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Analytical Measurements in Metabolic Changes of Bacterial Medium

M. SCRIPCARIU*, S. FLESCHIN**, A.A.BUNACIU**, V. MAGEARU**

**Drug Development Laboratory, Sos.Dudului, Nr.37, Bucharest-6, Romania.*
***Department of Analytical Chemistry; Faculty of Chemistry; University of Bucharest; 90, Sos Panduri, Bucharest 5, Romania*

Abstract

Continuous monitoring of a bacterial culture for pH, growth CO_2 and NH_3 in microbiological medium. Changes in the metabolic parameters of Proteus sp. cultures generally occurred about one or two hours before changes in growth were observed. The time of maximum CO_2 production preceded that of NH_3 elaboration by this organism. This type of monitoring system has great potential for the study of the metabolism of growing organisms as well as for the early detection of growth in liquid culture. Studies of the protozoan organism have suggested that the major source of substrate for the growth and energy production of the organism may be amino acids rather than carbohydrates. In such studies of the metabolism of microorganism, frequent determinations are necessary and the usual technique requires periodic sampling of the material under study.

These methods have the disadvantage of altering the volume of material, thus disallowing the use of small volumes. In order to obviate the sampling problems it is possible to utilize sensitive electrodes in "in situ" monitors. These electrodes also have the advantage of giving a continuous real time output of metabolic changes. Before applying this technique to an organism, it was necessary to prove its feasibility by monitoring growing cultures of an organism which has been well studied. The Proteus sp. organism is known to contain a very active urease and therefore, produces large amounts of ammonia in the presence of urea. The system is based a commercially available components and is simple for the monitoring if the carbon dioxide (CO_2), hydrogen ion activity (pH), ammonia and growth of organisms as measured by optical density. This system demonstrates a potential for wide application to a variety of microorganisms or other types of cell culture.

Materials and Methods

Apparatus

In these experiments the following electrometers were used: Orion model 801 I Beckman pH – 1, digital pH-meter, in conjunction with recorders, Texas Instruments. Ammonia electrodes were obtained from the Orion Research (95-10), combination pH electrodes from Corning (model 476050) and CO_2 electrodes from Orion.

A 250 ml Erlenmeyer flask was modified with two side holes, for electrodes. Trypticase soy broth (TSB) was purchased from Difco (lot 0670-01).

Growth of cells was monitoring by measuring the change in absorbance at 670-660 nm.

Potentiometric measurements for monitoring metabolic changes

Since the results desired in these experiments were the changes of NH_3 contents (NH_3 + NH_4^+) or CO_2 content (HCO_3^- + H_2CO_3) produced with time, direct measurements of electrode potential in millivolts required correction. Since the pH was changing as the cultures grew, the amount of NH_3 and CO_2 reaching the electrodes could change without a change in production by the growing organisms. The equation for the ammonia electrode was as follows:

Let

$$N = (NH_3)_q + NH_4^+ \quad (1)$$

$$E = E_o - S \log [P_{NH3}] \quad (2)$$

$$P_{NH3} = K_s \cdot (NH_3)_g \quad (3)$$

$$pK = pH + \log ([NH_4^+]/(NH_3)_g) \quad (4)$$

from eq. (4) it can be sown that

$$NH_4^+ = e^{2,303\,(pK - pH)}\,(NH_3)_g$$

and from eq. (2) and (3)

$$(NH_3)_g = (e^{2,303\,(Eo - E)/S})/\,K_S$$

therefore

$$N = [(e^{2,303\,(Eo - E)/S})/\,K_S] / [1 + e^{2,303\,(pK - pH)}] \quad (5)$$

and the change in N from initial condition (N_i) when the culture in inoculated in any time (N_t) is

$$\Delta N = N_t - N_i = \{[(e^{2,303\,(Eo - Et)/S})/\,K_S] / [1 + e^{2,303\,(pK - pHt)}]\} - \{[(e^{2,303\,(Eo - Ei)/S})/\,K_S] / [1 + e^{2,303\,(pK - pHi)}]\} \quad (6)$$

when

pK = $- \log K_{eq}$
pH = the pH at time t
pHi = initial pH
E_t = the electrode potential at time t
E_i = the electrode potential initially
E_o = the electrode constants in the TSB
S = Electrode slope
K_S = the solubility of NH_3 in water at 25^0C (moles/liter)

The slope measured as the difference in potential caused by a tenfold change in $(NH_3)_g$ and pH_i, pH_t and E_t. The pK was taken to be equal to 9,33 and k_S to be 18,8 moles/liters. E_o is possible to be evaluated in the TSB by two different methods:

1. By determination of N, with ammonia test, and E_o was calculated by rearranging (5)

$$E_o = E + S/2{,}303[\ln (N \cdot K_S) - \ln (1 + e^{2{,}303\,(pK-pH)})] \quad (7)$$

2. By standard additions. PH and potential reading in 100 ml of TSB were taken before and after addition 1M NH_4Cl.
   The initial N was calculated as follows: substrate eq. (2) at the initial condition from eq. (2) after the addition of NH_4Cl and rearrange:

$$e^{2{,}303(E2 - E1)/S} = P_{NH3\,(1)}/P_{NH3\,(2)} \quad (8)$$

from eq. 3

$$P_{NH3\,(1)}/P_{NH3\,(2)} = (NH_3)_{g1}/(NH_3)_{g2} \quad (9)$$

combination eq. (1) and eq. (4) gives:

$$N = (NH_3)_g\,[1 + e^{2{,}303\,(pK-pH)}] \quad (10)$$

substitution of eq. (9) and (10) into (8) and rearranging gives:

$$N_1/N_2 = [(1 + e^{2{,}303\,(pK - pH2)}/ (1 + e^{2{,}303\,(pK - pH1)}] \cdot 1/ e^{2{,}303(E2 - E1)/S} \quad (11)$$

but $N_2 = N_1 + \Delta N$ and therefore:

$$N_i = \Delta N / \{[(1 + e^{2{,}303\,(pK - pH2)}/ (1 + e^{2{,}303\,(pK - pHi)}] \cdot 1/ e^{2{,}303(E2 - Ei)/S} - 1\} \quad (12)$$

when
N_i, pH_i and E_i are the initial conditions,
S is the electrode slope,
$pK = - \log K_{eq}$,
pH_2 and E_2 are the condition after addition of ΔN amount of NH_4Cl.
For the CO_2 electrodes eq. (1)÷ (4) become:

$$C = H_2CO_3 + HCO_3^- \quad (13)$$
$$E = E_o + S \log [P_{CO2}] \quad (14)$$
$$P_{CO2} = K_S \cdot H_2CO_3 \quad (15)$$
$$pK = PH - \log [HCO_3^-/ H_2CO_3] \quad (16)$$

with similar derivation as those for ammonia electrode:

$$\Delta C = \{[(e^{2{,}303\,(Et - Eo)}/S)/K_S] \cdot [1+1/e^{2{,}303\,(pK - pHt)}]\} - \{[(e^{2{,}303\,(Ei - Eo)}/S)/K_S] \cdot [1+1/e^{2{,}303\,(pK - pHi)}]\} \quad (17)$$

and

$$E_o = E_1 - (S/2{,}303)[\ln (c \cdot K_S) - \ln (1 + 1/e^{2{,}303\,(pK - pH)})] \qquad (18)$$

Preliminary Results

Stability of Electrode Probes

The few probes were placed in various liquids and monitored to determine their stability. The results are shown in **Table 1**.

Table 1.

Type of probe	Time (h)	Solution used	Range observed
optical	18	dionized H_2O	0,02 A
CO_2	17	HCO_3^- - CO_2	3 mV
PH	16	TSB	7,20 ÷ 7,16
NH_3	15	TSB	4 mV

Conclusions

In a few experimental data, obtained from the growing cultures two technical problems were encountered; the seal of the ammonia electrode was not tight enough and the culture media entered the internal filling solution of the electrode. The second difficulty was ensuring the sterility of the system and is possible to test a newly designed culture vessel for longer-term studies.

With the development of other sensing electrodes and the use of enzyme coated electrodes, the ability to monitor the metabolism of bacterial cells, will be limited only by the physical size of the electrodes and the data handling required. It is expected that this technique will find a number of applications in studying the metabolism of organisms and cells in culture.

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