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APPARENT OXIDATION-REDUCTION POTENTIAL, ACID PRODUCTION, AND POPULATION STUDIES OF LACTOBACILLUS ACIDOPHILUS UNDER ANAEROBIC CONDITIONS

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In the two earlier articles by the authors (1935, 1936) investigations, on the growth of Lactobacillus acidophilus, were described in which the pH of the medium was kept constant at any desired value by means of an automatic regulator, and in addition the temperature and the composition of the gas mixture saturating the medium were controlled. The progress of the growth was followed in the later experiments by measurements of the acid production, apparent oxidation-reduction potentials ($E_a$ values), and the bacterial population. Some interesting inter-relations of these measurements were observed for which the second paper of this series may be consulted. In particular a connection was found between the $E_a$ value and the rate of acid production. The $E_a$ values were, however, found to be much affected by the presence of oxygen in the saturating gas.

It was considered, therefore, desirable to continue the investigations to include studies of bacterial growth under anaerobic conditions. However since it has been shown that carbon dioxide is essential for such growth, it was necessary to prepare an inert gas (nitrogen) free from oxygen but containing carbon dioxide. The method for obtaining such mixtures will first be described, together with an outline of changes made in the design of the culture flask. This will be followed by a discussion of the results of experiments, on the growth of L. acidophilus under
anaerobic conditions with automatic control at pH 6.0 during
which measurements of oxidation-reduction potentials, acid for-
matron, and population were carried out.

EXPERIMENTAL ARRANGEMENTS FOR ANAEROBIC
GROWTH

The method used for preparing oxygen-free mixtures of nitro-
gen and carbon dioxide depends upon the decomposition of solid

$$2 \text{NaHCO}_3 = \text{Na}_2\text{CO}_3 + \text{H}_2\text{O} + \text{CO}_2$$  \hspace{1cm} (I)

sodium bicarbonate at moderately high temperatures according
to the equation.

The tank nitrogen is first passed through a quartz tube containing
finely divided copper at 500°C. to remove the 0.2 per cent of
oxygen present as impurity. The gas then passes into the top
of a two-liter Pyrex bottle, shown in figure 1, where it is spread
by the cotton plug C before passing through the layer of sodium
bicarbonate, $S$, and finally passes through another cotton plug $C'$ before leaving through the tube $T_1-T_2$. The gas then flows to the culture flask as described in previous papers.

On its passage through the bicarbonate the nitrogen picks up carbon dioxide and water vapour, the latter being subsequently condensed out of the mixture. With a gas stream of 3 liters per hour and the bicarbonate at a temperature of 79.8°C., maintained by use of an oil thermostat, the resulting gas mixture contained 3 per cent of carbon dioxide. This percentage depends upon the rate of flow since it is considerably below the equilibrium value for reaction I at this temperature, as determined by Caven and Sand (1911). Experiment showed, however, that the composition of the issuing gas is quite constant over long intervals of time if the temperature and rate of flow are held constant. Precaution must be taken to prevent the moisture from condensing in the bottle and "caking" the solid reagents. By manipulation of the stopcocks $a$ and $b$ of figure 1, it is possible to by-pass the stream of nitrogen through the tube $T_2-T_3$ and at the same time relieve accumulated pressure in the bottle $B$ through the tube $T_4$. This arrangement was used in a test, to be described later, of the growth of the organism with and without carbon dioxide.

The oxygen-free mixtures of carbon dioxide and nitrogen pass into the modified culture flask shown in figure 2 through a sterile cotton plug in the tube $O$, enter the medium at the point $T$, and escape through a cotton plug inserted in tube $A$. In our early measurements of $E_h$, using the flask described in the first paper of this series and gas mixtures containing about 0.2 per cent of oxygen, it was observed that these potentials frequently rose a few millivolts for a short interval after each withdrawal of

1 It may be observed, by comparison of figure 2 of this paper with the corresponding figure of our first paper (1935), that three ground glass joints have been eliminated in the later model. This has reduced the cost of the apparatus and has increased the ease with which it may be sterilized and manipulated. The conical surface $E$ fits into the apparatus (figure 2 of our second paper (1936)) used for sterilization and insertion of the electrodes, and provision has been made in the hard rubber stopper, $R$, (figure 2 of the present paper) for separating the glass electrode $G$ from the gold, Au, and platinum, Pt, electrodes so that the metal electrodes may be cleaned with hot nitric acid.
a sample. This was probably due to the atmospheric oxygen which was drawn into the culture flask by the removal of the sample or was able to diffuse into the flask against the counter current of gas escaping through this opening. This contamination with atmospheric oxygen has been eliminated in the experiments reported in this paper by constricting the opening as shown at A, figure 2, with the result that the velocity of the gas escaping through this outlet is greatly increased. Samples are now taken from the culture flask with the aid of a pipet whose tip is ground to fit a stainless steel needle having a diameter somewhat less than that of the capillary outlet. Moreover, the sampling pipet is filled at a lower rate than that at which gas is being
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passed into the flask so that the liquid drawn from the flask is replaced by this gas and not by air drawn in through the opening A. The adoption of this technique has resulted in smoother $E_h$ time curves than those obtained in previous work.

Complete absence of the diffusion of atmospheric oxygen through the oil seal B provided for the stirrer S, figure 2, would be difficult to prove. However, it was observed that by allowing the gas entering at $T$ to escape through this oil, thus providing a counter current to such diffusion, no effect was observed on the $E_h$ measurements. Possible contamination with the oxygen dissolved in the solution of alkali that is added at intervals in controlling the pH has been eliminated by de-aeration of this solution with pure nitrogen, after which it was protected from atmospheric oxygen and carbon dioxide with the aid of a nitrogen-filled, collapsible, gas-tight bag.

EFFECT OF CARBON DIOXIDE ON L. ACIDOPHILUS CULTURES

Having taken the precautions described in the preceding section for the anaerobic growth of L. acidophilus in the presence of carbon dioxide, it seemed desirable to obtain additional confirmation of the necessity of this gas. Consequently a culture medium, through which pure nitrogen was passing, was inoculated in the usual manner with 0.07 per cent of a 17-hour culture. For 22 hours after inoculation there was no evidence of activity on the part of the culture. By turning stopcocks $a$ and $b$ of figure 1 an oxygen-free mixture of nitrogen and carbon dioxide (3.5 per cent) was then substituted for the nitrogen, and after an unusually long lag period the culture developed in a normal manner. The lag period, counted from the time of inoculation, was 32 hours, or 10 hours if counted from the time at which carbon dioxide was introduced. Had carbon dioxide been present at the time of inoculation, the lag period would have been only about 1 hour. It is not possible from this experiment alone to decide whether the long lag period following the introduction of carbon dioxide into the culture was due to a loss of viability on the part of the inoculum, or whether lack of carbon dioxide
during the first 22-hour interval induced the transition of the bacteria of the inoculum into a resting phase from which they slowly recovered in its presence.

Depriving a fully established culture of carbon dioxide has no appreciable effect upon the subsequent course of the growth cycle. Thus in one experiment, pure nitrogen was substituted for the nitrogen-carbon dioxide mixture 30 hours after inoculation. Following this alteration of the gas phase the acid production curve falls slightly below those for experiments in which the culture was not deprived of carbon dioxide but the differences are small. Gladstone, Fildes, and Richardson (1935) have emphasized, however, that an actively metabolizing culture may be producing carbon dioxide faster than it can be swept out. The bicarbonate ions which are present in solution following the original equilibration of the medium with the N₂–CO₂ mixtures also act as a reservoir of CO₂.

**APPARENT $E_a$ VALUES DURING ANAEROBIC GROWTH**

Our early measurements of the apparent oxidation-reduction potentials in bacterial cultures were made with the aid of gold electrodes. It was shown (1936) that two such electrodes agree closely with each other throughout an experiment. Since, however, many $E_a$ measurements (Hewitt (1935)) have been made with platinum electrodes one of the gold electrodes was replaced by platinum, as shown in figure 2, in order to make a direct comparison of the behavior of the two metals.

When placed in the medium as it comes from the sterilizer the gold potential, $E_a$ (Au), is usually about 250 mv. positive, using a value of +246 mv. for the potential of the 3.5 N calomel electrode against the normal hydrogen electrode. The platinum, apparently more responsive to the atmospheric oxygen dissolved in the medium, gives a value of +400 to +500 mv. During preliminary saturation with the nitrogen-carbon dioxide mixtures $E_a$ (Au) drops to about zero, which appears to be a value characteristic of the de-aerated sterile medium (compare, for example, Coulter (1928–29)). If the equilibration continues long enough, i.e., 2 days, the $E_a$ (Pt) also approaches this value.
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TABLE 1

Apparent oxidation-reduction potentials and acid production observed in a typical experiment* on the anaerobic growth of L. acidophilus with automatic pH control.

Temperature, 38°. pH, 6.0. Medium: 2 per cent neopeptone; 8 per cent galactose. Gas: CO₂ 2.6 per cent; N₂ 97.4 per cent. Titrating fluid: 1.94 N NaOH.

<table>
<thead>
<tr>
<th>TIME (hours)</th>
<th>Eₐ(Au) (millivolts)</th>
<th>Eₐ(Pt) (millivolts)</th>
<th>TIME (hours)</th>
<th>ACID PRODUCTION (milli-equivalent/liter)</th>
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<tr>
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<tr>
<td>99.75</td>
<td>-192</td>
<td>-198</td>
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* The data of this table were obtained in the same experiment as those of table 3 in a paper by Longsworth (1936). The two tables taken together consequently illustrate the results obtained in a typical experiment on the anaerobic growth of L. acidophilus.
The chief function of the initial saturation, however, is to establish the equilibrium concentration of bicarbonate ion in the sterile medium, as discussed in our first paper (1935). This requires less time than the stabilization of the $E_h$, particularly $E_h$ (Pt). Consequently, many of our cultures were inoculated before the $E_h$ (Pt) characteristic of the medium had been attained.

Table 1, which is self-explanatory, gives the $E_h$ values and the acid production, $A$, obtained during a quite typical experiment. In figure 3 the two sets of $E_h$ values and the rate ($dA/dt$) of acid production are plotted as functions of the time. It is evident that the $E_h$ (Pt) time curve differs markedly from that obtained with a gold electrode. The reasons for this difference remain obscure but are undoubtedly related to the fact that different metals attain equilibrium at different rates with the electromotively active materials in the solution with which they are in contact (see, for example, Michaelis and Flexner (1928)). If, as in this case, the relative concentrations of such materials
are constantly changing, different potential-time curves will be observed. The trends of both the $E_h$ (Au) and $E_h$ (Pt) time curves, after the $E_h$ (Au) reaches a minimum, were found in a number of experiments to be reproducible within a few millivolts.

It is an interesting fact that the first evidence of activity on the part of a culture after inoculation is a decrease in $E_h$. In cultures grown with pH control this drop begins several hours before alkali absorption or detectable changes in turbidity (optical density) are observed. As the rate of acid production, curve A of figure 3, increases, the $E_h$ (Au) drops rapidly to a minimum of about $-190$ mv. and then rises, with the rate of acid production, to a maximum of about $-150$ mv. soon after the maximum in $dA/dt$. This is followed by a decline of both the $E_h$ (Au) and $dA/dt$ until at 100 hours the $E_h$ (Au) has returned asymptotically to the minimum value of $-190$ mv.

A similar series of changes of potential have been observed by Gillespie and Rettger (1936) in L. acidophilus cultures grown without pH control. They ascribe the rise to a maximum and subsequent drop to changes of the pH of the culture. Our cultures, however, were grown at constant pH and the trends of $E_h$ (Au) observed by us cannot, therefore, be ascribed to such changes.

Regardless of the potential of the platinum electrode at the time of inoculation it is more "sluggish" than the gold during the initial downward drift of the $E_h$. At the time that the $E_h$ (Au) time curve is passing through a minimum there is always evidence of a plateau in the $E_h$ (Pt) time curve but the downward trend is resumed and a minimum is reached somewhat later than the time at which $dA/dt$ is a maximum. It is only in the old culture when the metabolic rate is low and the poising effect of the products of metabolism is high that the $E_h$ (Au) and $E_h$ (Pt) curves approach a common value of about $-190$ mv.

In any attempt to correlate $E_h$ measurements with the metabolism of the culture one is probably justified in ignoring the initial rapid drop of the $E_h$ since this is due to the replacement of the poising system of small capacity characteristic of the de-aerated sterile medium by one of increasing capacity character-
istic of the bacterial metabolism. The course of the $E_h$ (Au) time curve produced by the culture itself is probably represented by a curve through the points a, b, c, d of figure 3. The symmetry between this curve and that of the rate of acid production, $dA/dt$, suggests that the $E_h$ (Au) values are nearer than the $E_h$ (Pt) values to a measure of the instantaneous ratio of electromotively active oxidizing and reducing substances in solution.

![Figure 4](http://jb.asm.org/)  
**Fig. 4. Acid Production of Cultures of L. Acidophilus Grown with and without pH Control**  
Medium: 2 per cent neopeptone, 8 per cent glucose

The value of $-190$ mv., to which $E_h$ (Au) first falls and later, together with $E_h$ (Pt), returns, seems to be a characteristic of the organism at pH 6.0. It has been observed in all of our experiments in which the growth was under approximately anaerobic conditions, in spite of changes in the nature and concentrations of the constituents of the medium. Moreover, the same potential has been observed by Rogers and Whittier (1928) in the formation of lactic acid by *Streptococcus lactis*.

It is not possible, with the experimental data at hand, to
identify the particular system or systems of electromotively active materials responsible for the potentials observed. The only system that appears to be relevant and which has been adequately studied is the lactate-pyruvate system. Barron and Hastings (1934) have observed that

\[ E_a = -0.118 - \frac{RT}{2F} \ln \frac{\text{lactate}^-}{\text{pyruvate}^-} \]

at 35° and pH 6.0. If this system were contributing, reversibly, to the potential of -190 mv. observed in our culture it would indicate an apparently quite reasonable ratio of pyruvate to lactate of about 1 to 100.

The \( E_a \) values reported in our second paper (1936) were obtained in experiments in which the gas mixture contained about 0.2 per cent of oxygen. With this oxygen content and the rates of flow used by us, the actively metabolizing culture was apparently able to remove the oxygen (compare, for example, Hegarty (1936)) as fast as it passed into solution. Consequently the minimum \( E_a \) observed, -180 mv., was not very different from that attained in anaerobic growth. The presence of this amount of oxygen in the entering gas had the effect, however, of delaying the appearance of the minimum in the \( E_a \) time curve until \( \frac{dA}{dt} \) is a maximum, as shown in figure 4 of our second paper. Moreover, the final upward drift of the \( E_a \) time curve, in contrast with the downward drift of \( E_a \) (Au) observed in anaerobic growth, may be due to this trace of oxygen.

If the entering gas contains more oxygen than the culture can reduce, the minimum in the \( E_a \) time curve, although occurring soon after the maximum in \( \frac{dA}{dt} \), has a value much more positive than in anaerobic growth. Moreover, this minimum value, unlike that observed under approximately anaerobic conditions, depends upon the value of \( \left( \frac{dA}{dt} \right)_{\text{max.}} \). Thus, in three experiments in which the gas contained 2 per cent of oxygen the minimum values of \( E_a \) were +56, +100, and +159 mv. while the corresponding maximum rates were 8.0, 6.0, and 3.0 milli-equivalents
per hour per liter of suspension. These variations in \( \frac{dA}{dt} \) were the results of alterations in the composition of the medium.

We may therefore conclude from our determinations of \( E \) time curves at constant pH that, although much more remains to be done in this field, the trends of such curves are definitely related to the rates of acid production of these cultures. This does not exclude the possibility of correlations of such curves with the rates of cell multiplication, as suggested by Clifton, Cleary, and Beard (1934). However, in our work the best correlation is obtained with the acid production.

THE PHYSIOLOGY OF CULTURES GROWN WITH AND WITHOUT pH CONTROL

In the development of an acid-producing culture the amount of acid formed in a small interval of time, \( \Delta t \), is the product of that interval, the population (plate count) of the culture, \( C \), and the rate of acid production per unit of population, \( \Phi \). The last two factors may vary with the time. Thus, the total acid produced from the moment of inoculation at zero time to the time, \( t \), is given by the integral,

\[
A = \int_0^t \Phi \cdot C \cdot dt
\]  

Since the population and acid production of a normal culture are seldom limited by the available food supply it is customary to assume that accumulation of metabolic products has an inhibiting effect upon cell growth and the ability of the cell to convert carbohydrates into acid. These two cellular functions, although subject to the relation given above, should be considered separately.

In our first paper (1935) a comparison was made between the acid production of \( L. \) acidophilus cultures grown without pH control and at various pH values. When the pH was controlled at the favorable value of 6.0 the acid production was 200 per cent greater than in normal growth. This observation suggests that the hydrogen ion is one of the metabolic products exerting
an inhibiting effect upon either C or Φ, or both, although from measurements of A alone we were unable to choose between these alternatives. Our recent measurements of acid production have, therefore, been accompanied by population studies.

Typical results from our more recent experiments are shown in figures 4 and 5. In these figures the times, in hours, from the moment of inoculation are plotted as abscissae while the ordinates are, respectively, the acid production in milli-equivalents per liter of medium and the optical density.²

It may be seen from figure 4 that, with pH control, the acid production at 50 hours has been increased 800 per cent over that of a culture in the same medium but without pH control. Moreover, this ratio was maintained until the experiments were

² It has been shown by Longworth (1936) that for cultures of L. acidophilus grown with pH control measurements of optical density are proportional to the plate counts over an interval in which more than 90 per cent of the total growth occurs.
stopped at 100 hours, at which time the acid production was 77.5 milli-equivalents with normal growth and 712 with pH control. The latter value corresponds to 80 per cent conversion, computed as lactic acid, of the available sugar. The much larger increase resulting from pH control observed in these experiments than in the experiments described in our first paper can be attributed to the relatively rich medium employed in the more recent work.

The culture grown with pH control also develops a much larger population, as measured by the optical density, than in normal growth. As shown in figure 5, the increase amounts to about 300 per cent at 50 hours. Plate counts were also made at intervals throughout these experiments and yield curves, not shown, similar to those of figure 5, except that the increase in the plate count due to pH control is 200 per cent. The difference between this increase and the four-fold change observed in the optical density, figure 5, is due to the fact that there is a larger proportion of short chains and individual cells in a normal culture whose
pH has attained low values, as has recently been shown by Longsworth (1936).

From the data represented by figures 4 and 5 values of ψ, the rate of acid production per unit of optical density, may be computed. The results of these computations are plotted as ordinates against the time, in hours, as abscissae in figure 6, the open circles representing the values in normal growth and the solid circles corresponding to growth with pH control. In the early stages of growth, before the accumulation of appreciable concentrations of metabolic products, the values of ψ are approximately constant and independent of pH control. In the normal culture, however, ψ begins to decrease much earlier than in the culture at constant pH. It thus appears that the effect of pH control upon ψ is to delay the inhibiting action of the metabolic products on this property of the bacterial cell.

Although the hydrogen ion concentration (H+) has been considered the most important limiting factor in the lactic fermentation (Rahn (1932)) evidence has accumulated to the effect that the concentration of lactate ion (L-) is also a factor. These two variables are connected by the mass action relation

$$ (H^+) (L^-) = K_{HL} (HL) $$

in which the ionization constant has the value of $1.55 \times 10^{-4}$ (Borsook, Huffman, and Liu (1933)). The concentration of the undissociated acid (HL) is thus proportional to the product of both of these factors and has, as a matter of fact, been considered

\[ \text{In our second paper, and in the formulation of equation 1 of the present paper, the unit of population used in the definition of } \phi \text{ was determined from plate counts, } C. \text{ Since the optical density, } D, \text{ may, as already mentioned, be taken as a measure of the population a quantity } \psi, \text{ defined by the relation } \]

$$ \psi = \frac{dA/dt}{D} $$

will differ, in most instances, from \( \phi \) by a constant factor. The quantities \( \phi \) and \( \psi \) have essentially the same significance as the "fermenting capacity per cell" discussed by Rahn (1932). Equation 1 may thus be rewritten as

$$ A = \int_{0}^{t} \psi \cdot D \cdot dt $$

(1')
one of the chief inhibitors of growth and metabolism by Rogers and Whittier (1928) and others. In normal growth the production of lactic acid by the culture increases the values of both \((H^+)\) and \((L^-)\) whereas in growth at constant pH the former does not change. Undissociated lactic acid thus accumulates in both normal cultures and those grown with pH control, but more rapidly in the former case. The value of \((HL)\) for either case may be computed as follows. The observed quantities, which are the titratable acid, \(A\), and the pH, are related to \((H^+)\) and \((HL)\) by the equations,

\[
(HL) + (L^-) = A \tag{3}
\]

\[
pH = \log \frac{1}{(H^+)} \tag{4}
\]

Elimination of \((L^-)\) between equations 2 and 3 yields, for the concentration of undissociated lactic acid,

\[
(HL) = \frac{(H^+)}{K_{HL} + (H^+)} A \tag{5}
\]

It was of interest to determine whether there is a simple relation between \(\psi\) and \((HL)\) valid for the normal growth which also holds for growth with pH control. Among the many possible equations the following

\[
\frac{\psi}{\psi_0} = \frac{1}{1 + a(HL)} \tag{6}
\]

describes the change of \(\psi\) with the accumulation of lactic acid during normal growth. In this expression \(\psi_0\) is the value of \(\psi\) for a young culture and \(a\) is an empirical constant with a value of 350. The agreement, which is within the experimental error, between the observed and computed values is shown in figure 6 in which curve I is a plot of equation 6. In obtaining this curve the values of \((HL)\) were computed, using equations 4 and 5, from determinations of pH and \(A\) for the normal culture. The scattering of the points in the early stages of growth is due to the fact that the rate of acid production is low and the \(\psi\) values are therefore relatively inexact.
Using the same values of $\psi$ and $a$, but computing (HL) from the acid production data for the culture grown at pH 6.0, curve II of figure 6 was obtained. It may be seen that the observed values, indicated by the solid circles, follow curve II for a considerable part of the growth period. It must be recalled, however, that metabolism, as measured by the acid production, is much more rapid in the culture at pH 6.0 than in normal growth. Consequently by the time marked deviations from curve II are observed changes in the substrate, other than the accumulation of undissociated lactic acid, have probably occurred. These can affect the value of $\psi$.

Among such changes are increases of the salt concentration and depletion of the nutrient material. In normal growth the salt concentration retained its initially low value throughout the experiment while at constant pH the concentration of sodium lactate increased to a value of 0.7 N at 100 hours. Moreover, without pH control only 8 per cent of the sugar was utilized so that its concentration remained essentially constant. At pH 6.0, however, 80 per cent of this material was converted. Consequently these changes, which are relatively unimportant in normal growth, may affect the value of $\psi$ in growth at constant pH and their influence was not considered in equation 6. Winslow (1934) has shown that salt concentrations of the magnitude developed in growth at constant pH have an inhibiting effect upon bacterial growth and metabolism. Moreover, although most enzyme reactions are independent of the concentration of substrate until that is reduced below a critical value, the disappearance of the sugar in the culture medium must ultimately reduce $\psi$ to zero. It is not surprising, therefore, that the relatively simple relation represented by equation 6 can account for the changes in $\psi$ that are observed in cultures grown at pH 6.0 for only the first portion of the growth period.

In conclusion, then, it would appear that accumulation of undissociated lactic acid (HL), or in other words an increase of the ionic product $(H^+)(L^-)$, has an inhibiting effect upon both the growth and metabolism of L. acidophilus. If one of the factors $(H^+)$ is held at an appropriate value this effect is very
much reduced. However, even with pH control, alterations of the substrate and accumulation of other metabolic products ultimately have inhibiting effects.

SUMMARY

A method for the production of oxygen-free mixtures of carbon dioxide and nitrogen has been developed. With the aid of these mixtures and an apparatus for automatic pH control we have studied, at pH 6.0, the growth, acid production, and apparent oxidation-reduction potentials of cultures of *Lactobacillus acidophilus* under anaerobic conditions. The observation that carbon dioxide is essential for the growth of this organism has been confirmed.

Apparent oxidation-reduction potentials, $E_A$, in these cultures have been obtained with the aid of gold and platinum foil electrodes. Of these the gold electrode appears to respond more readily to changes in the oxidation-reduction balance of the system. During anaerobic growth the $E_A$ (gold) drops rapidly to a minimum of about $-190 \text{ mv.}$, then increases to a value of about $-150 \text{ mv.}$ after which it slowly returns to the minimum value, which appears to be a characteristic of the bacteria, having been obtained in all anaerobic cultures. The relation of these anaerobic $E_A$ measurements to possible metabolic processes in the culture has been discussed.

Using a more suitable medium than in previous experiments the measurements of growth and acid production at a constant pH of 6.0 have been compared with similar measurements in normal cultures. Control of the pH resulted in a four-fold increase of the bacterial population and a nine-fold increase of the acid production.

REFERENCES

BACTERIAL GROWTH AT CONSTANT pH