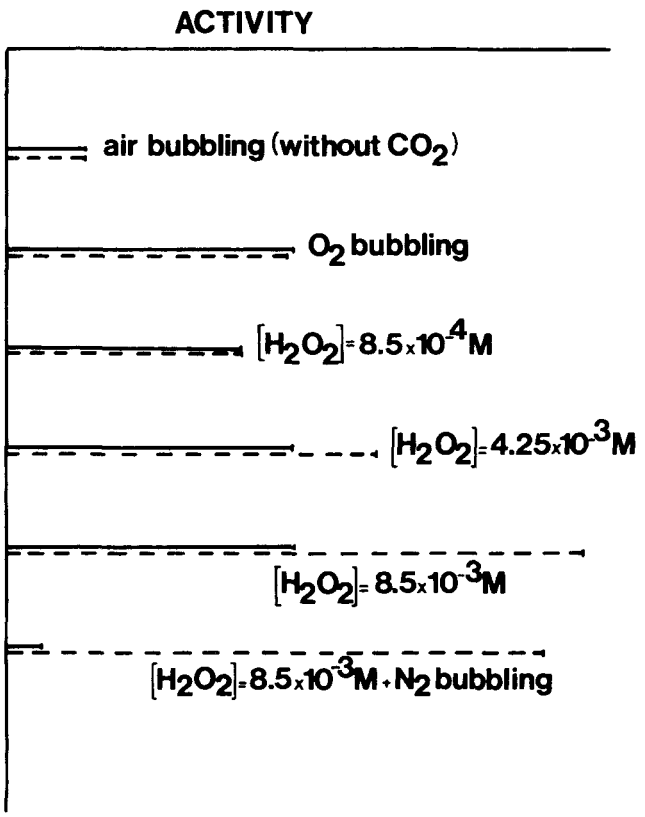


Fig. 1. Glucose-oxidase activity under different oxygenation conditions when free in a solution (——) (25 mg of crude enzyme in 100 ml) and immobilized in a membrane (- - -) (membrane area 40 $cm^2$, and thickness 40 $\mu$). The activity measured under air bubbling is used as a unit in both cases. The results deal with experiments performed with air and oxygen bubbling, and with an oxygenation through $H_2O_2$ molecules in presence and in absence of $N_2$ bubbling. In any cases glucose concentration is 20 mg/ml in 5 $10^{-3}$M phosphate buffer pH 8.

found of $1.57 \times 10^{-6}$ $cm^2$ $s^{-1}$ and $3.7 \times 10^{-7}$ $cm^2$ $s^{-1}$ for oxygen and gluconic acid respectively. The glucose oxidase kinetics were studied, first free in solution with catalase under air bubbling and under oxygen saturation and secondly immobilized in a membrane under air bubbling, under oxygen bubbling and with $H_2O_2$ as the source of oxygen (Figure 1). From the results given Figure 1, with the free enzyme the oxygenation with $H_2O_2$ and molecular oxygen are similar, and with the immobilized enzyme the $H_2O_2$ oxygenation is more efficient than the molecular oxygen. In the presence of $H_2O_2$, due to the catalase activity, there is a high oxygen concentration inside the membrane, even if the oxygen concentration is negligible (under $N_2$ bubbling) in the bulk solution. The physical existence of these local concentrations was checked with a catalase membrane coated on a $pO_2$ electrode (Radiometer . . .) (Figure 2). The electrode response is very quick and the observed $pO_2$ level is higher than 800 mm Hg. The yield of the use of the catalase-produced oxygen, that is to say the ratio between the consumed and produced oxygen, was studied as a function of $H_2O_2$ concentration (Figure 3). From the Figure 3 the yield under oxygen bubbling is not in the same order of magnitude. The described system is a solution for the oxygen mass transfer problem in immobilized enzyme systems with normal aqueous solutions. It is still more efficient when the oxygen mass transfer coefficient is small and the use of $H_2O_2$ is possible even if the oxygen solubility in the bulk solution is negligible. The solubility of oxygen in water is $10^{-3}$M, and obviously it is not

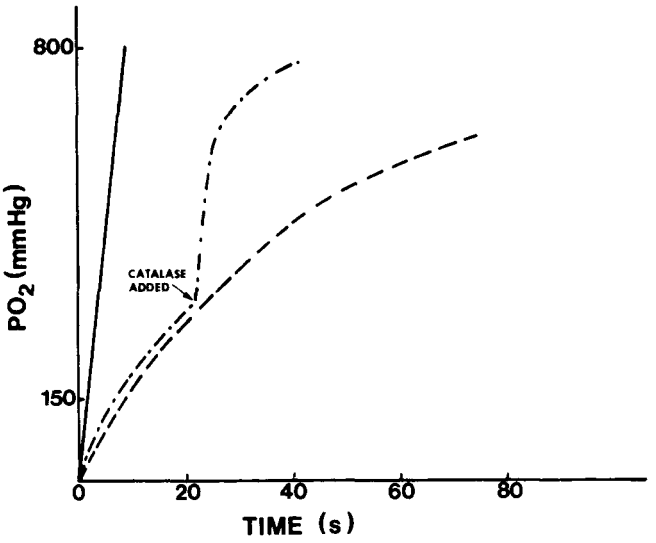


Fig. 2. $pO_2$ recorded as a function of time with a $pO_2$ electrode (1) immersed in a solution 8.5 $10^{-3}$M of $H_2O_2$ in absence (- - -) and in presence of catalase (-·-·-) (2) coated with catalase membrane and immersed in the same solution (——).

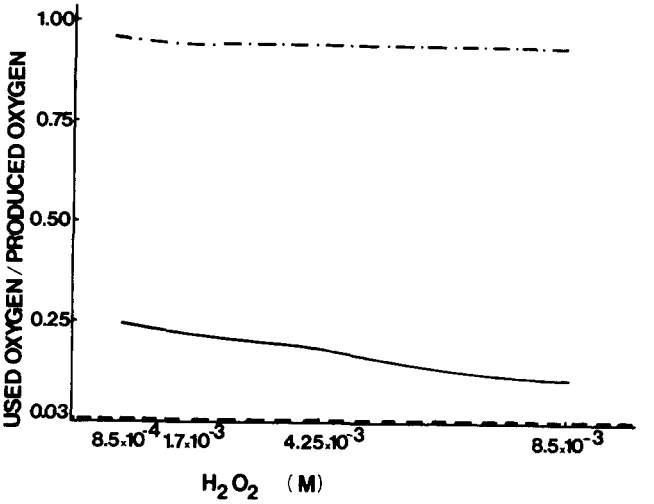


Fig. 3. Ratio between the oxygen used by glucose oxidase reaction and the oxygen produced by the catalase as a function of the $H_2O_2$ concentration for free (——) and immobilized (-·-) enzymes. The level of the same yield under oxygen bubbling is given (- - -) for both cases.

possible to transform more substrate in a packed bed reactor than this value when using molecular oxygen. Due to the concentration profiles, $H_2O_2$ can be used as a oxygen donor even if $H_2O_2$ exhibits or denaturation effects on the enzyme. During operation the $H_2O_2$ concentration can be negligible and the $O_2$ concentration high inside the support itself. The local $O_2$ concentration can be enough to compensate for the activity decrease due to the enzyme immobilization. Apparent activity yields of 110% with membranes and 150% with particles were experimentally observed. The yields are expressed with the enzyme activity measured under saturation conditions of $O_2$ as a reference.

From the high yield shown on Figure 3 and the $H_2O_2$ low cost, $H_2O_2$ as an oxygen donor is quite competitive in comparison with molecular oxygen (from the economical point of view).

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# The Criteria for Thermodynamic Stability


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Beegle, Modell, and Reid (1974b), in their recent discussion of the use of Legendre transforms to establish thermodynamic stability, have made an erroneous statement. The error is contained in the following sentences quoted from the paper.

". . . one can state that the necessary and sufficient criterion of stability is

$$y^{(m-2)}_{(m-1)(m-1)} > 0 \tag{21}$$

In other words, if $y^{(m-2)}_{(m-1)(m-1)}$ is positive, then all $y^{k-1}_{kk}$ $(k = 1, \ldots, m-1)$ are positive and the system is stable. The condition at which the system reaches the limit of stability is the one at which $y^{(m-2)}_{(m-1)(m-1)}$ becomes zero."

However,

1. The condition stated is not sufficient to assure stability; that is, the second sentence is not always true.

2. It is possible to have $y^{(m-2)}_{(m-1)(m-1)} = 0$ at points which do not lie on the limit of stability. While it is true that this condition is satisfied at all points which lie on the limit of stability, the converse is not true.

## A COUNTER-EXAMPLE

A ternary liquid-liquid equilibrium problem will be analyzed at this point to illustrate that (21) is not sufficient to establish stability. The excess Gibbs free energy is taken to be

$$\frac{G^E}{RT} = \alpha(x_A x_B + x_A x_C + x_B x_C) \tag{1}$$

According to Tisza (1951, 1961, 1966) and to Beegle et al. (1974b), at stable points four inequalities must be satisfied. The internal energy is used for $y^0$. That is,

$$y^0 = \underline{U}(\underline{S}, \underline{V}, N_A, N_B, N_C) \tag{2}$$

Then, at stable points (see Beegle et al. (1974a) for mechanics of handling the Legendre transforms)

$$y^0{}_{11} = \frac{T}{C_v} > 0 \tag{3}$$

$$y^1{}_{22} = -\left(\frac{\partial P}{\partial V}\right)_{T,N_A,N_B,N_C} > 0 \tag{4}$$

$$y^2{}_{33} = \left(\frac{\partial \mu_A}{\partial N_A}\right)_{T,P,N_B,N_C} > 0 \tag{5}$$

and

$$y^3{}_{44} = \left(\frac{\partial \mu_B}{\partial N_B}\right)_{T,P,\mu_A,N_C} > 0 \tag{6}$$

In the absence of other information, it is presumed that (3) and (4) are satisfied. For (5) and (6) we find, respectively, that

$$\frac{N}{RT} y^2{}_{33} = \frac{1 - x_A}{x_A} - 2\alpha[(1 - x_A)^2 - x_B x_C] \tag{7}$$

and

$$\frac{N}{RT} y^3{}_{44} = \frac{1 - x_B}{x_B} - 2\alpha[(1 - x_B)^2 - x_A x_C] - \frac{[-1 + \alpha x_C + 2\alpha(x_A x_B - x_C{}^2)]^2}{\frac{N}{RT} y^2{}_{33}} \tag{8}$$

Equation (8) is a specific instance of the "generalized derivative operator" introduced by Beegle et al. (1974b). That is,

$$y^{(k-1)}_{kk} = y^{(k-2)}_{kk} - (y^{(k-2)}_{k(k-1)})^2 / y^{k-2}_{(k-1)(k-1)} \tag{9}$$

which in this case becomes

$$y^3{}_{44} = \left(\frac{\partial \mu_B}{\partial N_B}\right)_{T,P,N_A,N_C} - \frac{\left(\frac{\partial \mu_A}{\partial N_B}\right)^2_{T,P,N_A,N_C}}{y^2{}_{33}} \tag{10}$$