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Oxidation of Glucose to Gluconic Acid by Glucose Oxidase in a Membrane Bioreactor

Ester Junko Tomotani, Luiz Carlos Martins das Neves, and Michele Vitolo*

University of São Paulo, School of Pharmaceutical Sciences, Department of Biochemical and Pharmaceutical Technology, Av. Prof. Lineu Prestes, 580, B-16, 05508-900, São Paulo, SP, Brazil, E-mail: michenzi@usp.br

Abstract

Glucose oxidase (GO) (EC 1.1.3.4) was used as catalyst for oxidizing glucose into gluconic acid utilizing a 10-mL Bioengineering Enzyme Membrane Reactor® or a 400-mL Millipore Stirred Ultrafiltration Cell (MSUC) coupled with a Millipore UF membrane (cutoff of 100 kDa) and operated for 12 h under an agitation of 100 rpm, pH 5.5, and 30°C. The effect of feeding rate (0.10, 0.15, or 0.20 min^{-1}), glucose (2.5 or 5.0 m*M*), and GO (1.0 or 2.0 mg/mL) concentrations on the catalysis were studied. A yield of about 75% was attained when the MSUC filled with 1.0 mg/mL of GO was fed with 2.5 m*M* glucose solution at a rate of 0.15 min^{-1}.

Index Entries: Glucose oxidase; membrane bioreactor; gluconic acid; stability; feeding rate.

Introduction

Bioconversions, defined as reactions promoted by enzymes, cells, or organelles in media with high or low water activity, have generated great interest in organic synthesis *(1)*. Among the advantages presented by biocatalysis are the high selectivity of the biocatalyst, the formation of less toxic effluents, and the possibility of carrying out coupled reactions. Biocatalysis, when its use is possible, competes advantageously with existing traditional processes such as extraction (which is highly dependent on the availability of raw materials and presents a low overall yield), fermentation (which requires the handling of a huge volume of medium and amount of biomass), and chemical synthesis (which is more pollutant, less selective, and high energy consuming).

Among all conceivable bioconversions, the oxidation of glucose into gluconic acid (GA) is one of great interest, not only owing to the high availability of glucose (attained from sucrose and starch, the main and abundant natural sources) but also to the high market demand for GA, a product largely employed in the food, pharmaceutical, and chemical industries *(2)*. The

*Author to whom all correspondence and reprint requests should be addressed.

oxidation of glucose into GA can be realized either by chemical catalysis (bismuth, palladium, platinum, or gold immobilized into active charcoal used as catalyst) *(3)* or by microbial conversion *(4)*. Fermentation is the main process for GA production, in which strains of *Aspergillus niger, Gluconobacter suboxydans,* or *Acetobacter methanolicus* are employed *(4)*. The preference for the biotechnological process is mainly owing to the good GA yield attained (>80%), the small amount of byproducts formed, the utilization of commercial-grade glucose, and the generation of environmentally inoffensive residues. Despite these advantages on the microbial process, intrinsic problems to be addressed include subsequent cell separation, as well as the discharge and destination of huge volumes of liquid after GA separation.

An alternative approach is the use of glucose oxidase (GO) (EC 1.1.3.4) as catalyst for glucose oxidation, which presents all the advantages described for the fermentation plus the low energy requirement, the high specificity for glucose, and the possibility of carrying out the conversion in a continuous reactor. GO (Molecular mass: 150–190 kDa) is formed by two chains of glycoprotein linked by disulfide bonds, with each chain having one ferrous ion and one FAD prosthetic group *(5)*. Moreover, it is largely employed in analytical procedures *(5–7)* and industrial processes *(6)* in both soluble and insoluble forms. Notable examples of reactors used for glucose/GA conversion catalyzed by GO that we operated continuously include packed-bed reactors *(6)*, fluidized-bed reactors *(8),* and membrane bioreactors *(9,10)*.

As a general rule, the packed- or fluidized-bed reactor operates with the enzyme linked to an insoluble inert support, whereas the membrane bioreactor can operate with the enzyme free in solution or linked to the membrane (the enzyme is confined on the surface or within the membrane). This feature is gradually shifting the industry preference from the traditional immobilized enzyme reactors (packed-bed reactor, continuous stirred tank reactor [CSTR], and fluidized-bed reactor) to the membrane bioreactor. This tendency is borne out by the use of the membrane bioreactor in a dozen processes, resulting in a large variety of products (cyclodextrins, fructooligosaccharides, cathecol, digests of casein and hemoglobin, among others) *(11–14)*.

The membrane bioreactor can be shaped either as a CSTR coupled with a semipermeable membrane or as a hollow-fiber reactor, i.e., a tank without stirring filled with a sheaf of straight-lined hollow-fiber tubes of semipermeable membrane. However, the membrane/biocatalyst arrangement can involve or not an interaction between them *(9)*. If they are linked, the membrane acts as catalysis and separation surface simultaneously; otherwise, it functions only as a separation surface. When the enzyme is in the soluble form, the membrane bioreactor can involve the recycle of the enzyme (the CSTR and the UF membrane module are connected in series) or not (the CSTR and the ultrafiltration (UF) membrane is adapted to the bottom of the CSTR as in a stirred ultrafiltration cell).

According to the literature, glucose oxidation by GO can be carried out through any of the aforementioned reactors *(9)*. Regarding the MB,

the preference is directed to the use of GO united to the membrane *(15)*. Nonetheless, it is notable that little information is available on membrane bioreactors employing soluble GO (mainly of the stirred UF-cell type), in spite of presenting operational characteristics of homogeneous catalysis, high activity per unit of volume, and absence of conformational and diffusional effects. If needed, the MB also allows working under aseptic conditions as well as with multienzymatic systems.

The present work deals with the use of a stirred UF-cell-type membrane reactor (Bioengineering Membrane Reactor®) for glucose/GA conversion catalyzed by GO. The effect of feeding rate (0.10, 0.15, or 0.20 min^{-1}), glucose (2.5 or 5.0 m*M*), and GO (1.0 or 2.0 mg/mL) concentrations on the catalysis were studied. Moreover, some kinetic parameters (pH and temperature effects, K_M, V_{max}, and K_i) related to GO were also determined.

Materials and Methods

Chemicals

GO from *A. niger* (cat. no. G 6766) and glucose were obtained from Sigma (St. Louis, MO). The 100-kDa UF membranes (PLHK07610 and PBHK07610, made of regenerated cellulose and polyethersulfone, respectively) were purchased from Millipore (Bedford, MA). All other chemicals were of analytical grade.

Membrane Reactors

A 400-mL Millipore stirred ultrafiltration cell (MSUC) (model 8400) and a 10-mL Bioengineering Enzyme Membrane Reactor (BEMR) (Bioengineering AG, Wald, Germany) were employed. The MSUC is a borosilicate glass cylinder sandwiched between a 316-L stainless steel lid and base, and the BEMR is a 316-L stainless steel cylinder, whose bottom has an inlet and an outlet for the external water bath for temperature control. The MSUC must be submersed in a water bath for temperature controlling. The diameters of the UF membranes employed in the MSUC and BEMR are 76 and 63 mm, respectively. Both reactors can be sterilized (autoclave up to 134°C by 30 min) and resist high temperatures (until 150°C) and corrosion by most substances (except strong acids, pH < 1.0; and alkalies, pH > 12.0). Moreover, they have safety valves (set to nominal 6 bar pressure limit) and can be coupled to a dosing pump, pressure probe, sterile filter, and bubble trap.

Membrane Reactor Tests

Ten milliliters of buffered (0.01 *M* acetic acid/acetate buffer, pH 5.5) GO solution (1 or 2 mg of GO/mL) was poured inside the BEMR, which had a UF membrane (PLHK07610 or PBHK07610) with a molecular mass cutoff of 100 kDa. The reactor was fed continuously with 2.5 or 5.0 m*M*

glucose buffered solution (0.01 *M* acetic acid/acetate buffer, pH, 5.5) at a rate of 0.10, 0.15, or 0.20 min^{-1}. The reaction was carried out for 12 h at 30°C and an agitation of 100 rpm. Pure oxygen was bubbled into the glucose solution, so that the dissolved oxygen (DO) concentration in the inlet solution remained between 6.5 and 7.0 mg/mL. Aliquots taken from the outlet solution were measured for the concentration of glucose and H_2O_2. The yield *(Y)* and the reaction rate *(r)* were calculated according to Eqs. 1 and 2. A total of 10 assays were realized, whose operational conditions are presented in Table 3.

A scale-up experiment (test 10) was carried out for 12 h using the MSUC, which was filled with 50 mL of buffered GO solution (1 mg/mL), and all operational conditions were fixed according to the best glucose oxidation performance established through the BEMR.

$$Y\ (\%) = ([G]_{consumed}/[G]_0)\cdot 100 \quad (1)$$

$$r\ (\text{mmol}/[\text{h}\cdot\text{mg}_E]) = \{(Q\cdot 60\cdot[H_2O_2])/1000\cdot m_E\} \quad (2)$$

in which $[G]_0$ is the inlet glucose concentration, $[G]_{consumed}$ is the $[G]_0 - [G]_{outlet}$, Q is the volumetric rate (mL/min), m_E is the weight of GO (mg), and $[H_2O_2]$ is the H_2O_2 concentration (m*M*).

When one considers the stoichiometry of glucose/GA conversion catalyzed by GO as 1 mol of glucose generating 1 mol of GA and 1 mol of H_2O_2 *(6)*, the following assumption is valid:

$$[GA] = [H_2O_2] = [G]_{consumed} = \{[G]_0 - [G]_{outlet}\} \quad (3)$$

Standard Assay for Measuring GO Activity

A standard assay for GO, carried out directly in a 1.2-mL cuvet of a spectrophotometer (Beckman-Coulter model DU 640), consisted of mixing 0.2 mL of GO solution (4 mg/mL in 0.010 *M* acetate buffer, pH 5.5), 0.5 mL of glucose solution (0.8 mg/mL in 0.010 *M* acetate buffer, pH 5.5), and 0.3 mL of 0.010 *M* acetate buffer (pH 5.5). The temperature was maintained at 35°C for all assays. Before mixing, pure oxygen was bubbled for 5 min into the glucose and buffer solutions. Glucose oxidation was followed by measuring the amount of H_2O_2 formed at $\lambda = 240$ nm *(16)*. The optical density (OD) related to H_2O_2 formation was recorded every 45 s for a total reaction time of 6 min. A blank constituted by 0.2 mL of GO solution and 0.8 mL of 0.010 *M* acetate buffer (pH 5.5) was employed to set the zero point of the spectrophotometer. One GO unit was defined as the amount of H_2O_2 (milligrams) formed per minute under the conditions of the test. The standard deviation (SD) and the coefficient of variation (CV) related to this method were 8.94×10^{-3} m*M*/min and 4.03%, respectively.

Characterization of GO

The pH, temperature, and substrate concentration of the standard reaction test related to GO were changed one by one at the intervals cited in the following sections. The inhibitory effect of H_2O_2 on GO activity was also evaluated.

Effect of pH on Activity and Stability

The effect of pH on the activity and stability of GO was determined always at 35°C by mixing the enzyme with buffer solutions at fixed pH (4.0, 4.5, 5.0, 5.5, 6.0, or 6.5). The buffers used were 0.010 *M* acetate buffer for pH 4.0, 4.5, 5.0, and 5.5 and 0.010 *M* phosphate buffer for pH 6.0 and 6.5. The stability against pH was determined by measuring the residual activity of GO after 1, 5, 10, 15, 20, 25, and 30 h of enzyme-buffer contact at 35°C.

Effect of Temperature on Activity and Stability

The activity of GO was determined by varying the temperature of the standard test (25, 30, 35, 40, 45, 50, or 55°C). A blank was prepared under the same conditions for each temperature employed. The activation energy (E_a, kJ/mol) was determined by the Arrhenius method, and the thermodynamic parameters were calculated by conventional equations (Eqs. 4–6) *(17)*. The stability of GO was evaluated by maintaining the enzyme solution for 1, 5, 10, 15, 20, 25, and 30 h in acetate buffer (0.010 *M*, pH 5.5) at each temperature cited. Then, the residual activity was measured as discussed under Standard Assay for Measuring GO Activity.

$$\Delta G = (R \cdot T / 2.303) \cdot \log(v \cdot h / k \cdot T) \tag{4}$$

$$\Delta H = E_a - R \cdot T \tag{5}$$

$$\Delta S = (\Delta H - \Delta G) / T \tag{6}$$

in which ΔG is the Gibb's free energy (kJ/mol), ΔH is the enthalpy (kJ/mol), ΔS is the entropy (kJ/[mol·K]), R is the gas constant (8.31 J/[mol·K]), T is the temperature (K), v is the enzyme activity, h is the Planck constant (3.978×10^{-32} Jmin) and k is the Boltzman constant (1.38×10^{-23} J/K).

Effect of Glucose Concentration on GO Activity

The kinetic parameters (K_M and V_{max}) of GO were determined through the conventional Lineweaver-Burk plot, by varying the glucose concentration of the standard test (2.2, 4.4, 6.6, 8.8, 11, 15, 20, or 25 m*M*).

Effect of H_2O_2 on GO Activity

In a 1.2-mL cuvet were mixed 0.2 mL of GO solution (4 mg/mL in 0.010 *M* acetate buffer, pH 5.5) and 0.3 mL of H_2O_2 solution (0.12 mg/mL in 0.010 *M* acetate buffer, pH 5.5). After 5 min at 35°C, 0.5 mL of buffered glucose

solution (2.2, 4.4, 6.6, 8.8, 11, 15, 20, or 25 m*M*) was added. Glucose oxidation was followed by measuring at λ = 240 nm the amount of H_2O_2 accumulated during 6 min, and the OD was recorded every 45 s. A blank constituted by 0.2 mL of GO solution, 0.3 mL of H_2O_2 solution, and 0.5 mL of 0.010 *M* acetate buffer (pH 5.5) was employed to set the zero point of the spectrophotometer.

Analytical Techniques

Determination of Glucose

The concentration of glucose was measured by using an enzymatic peroxidase/GO kit (Laborlab, São Paulo, SP, Brazil).

Determination of H_2O_2

H_2O_2 concentration was determined through the ultraviolet absorption method (λ = 240 nm) as described by Bergmeyer *(16)*. A standard curve was established by measuring the absorbance of H_2O_2 solution, whose concentration ranged from 0.18 to 18 m*M*. Ten volumes of H_2O_2 (pharmaceutical grade) were employed. The minimum square regression curve established was

$$ABS_{240nm} = 4.12 \times 10^{-2} \cdot [H_2O_2] - 6.97 \times 10^{-4} \qquad (r = 0.9995) \qquad (7)$$

in which $[H_2O_2]$ is the H_2O_2 concentration (m*M*).

The SD and CV related to this method were 0.86 m*M* and 0.33%, respectively.

Determination of DO

DO was measured using a conventional oximeter (DIGIMED, model DM4, São Paulo, Brazil). In all experiments the aqueous glucose solution was bubbled with pure oxygen, and the DO was always maintained between 6.5 and 7.0 mg/mL.

Results and Discussion

Effect of pH

It can be seen from Table 1 that the highest GO activity (3.28×10^{-3} U/mL) occurred at pH 5.5, which agrees with the value commonly cited in the literature *(5)*. Generally speaking, at this pH the ionic groups belonging to the amino acid constituents of the enzyme, mainly those located at the domain of the active site, had the proper ionization form for the catalysis (*17*). By leaving the enzyme dissolved in buffer solutions at fixed pH values (from 4.0 to 6.5 in the present study) for a long period, such as 30 h (Table 2), some alterations throughout the protein structure would be expected (as already mentioned, GO has two peptide chains, each having one FAD prosthetic group and one Fe^{+2} ion), affecting the enzyme activity and stability.

Table 1
Effect of pH and Temperature on Activity of GO[a]

pH	$Activity_{pH}$ $(U/mL) \times 10^3$	Temperature (°C)[b]	$Activity_{Temp}$ $(U/mL) \times 10^3$ [c]
4.0	1.47	25 (298)	3.56 (–2.45)
4.5	2.94	30 (303)	4.60 (–2.34)
5.0	3.26	35 (308)	5.00 (–2.30)
5.5	3.28	40 (313)	5.60 (–2.25)
6.0	2.86	45 (318)	6.20 (–2.21)
6.5	2.52	50 (323)	6.87 (–2.16)
7.0	1.97	55 (328)	5.33 (–2.27)

[a] Measurement was carried out in a 1.2-mL cuvet of a spectrophotometer at 35°C in which were mixed 0.2 mL of GO solution (4 mg/mL in 0.010 *M* acetate buffer, pH 5.5), 0.5 mL of glucose solution (0.8 mg/mL in 0.010 *M* acetate buffer, pH 5.5), and 0.3 mL of 0.010 *M* acetate buffer (pH 5.5).

[b] Numbers in parentheses are the values of temperature in degrees Kelvin.

[c] Numbers in parentheses are the values of the decimal logarithm of $Activity_{Temp}$.

Table 2
Variation in Residual Activity of EO, $(U/mL) \times 10^3$, Against pH and Enzyme/Buffer Contact Time at Fixed pH[a]

Time (h)	pH					
	4.0	4.5	5.0	5.5	6.0	6.5
0	3.35	3.31	3.30	3.30	3.33	3.31
1	3.75	3.84	3.02	3.74	4.76	3.28
5	4.11	3.62	4.81	3.44	3.56	3.88
10	3.68	3.00	2.72	2.81	3.34	3.33
15	3.88	2.87	3.43	3.91	4.31	3.37
20	4.54	3.91	2.92	3.28	3.98	3.30
25	4.30	4.32	3.52	3.28	3.28	3.22
30	4.57	4.01	4.03	3.38	2.81	3.15
Average	4.06	3.61	3.47	3.40	3.67	3.35
SD	0.43	0.51	0.67	0.33	0.63	0.51
CV	10.7	14.1	19.3	9.71	17.2	15.2

[a]Tests were carried out in a 1.2-mL cuvet of a spectrophotometer at 35°C in which were added 0.2 mL of GO solution (4 mg/mL in 0.010 *M* acetate buffer, pH 5.5), 0.5 mL of glucose solution (0.8 mg/mL in 0.010 *M* acetate buffer, pH 5.5), and 0.3 mL of 0.010 *M* acetate buffer (pH 5.5).

It is clear from Table 2 that the residual activity of GO varied with the pH of the solution and the GO/buffer contact time up to 30 h. In spite of the observed variation, the GO presented good stability at the pH interval studied, because the high (4.06×10^{-3} U/mL) and low (3.35×10^{-3} U/mL) residual activity average for pH 4.0 and 6.5, respectively differed 18%.

A difference in residual activity such as this could probably be owing to alterations in the complex molecular structure of GO, influenced in some manner by the hydrogen ion concentration, either at the level of tertiary and quaternary structures (reversible conformational modifications) or in the oxi-red state of FAD and Fe^{+2}. It could be speculated that the later alteration should result from side reactions, such as Fe^{+2}/Fe^{+3} (DO would be the oxidant) and/or $Fe^{+2}/FAD/Fe^{+3}/FADH_2$ (an internal oxidation/reduction reaction owing to the different potential of reduction presented by Fe^{+2} and FAD).

Effect of Temperature

From Table 1 it can be seen that the highest GO activity (6.87×10^{-3} U/mL) occurred at 50°C. Moreover, from 25 to 50°C the enzyme activity varied linearly with the temperature according to the following equation:

$$v = 1.25 \times 10^{-4} \times T^* + 6.25 \times 10^{-4} \qquad (r = 0.993) \tag{8}$$

in which T^* is the temperature (°C) and v is the GO activity.

By applying the conventional Arrhenius method to the data related to the logarithm of activity vs the inverse of temperature (K^{-1}) (Table 1), it was possible to establish the following equation:

$$\text{Log } v = -1011.00 \times (T^{-1}) + 0.9700 \qquad (r = 0.990) \tag{9}$$

in which T is the absolute temperature (K).

The activation energy (E_a), calculated through the inclination of Log $v \times T^{-1}$ (Eq. 9), was 19.4 kJ/mol. The derived thermodynamic parameters ΔH = 16.9 kJ/mol, ΔS = 0.105 kJ/(K·mol), and ΔG = –14.5 kJ/mol were calculated through Eqs. 4–6.

By applying the linear regression to the data presented in Table 3 [Log $v = f(t)$], a good fitness ($r > 0.990$) was found only for the data attained at 318 and 323K, whose equations were, respectively,

$$\text{(318K)} \quad \text{Log } v = -0.0057 \times t - 2.347 \qquad (r = -0.993) \tag{10}$$

$$\text{(323K)} \quad \text{Log } v = -0.019 \times t - 2.411 \qquad (r = -0.997) \tag{11}$$

in which t is time during which the GO solution was left under a set temperature.

Since there are two values of thermal inactivation constants (represented by the inclinations of Eqs. 10 and 11), it was possible to estimate the heat inactivation energy (E'_a) through the integrated form of Arrhenius equation *(17)*:

$$\text{Log } k_2/k_1 = [E'_a \times (T_1 - T_2)]/2.303 \times R \times T_1 \times T_2 \tag{12}$$

Thus, E'_a = 206.6 kJ/mol was calculated by introducing into Eq. 12 the correspondent values of k_1 (–0.0057 h^{-1}), T_2 (323K), T_1 (318K), k_2 (–0.019 h^{-1}),

Table 3
Variation in Residual Activity of EO, (U/mL) × 10^3, Against Temperature and Enzyme/Buffer Contact Time at Fixed Temperature[a]

Time (h)	Temperature					
	25°C	30°C	35°C	40°C	45°C	50°C
1	3.52/(–2.45)	3.91/(–2.41)	4.01/(–2.40)	3.99/(–2.40)	4.51/(–2.35)	3.55/(–2.43)
5	4.16/(–2.38)	3.75/(–2.43)	4.00/(–2.40)	3.86/(–2.41)	4.13/(–2.38)	3.05/(–2.52)
10	3.61/(–2.44)	3.44/(–2.46)	3.84/(–2.42)	3.77/(–2.42)	3.97/(–2.40)	2.63/(–2.58)
15	3.49/(–2.46)	3.31/(–2.48)	3.73/(–2.43)	3.73/(–2.43)	3.70/(–2.43)	1.99/(–2.70)
20	4.27/(–2.37)	3.28/(–2.48)	3.56/(–2.48)	3.61/(–2.44)	3.40/(–2.47)	1.55/(–2.81)
25	3.69/(–2.43)	3.26/(–2.49)	3.25/(–2.49)	3.56/(–2.45)	3.29/(–2.48)	1.35/(–2.87)
30	4.07/(–2.39)	2.81/(–2.55)	3.11/(–2.51)	3.56/(–2.45)	2.63/(–2.58)	1.28/(–2.98)

[a]Tests were carried out in a 1.2-mL cuvet of a spectrophotometer in which were added 0.2 mL of GO solution (4 mg/mL in 0.010 *M* acetate buffer, pH 5.5), 0.5 mL of glucose solution (0.8 mg/mL in 0.010 *M* acetate buffer, pH 5.5), and 0.3 mL of 0.010 *M* acetate buffer (pH 5.5). The logarithm of each residual activity is presented in parentheses.

and R (8.31 J/[K·mol]). The derived thermodynamic parameters, calculated through Eqs. 4–6, for the heat inactivation of GO were $\Delta G' = -9.46$ kJ/mol, $\Delta H' = 204$ kJ/mol, and $\Delta S' = 0.671$ kJ/(mol·K). An enthalpy of 204 kJ/mol indicates that GO inactivation probably went through the unfolding of its tertiary structure, because, as already proved *(18)*, such a mechanism occurs at $\Delta H'$ between 200 and 300 kJ/mol.

For temperatures below 318K the GO was quite stable, at least up to 30 h of submission to a fixed temperature (Table 3).

Effect of Glucose Concentration

The GO activities for various substrate concentrations are plotted in a Lineweaver-Burk graph (Fig. 1), from which $V_{max} = 75.2 \times 10^{-3}$ U/mL and $K_M = 29$ m*M* were calculated. The K_M values found in the literature vary from 10 to 33 m*M*, indicating that the value determined in our work fits quite well into the interval established for the GO from *A. niger (5,10,19)*.

Effect of H_2O_2

It can be seen from Fig. 1 that H_2O_2 acts as a reversible noncompetitive inhibitor *(17)*. The apparent V_{max} and K_M values for GO in the presence of H_2O_2 were 43.1×10^{-3} U/mL and 34.3 m*M*, respectively, whereas the inhibition constant (K_i), calculated by Eq. 13, was 1.22 m*M*:

$$v_i = (V_{max} \cdot [S])/(K_M + [S]) \cdot (1 + [I]/K_i) \quad (13)$$

in which v_i is GO activity in the presence of H_2O_2, [*S*] is the substrate concentration, and [*I*] is the inhibitor concentration.

A hypothesis to explain the reversible noncompetitive inhibition caused by H_2O_2 should consider that the Fe^{+2} is not part of the active site

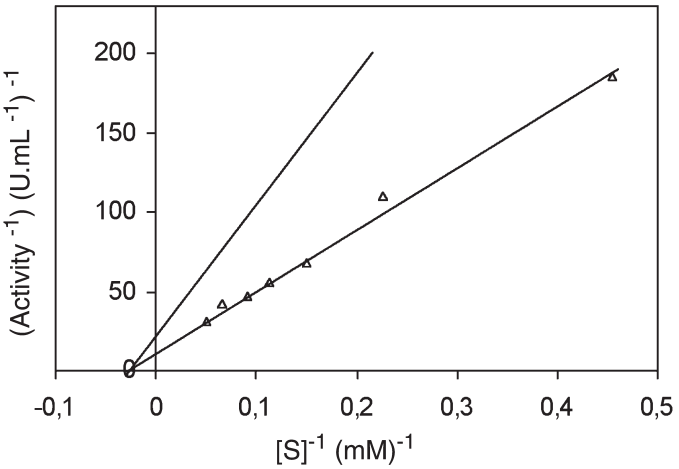

Fig. 1. Lineweaver-Burk plot for GO in (Δ) absence ($v^{-1} = 385.6 \times S^{-1} + 13.29$ [r = 0.993]) and (□) presence ($v^{-1} = 798.0 \times S^{-1} + 23.18$ [$r = 0.990$]) of H_2O_2.

of GO *(5)* and the occurrence of the reaction $2Fe^{+2} + H_2O_2 + 2H^+ \rightarrow 2Fe^{+3} + 2H_2O$. This reaction could be spontaneous, owing to the difference in the potentials of reduction in acidic medium presented by the pairs Fe^{+3}/Fe^{+2} (+0.771 V) and H_2O_2/H_2O (+1.77 V) *(20)*.

Membrane Bioreactor Tests

For illustrative purposes, the complete data related to tests 5, 9, and 10, in which the glucose conversion exceeded 50% (Fig. 2), are presented in Table 5. The mean conversion and productivity attained for all tests realized are presented in Table 4.

From Table 4, it is clear that the GO concentration, inlet glucose concentration, and feeding rate affected markedly the performance of the BEMR. The best result ($X_{mean} = 55\%$ and $r_{mean} = 12.4 \times 10^{-3}$ mmol/[h·mg$_E$]) occurred in test 5, which was conducted at 1.0 mg/mL of GO, 2.5 m*M* of glucose, and $D = 0.15$ min^{-1}.

Review of all the tests fed with 2.5 m*M* glucose (Table 4), except that carried out in the MSUC (test 10), reverted an up limit of 55% for the conversion attained (tests 5 and 9). This result would probably be owing to the inhibition promoted by the H_2O_2 on GO activity, as already referred. As can be seen from Table 5, the H_2O_2 concentration in the reaction medium ranged from 1.34 to 2.10 m*M*, agreeing well with the inhibitory concentration of 1.22 m*M* as predicted by K_i. Moreover, the residence times of 10 (D = 0.10 min^{-1}), 6.7 (D = 0.15 min^{-1}), and 5 min (D = 0.20 min^{-1}) employed were higher than the H_2O_2/GO contact time of 5 min, which was used in the inhibition study. Further research based on approaches such as increasing D and/or adding a peroxide-decomposing agent (e.g., catalase) in order to circumvent the inhibitory effect of H_2O_2 is necessary.

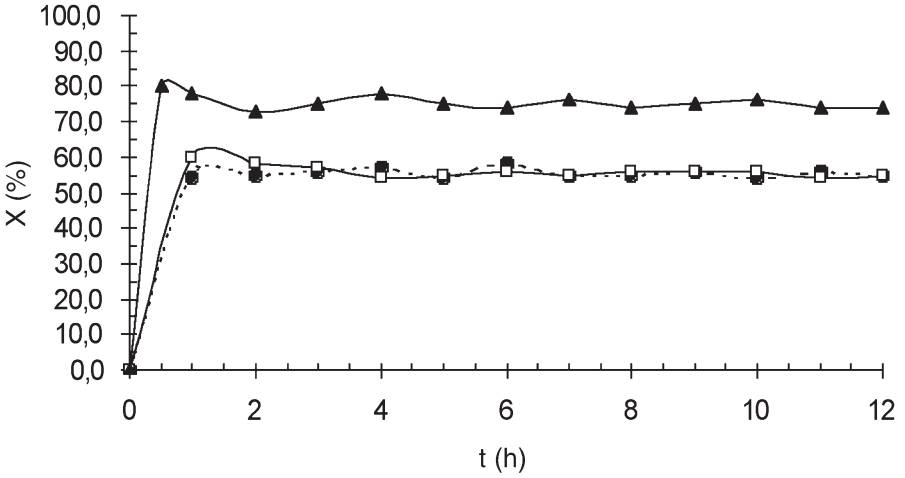

Fig. 2. Variation in conversion during continuous glucose oxidation catalyzed by GO for tests (•) 5, (□) 9, and (▲) 10. Tests 5 and 9 were conducted in the BMER, whereas test 10 was conducted in the MSUC.

Table 4
Conditions Under Which All Continuous Tests Were Conducted and Respective Average Conversion and Productivity Attained[a]

Test no.	[Glucose oxidase] (mg/mL)	[Glucose] (m*M*)	D (min^{-1})	X_{mean} (%)	r_{mean} (mmol/[h·mg_E]) × 10^3
1	1.0	5.0	0.10	26	3.60
2	1.0	2.5	0.10	45	7.60
3	2.0	2.5	0.10	44	7.00
4	2.0	5.0	0.10	25	3.10
5	1.0	2.5	0.15	55	12.40
6	1.0	2.5	0.20	46	10.0
7	2.0	2.5	0.15	41	8.90
8	2.0	2.5	0.20	40	11.6
9[b]	1.0	2.5	0.15	54	12.1
10[c]	1.0	2.5	0.15	75	46.0

[a]In all tests the pH, temperature, and agitation were maintained at 5.5, 30°C, and 100 rpm, respectively. Tests 1–8 were carried out in the BEMR coupled with a UF-regenerated cellulose membrane.

[b]The test was carried out in the BEMR using a UF polyethersulfone membrane.

[c]The test was carried out in the MSUC using a UF regenerated cellulose membrane.

It should be noted that tests 5, 9, and 10 had a quite stable steady-state phase by 10 h (Fig. 2), which corresponded to about 90 residence times, resulting in a significant operational performance for a continuous system *(21)*. More important, the performance of test 10 indicates that a fivefold scale-up allowed a period of stationary regime quite similar to that at small scale to be attained (Fig. 2). Furthermore, in test 10 the inhibitory effect of

Table 5
Variation in H_2O_2 Concentration, Conversion, and Productivity During Continuous Glucose Oxidation for Tests 5, 9 and 10[a]

	Test 5			Test 9			Test 10		
Time (h)	$[H_2O_2]$ (m*M*)	*X* (%)	*r* (mmol/[h·mg$_E$]) × 10^3	$[H_2O_2]$ (m*M*)	*X* (%)	*r* (mmol/[h·mg$_E$]) × 10^3	$[H_2O_2]$ (m*M*)	X (%)	*r* (mmol/[h·mg$_E$]) × 10^3
0	0	0	0	0	0	0	0	0	0
1	1.34	54	11.7	1.73	60	17.9	2.10	80	56.0
2	1.37	55	11.9	1.45	58	13.6	1.95	78	51.4
3	1.39	56	11.6	1.39	57	12.7	1.74	73	45.0
4	1.43	57	12.4	1.38	54	12.1	1.82	75	46.8
5	1.35	54	11.6	1.36	55	12.4	1.98	78	44.4
6	1.46	58	12.8	1.37	56	11.8	1.87	75	48.1
7	1.44	55	11.9	1.36	55	12.2	1.84	74	47.0
8	1.40	55	11.9	1.38	56	12.0	1.86	76	45.4
9	1.45	56	11.6	1.37	56	11.9	1.78	74	40.6
10	1.44	54	11.7	1.37	56	12.0	1.86	75	41.2
11	1.45	56	11.6	1.36	54	12.0	1.87	76	43.0
12	1.46	55	12.0	1.37	55	12.1	1.86	74	44.5
Mean	1.42	55	12.4	1.41	54	12.1	1.88	75	46.0

[a]Tests 5 and 9 were carried out in the BEMR, whereas test 10 was in the MSUC. In all tests the pH, temperature, and agitation were maintained at 5.5, 30°C, and 100 rpm, respectively.

H_2O_2 was minimized somewhat, because a conversion of about 75% (Fig. 2) and a mean productivity of 46.0×10^{-3} mmol/(h·mg$_E$) (Table 4) were attained. The different design of the MSUC compared with the BEMR could possibly explain this significant improvement in the performance of the process. Of course, these results stimulate further scale-up studies.

Finally, the type of UF membrane utilized, regenerated cellulose (test 5) or polyethersulfone (test 9), did not affect the performance of the process (Fig. 2). Indeed, this result could become useful when the oxidation of glucose is coupled in the same reactor with another bioconversion whose overall composition of the reaction medium has a less hydrophilic character, a case in which a UF polyethersulfone membrane would be more adequate.

Conclusion

The data presented lead to the general conclusion that the membrane bioreactor (either the Stirred Ultrafiltration Cell or Bioengineering System) is suitable for glucose oxidation by GO, as long as the inhibitory effect caused by H_2O_2 can be circumvented. As a starting point for scale-up studies, the following conditions could be taken into account: pH 5.5, 30°C, D = 0.15 min^{-1}, agitation of 100 rpm, 2.5 m*M* glucose, 1.0 mg/mL of GO, and DO between 6.5 and 7.0 mg/mL.

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