Bioelectrochemistry and Bioenergetics **6** (1979) 493–508
J. Electroanal. Chem. **104** (1979) 493–508

272 - Kinetic Model of a Depolarization-Type Bioelectrochemical Fuel Cell

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Revised manuscript received July 1st 1979

Summary

The present work concerns a depolarization type bioelectrochemical fuel–cell with a bioanode consisting of a *Micrococcus cerificans*/glucose resting–cell system. Its response is interpreted in terms of an oxygen supplying zone (electrode) and an oxygen–consuming zone (cellular mass) separated by the cell membrane areas. The influence of the substrate and microorganism concentrations in the kinetics of the overall process is evaluated, and the maximum efficiency for cell design is discussed.

Introduction

Biological fuel cells operate through the contribution of two electrochemical processes occurring simultaneously in different regions, one anodic and the other cathodic, with the participation of a living system either in both or in only one of them. The living system consists either of a growing cell suspension, or resting cells or an isolated enzymatic system.[1-6] According to the biofuel cell classification made by Gray Young *et al.*,[7] the depolarization type bioelectrochemical fuel cell uses either a microorganism (growing cell suspension or resting cells) or enzymatic systems to depolarize one half–cell by consuming the oxygen formed at the anode while air or oxygen is supplied to the other half cell.

For biological fuel cell design the determination of the quantitative relationships among the different kinetic parameters of the bioelectrochemical system is required.[8-18] The simplest type of biological fuel cell is the anodic depolarization biological fuel cell where the microorganism acts as a chemical depolarizer without interfering in the electrode processes.[7] This type of cell can be coupled to non–continuous fermentative systems.[19-21]

The aim of the present study is to interpret the mechanism of a biological depolarization type fuel cell in terms of a resistor model involving the interdependence of the biological and electrochemical parameters. A resting cell system is particularly suitable for this purpose taking into account that biological variables involved (cell and substrate concentration) can be easily controlled.

Experimental

The schemes of the H–type cell and of the auxiliary measurement circuits are depicted in Fig. 1. The cell is made of Pyrex glass with ground glass joints. The anodic and cathodic compartments locate platinized platinum electrodes isolated by cellulose acetate membranes. The black Pt electrodes (*ca.* 10.5 cm^2 apparent area) are anodized at 0.23 A during 10 min in 2 *N* H_2SO_4 at room temperature before each run. The composition of the electrolyte containing the cell suspension expressed in g/l is: NaCl (0.2); $MgSO_4 \cdot 7\,H_2O$ (0.2); KH_2PO_4 (1); K_2HPO_4 (2); Na_2HPO_4 (1). The electrolyte is buffered at pH 7.

The cell suspension of the anodic compartment is continuously pumped at 150 cm^3/min through an O_2–monitoring electrode.[22,23] The potential *vs.* current relationships were obtained under constant load discharge conditions. The current *vs.* time relationships were obtained by using a 1000 Ω external resistance between the anode and the cathode of the electrochemical cell. Under these circumstances it should be emphasized that, before each run, the working electrode pretreatment already referred was systematically repeated. Anodic and cathodic potentials are potentiometrically measured against saturated calomel electrodes (S.C.E.). The concentration of glucose is evaluated using the *o*–toluidine method and the number of viable microorganisms is determined by plate–counting methods.

The cells are cultivated at 30 °C on agar slant tubes with the addition of the electrolyte and yeast extract (5 g/l), $(NH_4)_2SO_4$ (1.5 g/l) and glucose (4 g/l). After 24 hr the cells are transferred to ERLENMEYER flasks under stirring. When the culture reaches the logarithmic region of the growth curve (4 to 5 hr), the cells are centrifugated and washed with the electrolyte before preparing the suspensions of different concentration.

The obligated aerobic *M.c.* strain is generally grown with insoluble carbon sources such as hydrocarbons,[24-29] With this media, however, the determination of the number of cells is inaccurate. The *M.c.* strain is then successfully adapted to grow with soluble carbon sources such as glucose in the presence of yeast extract. A resting cells system without the addition of yeast extract is employed to keep a controlled cell concentration and to avoid turbidity changes of the medium.[30]

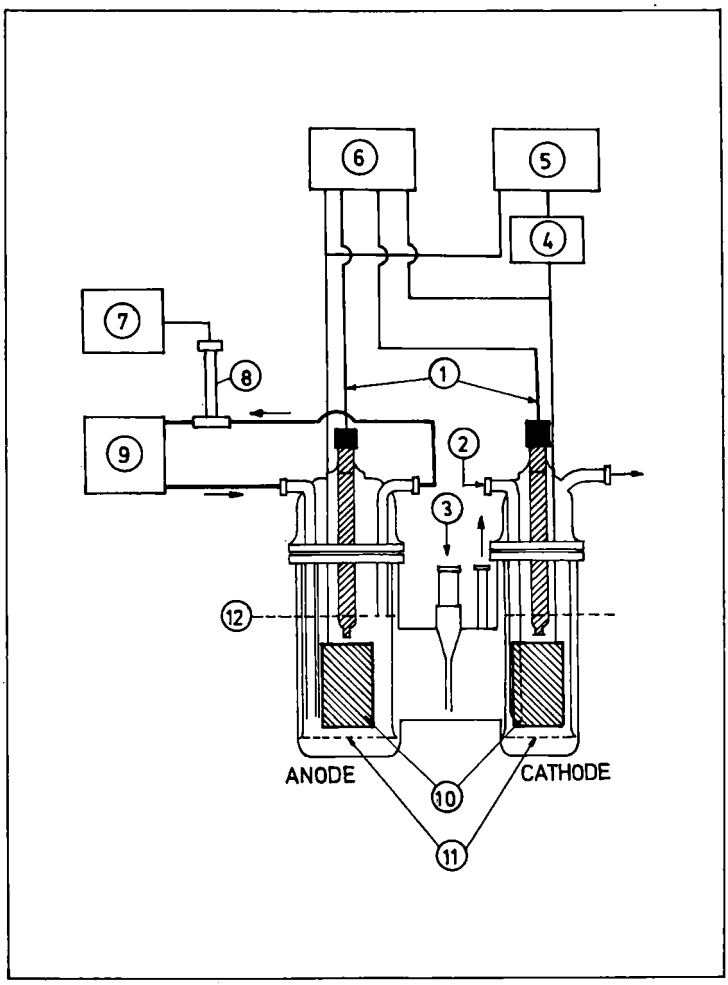

Fig. 1.
Experimental set-up of cell and measurement circuitry. 1 : Calomel electrodes ; 2 : Oxygen inlet ; 3 : Nitrogen inlet ; 4 : Charge resistor ; 5 : Ammeter ; 6 : Potentiometer ; 7 : *x–t* recorder ; 8 : Oxygen probe ; 9 : Peristaltic pump ; 10 : Platinized platinum electrodes ; 11 : Cellulose acetate membranes ; 12 : Electrolyte level.

Results

The galvanic cell containing the sterile medium without glucose (blank) attains a residual current (I_0) of *ca.* 10 μA. In the presence of glucose it decreases to 2 ± 0.8 μA remaining at this value for at least 24 hr. Under these circumstances the open circuit potential is 0.3 ±

0.015 V (*vs.* S.C.E.). The electrolyte saturated with O_2 at atmospheric pressure exhibits at 0.3 V an O_2 limiting current equal to 10 μA, which is taken as the 100 % O_2 threshold.

The resting cells are then added to the anodic compartment and left without glucose for 30 min (Fig. 2). Then, the experiment is initiated ($t = 0$) by adding different amounts of substrate to the system and following the changes of the electrochemical parameters and the O_2 concentration, the latter decreasing very fast. The current (I) reaches a maximum value (I_m) decreasing afterwards linearly when the resting cells consume glucose and when the latter is depleted, the current approaches exponentially that of the blank. Otherwise, after the glucose addition, the concentration of dissolved O_2 decreases to a low limiting value which depends only on the microorganism concentration and it

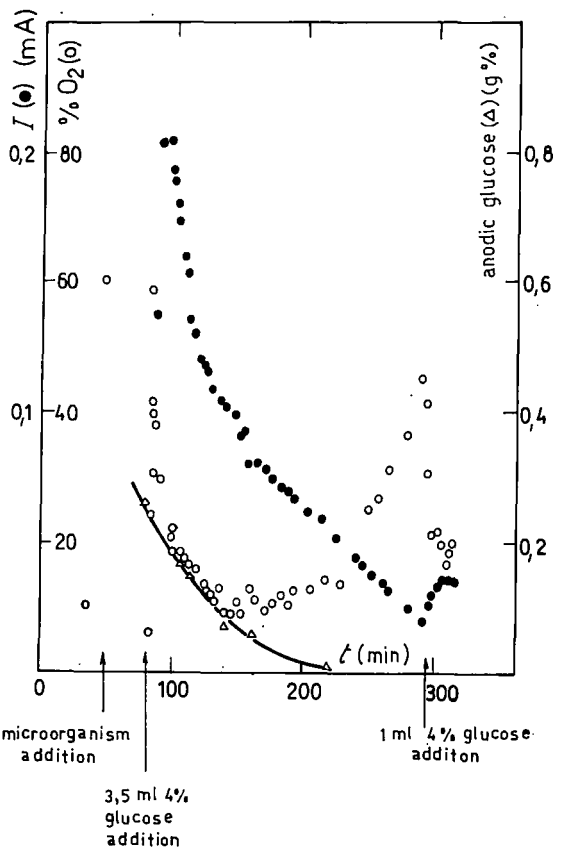

Fig. 2.

Time dependence of I (●), oxygen percentage (○) and anodic glucose concentration (Δ). Microorganism concentration : 3×10^7 cell/cm^3.

remains practically constant as long as glucose is present in the solution. When glucose is depleted, the O_2 concentration increases to reach *ca.* 50 % O_2 concentration at 294 min. At this point a new addition of glucose is made and immediately a decrease of the O_2 concentration is observed again. The anodic potential decreases as the current drawn from the biocell increases, otherwise, the cathodic potential remains practically unaltered.

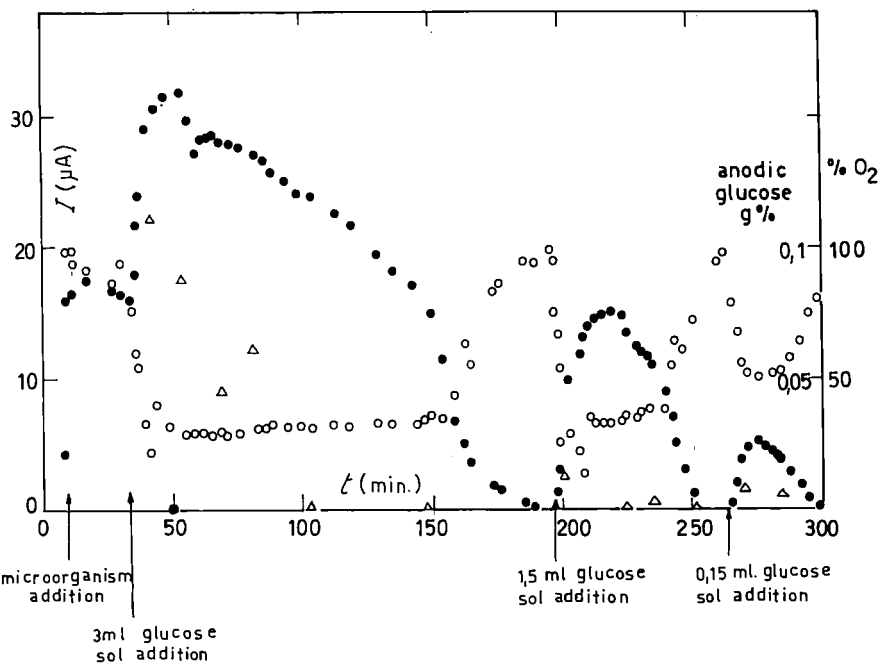

Fig. 3.
Repetitive experiments after successive glucose additions : (●) *I vs.* time ; (○) % O_2 *vs.* time ; (△) % anodic glucose *vs.* time. Microorganism concentration : 6×10^6 cell/cm^3.

Experiments made by adding new amounts of glucose to the exhausted medium show that in spite of the constant number of cells, the efficiency of the galvanic cell decreases after the successive glucose additions (Fig. 3). This is apparently related to the systematic damage of the biological material during the cell operation which cannot be attributed to a partial lysis of the cells.

The current drainage through the load resistance increases very remarkably when cells are added to the substrate as compared to the plain substrate solution. This clearly shows the importance of the biological agent in the performance of the electrochemical cell. Under each parti-

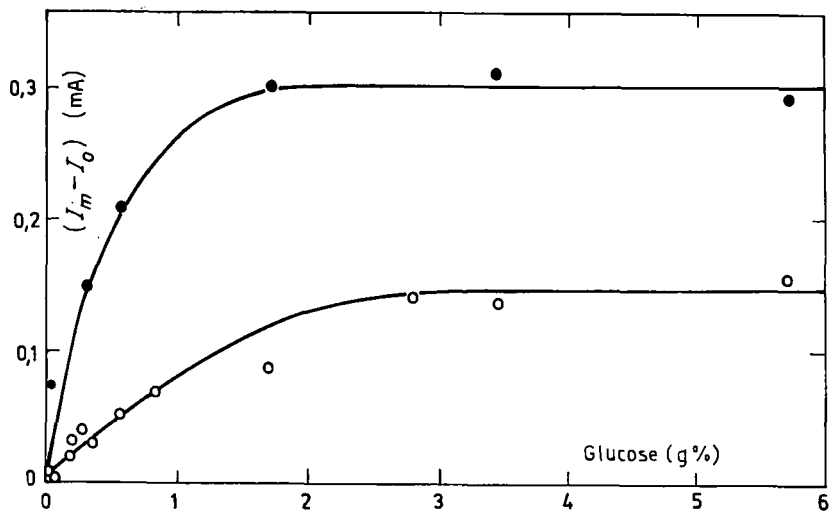

Fig. 4.
Maximum current (I_m–I_o) *vs.* glucose concentration. Microorganism concentration: (●) 1.4×10^8 cell/cm³; (○): 1.4×10^7 cell/cm³.

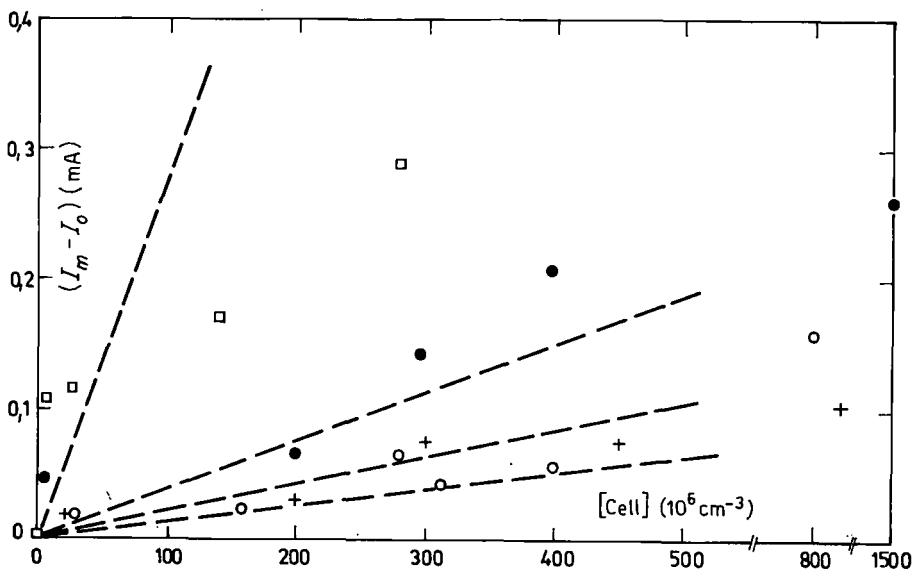

Fig. 5.
Maximum current (I_m–I_o) *vs.* microorganism concentration. Initial glucose concentration [g]: (○) 0.129 g %; (+) 0.250 g %; (●) 0.363 g %; (□) 3.5 g %.

cular set of experimental conditions, due to the gradual deterioration of the biological system, the maximal current value was taken as a measure of the electrochemical cell performance.

Other types of experiments are made, namely, those with a constant number of cells and a variable concentration of glucose and those with a constant glucose concentration and different microorganism concentrations (Figs. 4–5). To avoid irreproducibility derived from the different inocula a liquid culture previously obtained from a 24 hr culture tube at 30 °C is used. Control experiments show no appreciable cell damage in the diluted media before adding the cells to the bioanode compartment.

For a constant glucose concentration the current maximum and the rate of O_2 consumption depend directly of the microorganism concentration (Fig. 6).

After reaching I_m, a linear log I *vs.* time relationship is observed which holds until the O_2 concentration at the bioanode begins to increase due to glucose depletion. The time to attain the maximum current depends inversely on the glucose concentration and on the microorganism concentration (Table 1).

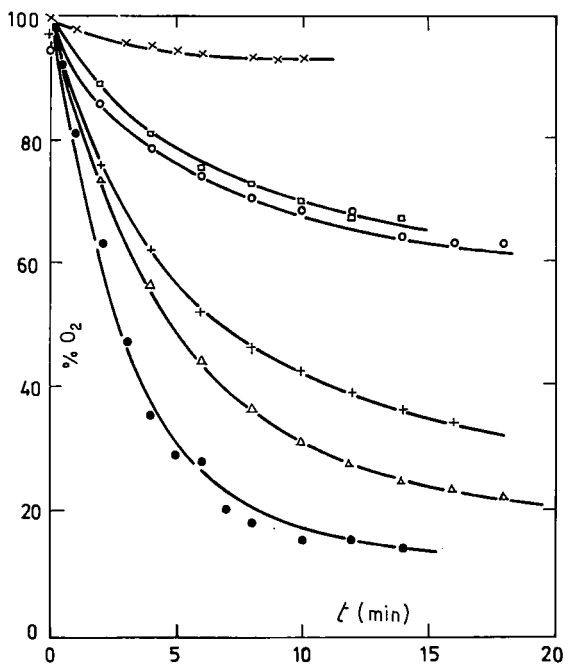

Fig. 6.

Time dependence of the oxygen concentration for different microorganism concentrations: (●) 1.5×10^9 cell/cm³; (△) 4×10^8 cell/cm³; (+) 3×10^8 cell/cm³; (○) 2.8×10^8 cell/cm³; (□) 2×10^8 cell/cm³; (×) 2.9×10^6 cell/cm³. Initial glucose concentration [G_0]: 0.363 g %.

Table 1. Time to reach I_m counted from $t = 0$ for different glucose and cell concentrations.

Initial glucose concentration (g %)	Cell concentration (cells/cm^3)	Time to reach I_m (min)
0.363	4×10^8	20
0.363	3×10^8	15.8
0.363	2.8×10^8	15.8
0.363	2×10^8	11.5
0.363	2.9×10^6	7.6
5.714	1.4×10^8	8.5
3.425	1.4×10^8	6
1.714	1.4×10^8	7.2
0.571	1.4×10^8	10
0.307	1.4×10^8	11
0.057	1.4×10^8	12

The current *vs.* potential (*I vs. U*) plot (Fig. 7) was obtained differently from the conventional polarization curves. In this case a constant charge resistor (1000 Ω) was used to record the *U vs. t* and the *I vs. t* displays. The pair of the instantaneous *I–U* values were read from the *U–t* plot and from the *I–t* plot at different *t*. The *I vs. U* plot, therefore, reflects the whole evolution of both the biological and the electrochemical systems. Thus, under working conditions the bioanode potential becomes more negative than the corresponding equilibrium potential. The *I vs. U* plot apparently shows a predominantly ohmic–type behaviour. The increase of microorganism concentration shifts the bioanode potential towards more negative potentials as it would be expected for a decrease of the oxygen concentration due to the presence of the microorganism. For a microorganism concentration lower than 5×10^7 cells per cm^3 the current depends linearly both on the glucose and on the microorganism concentrations (Fig. 8). For glucose concentrations lower than 1.5 g/l, I_m also depends linearly on glucose concentration (Fig. 4). At higher concentrations a saturation effect is observed indicating that the bioelectrochemical process is controlled by the microorganism concentration.

It is noteworthy that the cell response is only achieved whenever microorganisms, substrate and oxygen are present. Only under these circumstances the bioelectrochemical reaction takes place. This discards the possible discharge of glucose as being the responsible of the cell potential.[31]

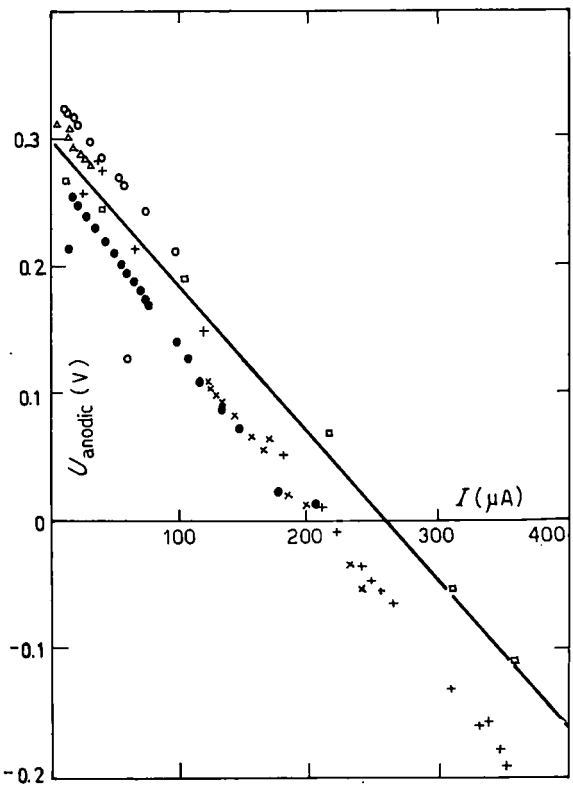

Fig. 7.
Anodic potential *vs.* I plot. (●) microorganism concentration : 3×10^7 cell/cm³, initial glucose concentration [g_0] : 0.26 g % ; (○) 9.6×10^6 cell/cm³ and 0.23 g % ; (△) 7.7×10^6 cell/cm³ and 0.28g % ; (+) 9.2×10^6 cell/cm³ and 0.6 g % ; (×) 3.5×10^8 cell/cm³ and 0.19 g %. (□) Estimated values.

Discussion

The possible kinetic control at the bioanode

The foregoing results show that at the bioanode the current output involves both glucose and O_2 consumption and depends on the cell per cm³ N and glucose concentration [g]. The overall anodic process involves the rate of transport of g and O_2 through the different physical and biological interfaces. The complementary reaction at the cathode is attributed to the electroreduction of O_2.

From the kinetic standpoint the bioelectrochemical process at the bioanode involves three regions, namely, the electrode|electrolyte interface acting as the electrochemical O_2 supply region, the microorganism

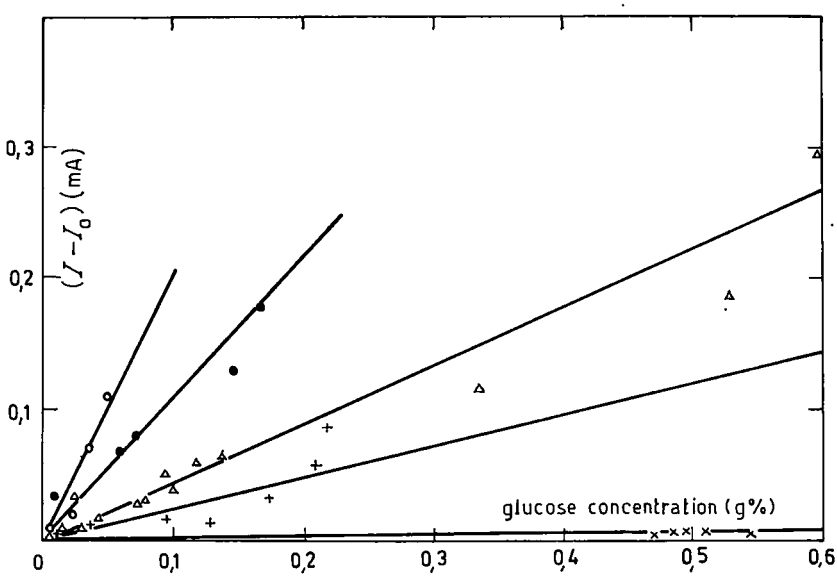

Fig. 8.
Current substrate concentration relationships. Microorganism concentration : (×) 2×10^6 cell/cm³ ; (△) 9.2 to 9.6×10^6 cell/cm³ ; (+) 7.7×10^6 cell/cm³ ; (●) 3×10^7 cell/cm³ ; (○) 3.5×10^8 cell/cm³.

mass which is the O_2 consumption region and the cell membranes. The substance itself cannot be considered responsible for the current production because in the absence of the bacteria practically no current is produced.[31] Under limiting circumstances the anodic process may be rate controlled either by the electrochemical reaction or by the biological process itself. Under intermediate conditions the cell behaves as an oxygen concentration type cell where the electrochemical reaction at the anode is assisted by a biological agent (M). Thus,

$$2\,OH^- + M \overset{1/R_1}{\rightleftharpoons} H_2O + \frac{1}{2}\,O_2 + M' + 2\,e^- \qquad (1)$$

and for the cathode :

$$\frac{1}{2}\,O_2 + H_2O + 2\,e^- \rightleftharpoons 2\,OH^- \qquad (1')$$

where M yields M′ during the anodic reaction and $1/R_1$ represents the reaction rate of the anodic step (1) and R_1 stands for the specific reaction resistance. The latter consists at least of the transport of the reactive species from the bulk of the solution towards the electrochemical interface

at the rate v_1, the proper electrochemical reaction yielding O_2 at the rate v_2, and the transport of reaction products from the electrochemical interface outwards at the rate v_3. If either v_1 or v_3 are smaller than v_2, the rate of the reaction (1) becomes rate-determining by the transport of reacting species either from or towards the electrochemical interface.[32]

The biological process involves the transport of O_2 and g through the cellular membrane to the interior of the cells. These transport processes occur through the two interfaces of the cellular membrane. Thus, at the outer solution|membrane interface O_2 and g are transferred from the solution, namely :

$$O_2 \text{ (solution)} \overset{1/R_2}{\rightleftharpoons} O_2 \text{ (membrane)} \tag{2}$$

$$g \text{ (solution)} \overset{1/R_3}{\rightleftharpoons} g \text{ (membrane)} \tag{3}$$

The transport of g (R_4) and O_2 (R_5), through the cellular membrane is produced by the corresponding concentration gradients across the membrane. The glucose transport is probably related to a facilitated diffusion process.[33,34] At the membrane|inner solution interface the corresponding transport phenomena can be expressed as follows :

$$O_2 \text{ (membrane)} \overset{1/R_6}{\rightleftharpoons} O_2 \text{ (cell interior)} \tag{4}$$

$$G \text{ (membrane)} \overset{1/R_7}{\rightleftharpoons} G \text{ (cell interior)} \tag{5}$$

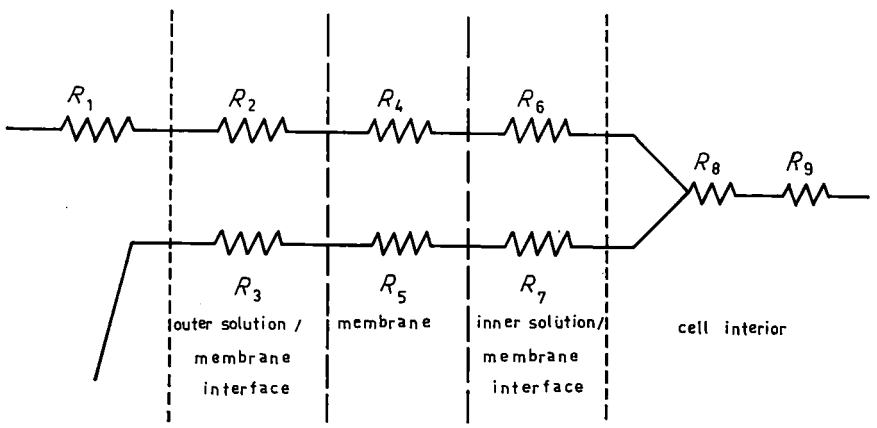

Fig. 9.
Simple equivalent circuit for the bioanode.

Finally, inside the cell, the g oxidation is biologically accomplished. The total biological reaction is :

$$g + O_2 + \text{Enzimatic system} \overset{1/R_8}{\rightleftharpoons} \text{Oxidation products}, \qquad (6)$$

the reaction products diffusing out :

$$\underset{\text{(cell interior)}}{\text{Oxidation products}} \overset{1/R_9}{\rightleftharpoons} \underset{\text{(cell exterior)}}{\text{Oxidation products.}} \qquad (7)$$

Therefore, the bioelectrochemical reaction can be conceived as the sum of successive stages which are represented by an equivalent electrical circuit (Fig. 9). The current output through this circuit depends on the sum $\sum_i R_i$, including R_c, the internal galvanic cell resistance. The kinetic analysis involves various limiting cases which are considered below disregarding for the moment any ohmic–type polarization.

When $R_1 \gg \sum_{i=2}^{n} R_i$, the rate of the reaction at the bioanode is the rate determining step and this implies either a net electrochemical activation control or a diffusion control due either to the reacting species or to the reaction products. In the former case, the rate of either reactions (1) or reaction (1′) fit the kinetic laws of activated processes namely :[23]

$$I_a = k' A \; c_M{}^x \; c_{OM}{}^y - \theta \exp\left(\frac{\alpha_a F \eta_a}{RT}\right) \qquad (8)$$

or

$$I_c = k'' A \; (1 - \theta) \; p_{O_2}{}^z \exp\left(\frac{\theta_c F \eta_c}{RT}\right) \qquad (9)$$

respectively, where k' and k'' are formal rate constants, A is the electrode area, the η's are the charge transfer overpotentials referred to the O_2 reversible electrode, the α's are the transfer coefficients assisting the reaction either in the anodic (a) or in the cathodic (c) direction, θ is the degree of electrode area available, the c's refer to the concentrations at the electrode surface of the species indicated by the subindices, p denotes the oxygen gas pressure ad x, y and z are the corresponding reaction orders. The charge transfer overpotentials, in the case of the oxygen electrode are the kinetically deciding parameters.

If $R_1 \ll \sum_{i=2}^{n} R_i$, the resistance of the electrochemical O_2 production is smaller than the resistance of any of those processes involving the O_2 consumption by the biological system. Then, the metabolic characteristics of the biological species regulates the O_2 level in the system.

Under quasi–stationary state the amount of O_2 generated at the electrode equals the amount of O_2 incorporated to the cellular mass. Therefore, the corresponding fluxes, respectively $\boldsymbol{J}_{O_{2,e}}$ and $\boldsymbol{J}_{O_{2,c}}$ are equal. Under these circumstances the rate of O_2 electroformation can be expressed by means of the simple convective–diffusion equation :

$$\boldsymbol{J}_{O_{2,e}} = D_{O_2} \frac{c_{O_{2,e}} - c_{O_{2,s}}}{\delta_N} = \boldsymbol{J}_{O_{2,c}} \tag{10}$$

where $c_{O_{2,e}}$ is the O_2 concentration at equilibrium determining the bio-anode potential, $c_{O_{2,s}}$ is the saturation concentration and δ_N is the apparent diffusion layer thickness. Independently of the controlling step, the O_2 and the g fluxes at the outer solution |membrane interface, at the membrane|inner solution interface are equal :

$$\boldsymbol{J}_{R_2} = \boldsymbol{J}_{R_4} = \boldsymbol{J}_{R_6} = \boldsymbol{J}_{R_8} = \boldsymbol{J}_{R_9} \tag{11}$$

These fluxes may be expressed by an equation such as :

$$\boldsymbol{J}_i = k_i A' \Delta c_i \tag{12}$$

where A' is the total membrane area available which is proportional to N, and k_i is the permeability coefficient of the i–species

$$k_i = \frac{D_i \beta_i}{l} . \tag{13}$$

where D_i is the diffusion coefficient at the membrane ; β_i is the partition coefficient, and l is the average membrane thickness.

In the interior of the cell, the combustion reaction is a function of both the cellular g concentration and the O_2 concentration. For the overall process, expressed by $\boldsymbol{J}_8$, independently of the consecutive processes of g incorporation and oxidation, it can be assumed that the enzyme system involved in reaction (8) is in the neighborhood of the membrane. Then, $c_{g,\text{cell}} = c_{g,e}$ and the total reaction pathway can be concisely expressed by

$$g + E + \frac{1}{2} O_2 \rightleftarrows gE + \frac{1}{2} O_2 \rightleftarrows E + \text{Products.} \tag{14}$$

where E represents the enzymatic system participating in the glucose oxidation.

The cellular combustion fits the MICHAELIS–MENTEN equation :

$$\boldsymbol{J}_8 = \frac{N \boldsymbol{J}_{8,\max} c_{g,e}}{K_M + c_{g,e}} \tag{15}$$

where K_M is the reciprocal of a complex affinity constant involving g input and conversion. Hence, under a quasi-stationary state $\boldsymbol{J}_8 = \boldsymbol{J}_{O_2}$ and the rate of O_2 production at the electrode is controlled by the conversion of g to products in the interior of the cell.

Kinetic interpretation of the cell response

The steady current, I, floxing through the cell can be expressed either in terms of O_2 production or cellular combustion :

$$I = n\boldsymbol{F}A\,\boldsymbol{J}_{O_2} = \frac{N\,\boldsymbol{J}_{8,\max}\,c_{g,e}}{K_M + c_{g,e}} \tag{16}$$

where n is the number of charges per mole of reacting species, and $\boldsymbol{F}$ is the FARADAY constant. For the system involving a large N, before g addition $c_{g,e} = 0$, $\boldsymbol{J}_8 = 0$, $I = 0$ and $c_{O_2,e} = c_{O_2,i}$. After g is added, the microorganism consumes O_2 and an O_2 gradient is established. Then I is given by :

$$I = n\boldsymbol{F}AN\,k_{O_2}\,\Delta c_{O_2}. \tag{17}$$

Depending on the amoung of g added, $c_{g,e} \gtrless K_M$. When $c_{g,e} \gg K_M$, I_m, independently of the g concentration, $\boldsymbol{J}_8 = \boldsymbol{J}_{8,\max}$ and $\Delta c_{O_2} = \text{const.}$ When $c_{g,e} \ll K_M$, I depends on the g concentration and, under steady state conditions, it is linearly related to c_g.

For a constant N, once g is depleted, a new addition of g implies that the current again increases to attain a new steady state. Then, the shorter the time required for attaining I_m, the larger the $[g]/N$ ratio. For a particular g concentration, the slope of the percentage of dissolved O_2 *vs.* time relationship depends only on N and the slope of the I_m *vs.* N plot depends on the external g concentration. If the current output is low and the O_2 uptake by the microorganism becomes larger than the contribution of the electrochemical reaction the rate is controlled by the dissolved O_2 concentration. When N is large, the O_2 transport from the electrode or the g transport to the cellular mass results scanty influenced by the viscosity variation, since the latter enters the kinetic equation just with a 1/6th exponent.[32]

A deviation from the linear relationship when N is high may probably be due to a diminution of the active membrane area (A') by the increasing cell contacts which affects directly the g and O_2 transport. This effect is partially compensated by stirring.

The total overpotential (η_T) of the working biocell can be expressed as the sum of the charge transfer (η_a), concentration (η_c) and ohmic (η_o) overpotentials. For the ohmic cell resistance, however ($R_c = 1500\ \Omega$), the biological fuel cell response depends markedly on the ohmic overpotential. For low values of R_c the current depends linearly on the microorganism concentration and on the concentration of the oxidizable substrate. On the other side, each biological species has its own maximum

rate of consumption at a critical concentration. Therefore, to achieve such a situation when designing a depolarization type bioelectrochemical fuel cell, there is an optimal cell concentration for which the cell membrane area restriction by contact between microorganism is avoided. For this type of depolarization bioelectrochemical fuel cell the oxidizable substrate must produce under its metabolic pathway the larger oxygen uptake.

Acknowledgments

The Institute (INIFTA) is patronized by the following Institutions : Universidad Nacional de La Plata, Consejo Nacional de Investigaciones Científicas y Técnicas and Comisión de Investigaciones Científicas (Pcia. de Bs.As.). This work was partially supported by the Regional Program for the Scientific and Technological Development of the Organisation of the American States.

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