CONDITIONS AFFECTING THE MEASUREMENT OF OXIDATION-REDUCTION POTENTIALS OF SOILS

by

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A Thesis submitted to the faculty of the Department of Agricultural Chemistry and Soils in partial fulfillment of the requirements for the degree of Master of Science in the Graduate College University of Arizona 1940

Approved: J. F. Bronner, May 21, 1940.

Directors of Research
ACKNOWLEDGMENTS

The author wishes to express his appreciation to the staff of the Department of Agricultural Chemistry and Soils for their cooperation during the course of this work; especially to Drs. T. F. Buehrer and Wm. P. Martin, the research directors. Their aid and suggestions are gratefully acknowledged.

The author also wishes to express his indebtedness to the University of Arizona for the award of the Paul Steere Burgess Fellowship which made the completion of this work possible.
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CONDITIONS AFFECTING THE MEASUREMENT OF
OXIDATION-REDUCTION POTENTIALS OF SOILS

By Bartley P. Cardon

INTRODUCTION

The fundamental relationship of oxidation and reduction to soil fertility has long been recognized by soil investigators. By means of a series of oxidations and reductions the plant is able to secure food and energy for its growth and development. It is now generally conceded that aerobic decomposition of organic matter results in the accumulation of plant nutrients and the destruction of toxic substances, while under anaerobic conditions, toxic compounds tend to accumulate. The extent to which a soil is aerated will in large measure determine whether the soil is in an oxidized or reduced condition. Since Gillespie (20) first demonstrated that waterlogged soils have a low oxidation-reduction potential, this phenomenon has been the subject of numerous investigations. All agree that the exact nature of the equilibrium causing the potential is very complex and difficult to determine. In any given soil it is without doubt the resultant of a number of reactions. Compounds are continually being dissimilated as a result of the
metabolic processes of the microorganisms inhabiting the soil. Each reaction is undergoing change at its own rate; therefore any equilibrium is a dynamic one, changing with time until the unstable constituents taking part in the equilibrium have reached a more stable state.

The theoretical considerations underlying the determination of the redox potential have been well developed. The theory and equations can be obtained from any standard physical chemistry text, as well as from numerous articles, principally those of Clark (12,14), Hewitt (24), and Michaelis (34). Reviews of the literature have been given by Parks (35), Sturgis (39), Bradfield and co-workers (3), and others, and consequently will not be repeated here. However, it should be pointed out that the different investigators disagree as to the value of a determination of the redox potential for indicating the inherent fertility of soils. One group headed by Bradfield (3), Pearsall (36), and others, consider it a valuable index of fertility, while another group, including such investigators as Stephenson (38), Heintze (22,23), Willis (44,45,43), and Volk (42) conclude that the redox potential is of little value in determining soil fertility.

Most of the work done on the redox potential of soils has been concerned with the method of measurement and the changes which occur under waterlogged conditions. The results obtained by the different investigators are in
some instances conflicting and difficult to explain. In order to discern more accurately some of the changes which occur in the decomposition of soil organic matter, it seemed desirable to work with pure, simple, and definitely known organic compounds. Such studies have been made in solution by numerous investigators, notably Conant and co-workers (16), and Clark and co-workers (14), who worked with a large variety of dyes and other organic compounds. Very little work has been done, however, on the behavior of such compounds in the presence of soils. The present investigation was therefore undertaken in the hope of correlating some of the results obtained with those in which complex plant materials and manures were allowed to decompose under controlled conditions.

EXPERIMENTAL METHODS

Vacuum Tube Circuit

In general the apparatus and method of measurement used for the determination of the redox potential was the same as that developed by Buehrer, Martin, and Parks (9); however, there were several details of the method that were still in doubt. Preliminary experiments with certain western soils in the presence of little or no decomposing organic matter showed that they were so poorly poised that the electrodes used were very easily polarized. This polarization effect occurred so readily
in some of the soil suspensions that it was almost impossible to obtain or duplicate any potential measurements. Variations as high as 100 millivolts sometimes occurred between two electrodes in the same soil suspension. Since a vacuum tube amplifying unit would reduce the amount of current necessary to make a reading (and therefore minimize the polarization of the electrodes), it was decided to construct such a circuit for use in this connection.

The theory and operation of vacuum tube circuits has been well developed for use in pH measurements using the glass electrode. The literature on the subject, of which the articles of DeEds (17), Cameron (10), Fosbinder (18), Woontom (46), and Goodhue and co-workers (21) are typical, gives the details of the construction of such circuits.

Several of these circuits were constructed and discarded because they appeared to be unsatisfactory for this purpose. The one finally adopted and used is a modification of several circuits tried before. A working diagram and the manner in which the unit is connected to the potentiometer is shown in Fig. 1. Besides being easy to construct and operate, the set is very economical; but tube characteristics are more important than the actual circuit in determining the amplification that may be expected.
FIG. 1 POTENTIOMETER AMPLIFIER CIRCUIT FOR REDOX MEASUREMENTS
The unit to be described involved a type 32 tetrode tube. The control grid bias "A" is a \(1\frac{1}{2}\) volt battery. Potentials of \(22\frac{1}{2}\) volts and 45 volts are placed in the screen grid and plate circuits, respectively. The plate current is balanced out by means of the zero shunt which is composed of a \(1\frac{1}{2}\) volt battery placed in the circuit in opposition to the plate current, one 25,000-ohm fixed resistance, and one 5,000-ohm rheostat.

In actual operation the amplifying unit is only used to obtain the potential of the unknown cell. The auxiliary galvanometer "11" is used to balance the working battery "\(E_w\)" against the standard cell "\(E_s\)". This is done by moving the double pole double throw switch "3" so as to include "\(E_s\)" and exclude the unknown cell "\(E_x\)" and move switch "4" to the left. After "\(E_w\)" has been checked against "\(E_s\)", "\(E_x\)" is connected into the circuit in place of "\(E_s\)".

The amplifying unit is balanced before it is connected into the potentiometer circuit. Before balancing, the filament is first allowed to become incandescent. The control grid circuit is formed by closing the shorting switch "3". Before the plate and screen grid circuits are closed the galvanometer is protected by means of the variable shunt resistance "9". To nullify the plate current, this resistance is increased to force a little current through the galvanometer. The 5,000-ohm
rheostat is then moved to balance the plate current. When this is perfectly balanced the resistance "9" can be disconnected entirely if desired.

In determining the potential of the unknown cell the amplifying unit is connected to the potentiometer in place of the galvanometer "11" by means of the double pole double throw switch "4". The shorting switch "3" is then opened. If the potential of the unknown cell "Rx" is not balanced by the graduated potentiometer resistance "2," it will change the charge on the control grid "6" which will cause a relatively large change in the plate current, thus causing the galvanometer "10" to deflect. The potentiometer reading is adjusted to zero again by varying the resistance "2". The potential of the unknown cell is obtained by the reading on the potentiometer.

**Carbon Electrodes**

Even with the use of the vacuum tube set, there was still considerable polarization in some of the soil samples. In these samples it was impossible to remove an electrode, wash it, then place it back in the soil suspension and duplicate its previous reading. For this reason a carbon electrode was tried. Such electrodes were made by removing the wood from around the lead of large soft-ledged pencils, until about one half inch was exposed on
each end. To one end was fastened a screw clamp which was in turn connected to an insulated wire leading to the potentiometer. The wood at the base of the other end was very carefully coated with paraffin to prevent its being wetted by the solution.

Two of these electrodes were then inserted in a rubber stopper and then immersed in the soil suspension. When readings were taken there was no evidence of polarization. The potentials obtained were very sharp and clear and could be read to tenths of millivolts accurately. The different electrodes, however, did not agree. The carbon was easily poisoned and very difficult to clean. If an electrode were placed in a solution which had a potential of about 200 millivolts, removed and cleaned, then placed in a solution with a potential of about 400 millivolts, the reading would usually be in the vicinity of 200 millivolts, and show a gradual rise. Since such variability must be eliminated if an electrode is to be suitable for measuring the changing potential of a soil, these electrodes were discarded.

**Platinum Foil Electrodes**

In a soil suspension in which the platinum wire electrodes were easily polarized, it was found that four electrodes connected in parallel showed no evidence of polarization. This seemed to indicate that the polariza-
tion was a surface phenomenon and could probably be eliminated by using electrodes with larger surfaces.

An electrode was constructed somewhat similar to that used by Parks (35), but using in place of the platinum wire a piece of foil one centimeter square. When this electrode was placed in the soil suspension and potential readings made, there was no evidence of polarization. Only rarely did the electrodes constructed in this way exhibit any polarization effect. Consequently it was seldom necessary to use the vacuum tube amplifying unit.

The cleaning of the platinum foil was, however, very difficult. The methods advocated by Bradfield and workers (3), Peck and Batjer (37), and Parks (35), proved inadequate. Newly constructed electrodes would agree very closely, but after using and cleaning them several times with an alcohol or bunsen flame, they became so badly "poisoned" that two electrodes would not agree within 25 millivolts. Reverse electrolysis in a solution of 0.1 N sulphuric acid was tried. The results were similar to those obtained with hot chromic-sulphuric acid solution as used by Parks (35). It was finally concluded that the alcohol and bunsen flames were not hot enough to remove completely all of the impurities from the surface of the platinum foil. Ignition in an oxygen-natural gas flame proved to be very satisfactory. Electrodes
cleaned in this way did not show a lag effect as found by Bradfield and workers (3), Volk (40), and others. The electrodes could be removed from a solution of low potential, cleaned, and placed in a solution of high potential and the correct potential could be obtained in five minutes; regardless of whether the solution was strongly or poorly poised.

Extreme care must be exercised in the cleaning of the electrodes. Before heating they must first be thoroughly washed with distilled water and then alcohol to remove the salts and other soluble substances on the surface of the platinum. The heating removes all traces of an oxide or organic film on the surface of the electrode. In cleaning the electrodes by this procedure, they must be brought very gradually to a temperature just below the melting point, before the platinum foil is ignited. As it is necessary to ignite to a bright yellow heat, the flame must be carefully adjusted or some volatilization of the platinum will occur. Regardless of the care taken in their construction and cleaning, some of the electrodes were never satisfactory. These were usually rebuilt or discarded.

Some electrodes which gave perfect readings in the well poised quinhydrone or ferro-ferricyanide solutions were very erratic in a weakly poised soil suspension; especially one in which the organic matter was undergoing
fairly rapid decomposition. In some cases this instability was caused by minute cracks in the glass. Very slight leaks in the glass can be sealed by filling up the inside bottom of the electrode with "picein". This device will also prevent leaks caused by the improper sealing of the glass to the platinum wire.

Lewis and Randall (31) stress the fact that surface strains in the platinum foil will make an electrode unstable. To determine the magnitude of this effect several electrodes were raised from the soil suspension, the platinum foil quickly bent several times and the electrodes lowered back into the soil suspension, the potential being read immediately. In all cases the potential dropped an amount varying from 20 to 100 millivolts. It was very unstable, however, and gradually approached its former value. These electrodes could be rendered stable by again heating them to bright redness.

The platinum foil used in the construction of the electrodes must be very pure. The electrodes made from platinum that had been used for other purposes were never reliable. This was probably caused by the fusion of a reduced metal or similar substance with the platinum which was formed by reduction during the previous ignition. The cleaning apparently did not remove these impurities; consequently the electrodes had to be discarded.
Final Procedure in Constructing Electrodes

The electrodes finally adopted for use were constructed in the following manner. One end of a 3 cm. length of 25 B. and S. gauge (0.018 inch dia.) platinum wire was welded to a platinum foil 0.008 inch thick and having a surface area of 2 sq. cm. The other end was welded to a 10 cm. length of 22 gauge (0.025 inch dia.) Cu wire. This was inserted into an 8 cm. length of pyrex glass tubing in such a position that the platinum foil extended about 0.4 cm. from the end of the tube. This end of the glass tube was then fused around the short length of a platinum wire, making sure that the glass was not fused to the Cu wire or the platinum foil. A small piece of pinein was then inserted into the electrode and melted until it covered the inside bottom of the tube. The Cu wire projecting from the other end of the electrode was soldered to a length of insulated wire leading to the potentiometer. This joint was taped very tightly with splicing compound to prevent any current leakage.

In all of the determinations the electrodes were cleaned just before they were used. Care was taken to prevent the platinum foil from being bent or even touched before it was inserted into the soil suspension.
**Connecting Bridges**

Numerous types of bridges for connecting the calomel cell with the soil suspension have been used. Because of the mechanical advantages involved, the use of the saturated KCl-agar bridge is desirable. Several investigators, however, have found them unsatisfactory. Allyn and Baldwin (2) found that agar had a slight reducing action in bacterial suspensions. Burrows and Gordon (11) concluded that it was necessary to renew the solution in the bridge between each measurement. They used a liquid KCl bridge, flushing it clean after each determination. Parks (35) found that a saturated KCl-agar bridge had the least disturbing influence on both the Eh and pH of the soil suspension.

ZoBell (47) constructed a calomel cell using as a bridge a saturated solution of KCl which made contact with the cell under measurement through asbestos wicks. To determine its probable applicability, a slight modification was made in the construction of this bridge. It consisted of a piece of ordinary glass tubing bent in an inverted U-shape. Both ends of the tube were fused around short fibers of asbestos. The bottom of a short test tube was connected to the center of the arch of the inverted "U" to facilitate the filling of the tube with saturated KCl solution. A rubber stopper was placed in the mouth of the test tube to prevent evaporation. It
was used in the same manner as the agar bridges. Subsequent tests showed that this bridge was no improvement over the agar type; moreover the construction and care were much more difficult. Consequently the asbestos wick bridge was discarded in favor of those made of saturated KCl and agar.

**Stirring**

Very little work has been done on the effect of stirring on the redox potential of soil suspension. Parks (35) concluded that shaking just before reading was sufficient. Preliminary experiments with and without stirring indicated that in those solutions or suspensions in which the equilibrium was fairly stable, stirring made very little difference.

In those suspensions, however, in which there was actively decomposing organic matter, the effect of agitation was apparent. In some respects this effect is comparable to the stirring of the suspension in the determination of the pH as found by Wiegner and Pallmann (45A). The equilibrium of a suspension containing rapidly decomposing organic matter appears to shift rapidly. If the soil is not kept in suspension, the attainment of equilibrium is very much retarded. This is exactly what happens when the soil particles are allowed to settle out at the bottom of the suspension. Consequently in this type of
sample, stirring is absolutely essential. It was observed also that when the suspensions were stirred, the potential soon attained a constant value.

Because of the large number of measurements made, it was necessary to provide several stirrers, which was accomplished by connecting them in series and driving all from one motor. The individual stirring unit is shown in Fig. 2. The bearing, which is marked in black, is composed of brass. The pulley and hollow shaft are made from one piece of iron. A \( \frac{1}{4} \) inch hole is made through the vertical center of the pulley and shaft, to hold the glass stirrer. The set screw at the top of the pulley holds the glass rod tightly in place. The glass stirrer was made from a \( \frac{1}{4} \) inch pyrex glass rod. A one-inch length of this rod was welded across the end of a twenty five-inch length to form a "T". The short arms of the "T" were then heated to the softening point and flattened into paddles of the desired shape and pitch.

The power to turn the stirrers was supplied by a Franz Premier vacuum cleaner motor. The type of motor here used had a vertical drive shaft. A one-inch pulley was attached to this shaft and the height of the motor adjusted so that it was level with the three inch pulley of the first stirring unit. Five stirrers were used, which were mounted on a frame over the water bath so that the samples could be kept at a constant temperature while they were
FIG. 2  STIRRING UNIT
being stirred. A resistance of 360 ohms was placed in series with the motor to reduce its speed.

In some of the studies it was desirable to stir the sample but still keep it under anaerobic conditions. The apparatus developed and used for this purpose is shown in Fig. 3. The bell shaped glass tubes were sealed to a ½ inch glass rod and 3/4 inch glass tubing of the desired length. The two were placed together as shown in the diagram and a paddle was built on the end of the glass rod as described before. Enough Hg was added to insure the seal's being air tight. This unit was then mounted in a rubber stopper, the electrodes grouped around it and the rubber stopper was then placed very tightly in the suspension bottle. Paraffin was used to seal up any cracks.

**Temperature and pH**

Because of variations caused by changes in the temperature all samples were placed in a constant temperature bath and kept at 30° C during the course of measurement. The pH was determined at the time of Eh measurement by means of a Beckman pH-meter. All observed Eh values were calculated to the normal hydrogen electrode taken as zero and to a constant reference pH value of 7.00.
THE EFFECT OF GASES ON REDOX POTENTIALS

Oxygen Electrode

In working with simple inorganic solutions it has been found that the gas phase under which the solution is kept will in part determine the value of the potential. The potential of a solution is a measure of free energy change of a reversible chemical reaction which has a certain tendency to occur at the electrode surface. It is a measure of the tendency of a system in equilibrium to undergo a reversible change from one state to another. There are several types of electrode reaction which are essentially reversible, and the potential value under definite conditions can be accurately determined. Consider a cell composed of a zinc electrode immersed in a zinc chloride solution. There is a tendency for the zinc metal to lose two electrons and go into solution as zinc ions, opposed by the reverse tendency for the zinc ions to take up two electrons and deposit as the metal. The balance between these two tendencies determines the potential of the cell.

A similar type of reversible reaction occurs when an inert electrode such as platinum is placed in a solution of ferric and ferrous salts. The electrode reaction in this case is essentially an electronic change in which ferric is converted into ferrous ion, or vice versa. If
a platinum electrode is placed in a potassium chloride solution, a very definite potential can be measured. There is, of course, a liquid potential which exists at the junction of the saturated KCl solution of the agar bridge and the potassium salts in solution, but the magnitude of this potential is relatively small. In an aqueous solution of KCl there are present potassium-ion, chloride-ion, hydrogen-ion, and hydroxyl-ions. In view of the fact that a potential is obtained in such a solution with a bright platinum electrode, there must be one or more ions in the solution which take part in the electrode process. In the present case potassium ion, chloride ion, and the hydrogen ions are precluded from the electrode process because neither their elementary forms nor any other possible oxidation states are present. Hence the only ion left to take part in the electrode reaction is the hydroxyl ion.

The only electrode reaction in which hydroxyl ions could participate is one involving gaseous oxygen and water. The nature of the process and its dependence on the pH have been discussed by Latimer (30) who writes the reaction in neutral, acid and alkaline solutions as follows:

1. Acid solution.

\[ 2\text{HOH} = \text{O}_2 + 4\text{H}^+ + 4\text{e}^-; \quad E^0 = -1.229 \text{ v.} \]
2. Neutral solution.

\[ 2\text{HOH} - \text{O}_2 + 4\text{H}^+ (10^{-7} \text{m}) + 4e^- ; E^0 = -0.815 \text{ v.} \]

3. Basic solution.

\[ 4\text{OH}^- = \text{O}_2 + 2\text{HOH} + 4e^- ; E^0 = -0.401 \text{ v.} \]

These reactions would hold for any system in which oxygen participates in the electrode reaction. The above potential values are theoretical, having been calculated thermodynamically from various solubility and calorimetric data. In the measurement of the oxygen potential the characteristics of the electrode are such that an actual determination is very difficult, if not impossible, (31). Such electrodes give very erratic potentials, and small amounts of current cause polarization, which give rise to a counter electromotive force. Hoar (25) investigated the kinetics of the oxygen electrode process. By an extrapolation of the anodic and cathodic polarization curves, he secured reasonable agreement between the value obtained from the graph and the theoretical values.

Because of the possibility of an oxygen potential, certain investigators such as Volk (41) carried out the measurements under an atmosphere of nitrogen. Knight (26) also showed that the potentials of these broth solutions could be poised at any desired value by varying the ratio of the oxygen to nitrogen which was bubbled through them. Boyd and Reed (5) stated that in broth solutions there were gas-metal electrode potentials operating at the
noble electrodes and these, in large measure, determine the potential of the system. In a more recent publication (6), they demonstrated that in addition to these gas potentials, there were non-gaseous electromotively active systems. Parks (35) found that nitrogen caused a marked drop in the potential when it was bubbled through a soil suspension.

French and Kahlenberg (19) did considerable work in determining the effect of gases on various electrodes when immersed in a KCl solution. The variations obtained using a platinum electrode with various inert gases, such as nitrogen, methane, and helium, were such that they could not be explained on the basis of oxygen elimination. These investigators found that each gas had a definite and characteristic effect upon the platinum electrode. The time required to establish equilibrium with oxygen was much slower than was true for the other types of electrodes. They concluded that in the case of the platinum electrode the action of the inert gases is not merely due to the dilution of the oxygen but rather that each gas exhibits a specific and pronounced effect upon the potential of the platinum electrode. No attempt was made by French and Kahlenberg (19) to explain the mechanism of these effects.
The Effect of Gases on the Potential of
the Platinum Electrode in Various Solutions.

The foregoing results indicate the possibility of
the existence of an oxygen electrode potential, manifest-
ing itself in the soil suspension and to a large degree
influencing the potential that is being measured. It
seemed desirable, therefore, to determine the magnitude
of this effect and from the results obtained to draw some
conclusions as to the proper method of measurement of
redox potentials of soils.

Studies on this gas effect were accordingly carried
out with buffer solutions and sterile soil suspensions.
This procedure eliminated two variables: (a) the effect
of pH change and (b) changes caused by microbial growth.
A series of Sorensen's standard buffer solutions was
prepared according to Clark (13). The pH values of these
solutions ranged from 2.30 to 8.48. The soil suspensions
were prepared in a ratio of one part of soil to five of
water, and sterilized. The change of Eh value with time,
brought about by bubbling oxygen, nitrogen, and air through
these buffer solutions was then determined. In these
determinations duplicate platinum foil electrodes were
used. These electrodes were inserted into a rubber stop-
per along with an inlet and outlet tube for the gases.
The rubber stopper was then tightly inserted into the jar
containing the solution. Connection was made with the
saturated calomel half-cell by means of a KCl-agar bridge. The stopper was then sealed tightly with hot paraffin. Gas was bubbled through the solution continuously until equilibrium was reached. The Eh and pH determinations were made periodically while the gas was being passed.

The gases used in the experiment were commercial oxygen and nitrogen. Traces of oxygen in the nitrogen were removed by means of pyrogallic acid-potassium hydroxide solution, followed by washing with sulfuric acid and distilled water.

These gas potential studies involved both organic and inorganic buffers; a sterile desert soil, a sterile humid soil, a weak organic acid solution and a neutral salt solution. The results of these experiments are given in Figs. 4 to 10. It may be observed that in all cases nitrogen caused a drop in the potential which gradually leveled off to an apparent equilibrium. Under an atmosphere of oxygen the potential tended to rise slightly or remain essentially constant. In nearly all cases the air-curve was found to lie between that of pure oxygen and pure nitrogen, and the potentials for air approximated more closely to those for pure oxygen.

It is remarkable that the effect of nitrogen is identical in all cases, at least in direction if not in magnitude; and furthermore, that it manifests itself in a wide variety of solutions in which the measurements
MIXTURE OF SUCCINIC AND FUMARIC ACIDS
pH - 2.31

NaCl
pH - 7.22

FIG. 4

TIME OF MEASUREMENT
PHOSPHATE BUFFER

pH — 5.45

TIME OF MEASUREMENT

FIG. 5
PHOSPHATE BUFFER

pH—7.45

TIME OF MEASUREMENT

MV.

[Graph showing the relationship between pH and time of measurement under different conditions (AIR, O₂, N₂).]

PHOSPHATE BUFFER

pH—7.45
FIG. 7

NaOH-GLYCINE-NaCl BUFFER
pH - 7.00
FIG. 8

BORATE BUFFER
pH — 8.48

MV.

TIME OF MEASUREMENT

18 HOURS

O₂

N₂

HOURS
CLARION SILT LOAM (STERILE)

pH - 5.13

FIG. 9

TIME OF MEASUREMENT

O₂

AIR

N₂
Figure 10

Palos Verdes Sandy Loam (Sterile)

pH = 6.20
were made. This result is found to be in striking agree-
ment with those obtained by French and Kahlenberg (19)
with different electrodes in a potassium chloride solu-
tion. It is also significant that whereas the authors
just referred to found the effect to be true for a wide
variety of metal electrodes in potassium chloride solu-
tions, the present studies show that it is also true for
a given electrode in a variety of solutions.

The equilibrium and pH values found in these ex-
periments are shown in Table I.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>THE pH AND THE FINAL EQUILIBRIUM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solutions</th>
<th>pH</th>
<th>Equilibrium Eh Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O₂</td>
</tr>
<tr>
<td>Succinic Fumaric Acid</td>
<td>2.51</td>
<td>0.632</td>
</tr>
<tr>
<td>Phosphate Buffer</td>
<td>5.45</td>
<td>0.683</td>
</tr>
<tr>
<td>Phosphate Buffer</td>
<td>7.45</td>
<td>0.450</td>
</tr>
<tr>
<td>Borate Buffer</td>
<td>8.48</td>
<td>0.432</td>
</tr>
<tr>
<td>Glycine - NaCl - NaOH</td>
<td>7.00</td>
<td>0.508</td>
</tr>
<tr>
<td>NaCl Solution</td>
<td>7.22</td>
<td>0.424</td>
</tr>
<tr>
<td>Clarion Silt Loam</td>
<td>5.30</td>
<td>0.470</td>
</tr>
<tr>
<td>Palos Verdes Sandy Loam</td>
<td>7.20</td>
<td>0.475</td>
</tr>
</tbody>
</table>

The data of Table I show that the equilibrium value
under an atmosphere of nitrogen is not the same in each
case. If an oxygen potential were operating in these
solutions, the drop caused by the nitrogen could be partially explained on the basis of the nitrogen serving to dilute or remove the oxygen. If the oxygen was completely removed, all possibilities of an oxygen potential would be eliminated. Theoretically the only potential left should be a solution potential. However, the potentials of the different systems under an atmosphere of nitrogen seemed to reach an equilibrium value after several hours of bubbling. This value was different for each buffer and for the sterile soil suspension. All three of the reaction equations given by Latimer (30) indicate that a decrease in the partial pressure or solubility of oxygen would make the potential more negative. If the extent to which nitrogen will remove oxygen from a solution when the gas is bubbled through it, depends on the nature of the solution, these different equilibrium values could perhaps be explained. However, the rather stable equilibrium value obtained under the atmosphere of nitrogen seems to indicate that nitrogen is concerned in the electrode process. The results of French and Kahlenberg (19) also pointed to this conclusion. This effect might conceivably be due to an equilibrium between nitrogen gas dissolved in the solution and nitrogen gas dissolved in the platinum metal.

In an organic oxidation-reduction system, the effect of the hydrogen-ion concentration upon the redox potential
is indirect. The hydrogen-ion concentration merely affects the relative amounts of reduced and oxidized substances which take part in the electrode reaction. In an inorganic system, if the hydrogen is to enter into the electrode reaction, hydrogen or oxygen gas must be present. This is the principle upon which the hydrogen electrode is based. In case all of the oxygen and hydrogen were eliminated by bubbling nitrogen through the solution, the pH of the solution should not affect the final Eh value obtained under an atmosphere of nitrogen. The data of Table I show that this is not exactly true. There still seems to be a relationship between these two quantities since the Eh tends to increase with the pH. This seems to indicate that the final equilibrium value under an atmosphere of nitrogen is due to the incomplete removal of oxygen.

In every case the change in Eh with time caused by bubbling nitrogen through a solution was completely eliminated when a little ferro-ferricyanide or quinhydrone was added to the solution. The potentials of each of these systems completely masked any potential change which previously occurred during the bubbling of nitrogen. This very strikingly illustrates the possibility of one strong electrode reaction in the soil completely masking the effect of any weaker reactions. If the potential obtained under an atmosphere of nitrogen
is such that it will mask the potential of the soil sus-
pension, the use of this gas is certainly undesirable.
We are therefore led to the conclusion that the practice
of measuring the redox potentials of soils under an at-
mosphere of nitrogen, as advocated by some soil investi-
gators, such as Volk (40,41,42) is probably not justi-
fi able. Furthermore, it is quite probable that the er-
ratio results obtained by this procedure, which have led
investigators in this field to doubt the utility and app-
plicability of redox potential data to the evaluation
of soil fertility factors, may be due to this cause.

There are other reasons why measurement of the redox
potential under an atmosphere of nitrogen is undesirable.
First, Parks (35) has shown that soil suspensions show a
drift of potential in an atmosphere of air after about
twenty minutes. Since it requires from 2 to 10 hours
for nitrogen to come to apparent equilibrium with the
suspension, the Eh value of the soil will doubtlessly
have changed considerably before any comparable readings
can be obtained. Secondly, measurements under an atmos-
phere of nitrogen will cause marked changes in the micro-
bial activity, both in the rate of decomposition, and the
types of microorganisms that are active. Thirdly, any
enzyne systems such as gluthathione or cytochrome b,
which are autooxidizable, will, under anaerobic condi-
tions, tend to revert to the reduced state. Hence,
bubbling nitrogen through the suspension may actually change the real potential of the system.

The foregoing reasoning leads to an important recommendation which should be made regarding the conditions under which the redox potentials of soils should be measured. The system involving an atmosphere of air was found to give the most stable and reproducible potentials. Hence it is desirable to make such measurements in the presence of air, and as previously shown in this thesis, with vigorous and constant mechanical stirring. These conditions were accordingly used in all subsequent measurements on incubated soils.

It is recognized that even under these conditions there may be a slight oxygen effect, but it may be safely assumed that such an effect will be small because of the slight solubility of air in water. In any event, the values obtained for a series of soils, though perhaps not of absolute significance, will be comparable, and the redox potential may then give a reliable indication of the changes occurring in the soil during organic matter decomposition and its effect on soil fertility.
INCUBATION STUDIES

McGeorge (32) and Breazeale and McGeorge (4) have shown that western soils with a low organic matter content puddle easily under irrigated conditions and that when puddling occurs anaerobic conditions prevail. When organic compounds decompose in the soil there is liberated a wide variety of dissimilation products. But the compounds formed under aerobic conditions are quite different from those formed under anaerobic conditions. In the studies on the influence of decomposing organic compounds upon the Eh value of soils, therefore, it seemed desirable to carry out this decomposition under both puddled and unpuddled conditions.

Method of Incubation

In order to obtain comparable results, one soil only, a Palos Verdes sandy loam, was used in these experiments. A description of this soil will be found in the Soil Survey of the Tucson Area (39A). The particular sample used was obtained about one half mile west of the junction of Oracle and Ina roads. This junction is approximately four miles north of Tucson on the Oracle road. The physical characteristics of this soil, according to Caster (11) are:
Apparent density 1.75
Specific gravity 2.54
Volume expansion 10.90%
Pore space 38.00%
Water Holding Capacity 24.00%
Moisture Equivalent 13.40%
Moisture at Saturation 24.00%

This is a virgin, non-calcareous soil. Its organic matter content is less than 0.1%.

The incubation studies were arranged as follows:

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<tbody>
<tr>
<td>1.</td>
<td>SoPo00</td>
<td>11.</td>
<td>SoPo10</td>
<td>21.</td>
</tr>
<tr>
<td>2.</td>
<td>SoPo01</td>
<td>12.</td>
<td>SoPo10</td>
<td>22.</td>
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<tr>
<td>5.</td>
<td>SoPo04</td>
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<td>7.</td>
<td>SoPo06</td>
<td>17.</td>
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</tr>
<tr>
<td>10.</td>
<td>SoPo09</td>
<td>20.</td>
<td>SoPo10</td>
<td>30.</td>
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</table>

in which So and Sl = soil sterilized and unsterilized, respectively,

P0 and Pl = soil puddled and unpuddled, respectively, and

Oo, O1, ..., Og = no treatment with organic matter, cystine, starch, gum arabic, cellulose, urea, benzoic acid, succinic acid, and casein added at the rate of 0.5% respectively.

The procedure for the preparation of the incubation samples was as follows: Fifty grams of soil were placed in each of 36 glass tumblers, the experiment being replicated eight times. Each eight replicate samples were then given a treatment to correspond to one of those listed above. When organic materials were added, they were incorporated thoroughly with the soil in the dry
state and at the rate of 0.25 grams per 50 grams of soil. Sufficient moisture was added to bring the soil samples up to 60 per cent of their water holding capacity, and they were maintained at this level throughout the course of the experiment by frequent additions of distilled water. The samples were incubated at 30° C for various lengths of time, when some of them were removed for the redox potential measurement.

The proper samples were puddled at the time the moisture was added, and every two days thereafter. It was accomplished by working the soil with a spatula until sticky. Sterilization was carried out in an autoclave at 15 lbs. pressure for two hours.

Samples were removed for analysis at the end of one, two, four, seven, twelve, and twenty days. Upon removal each sample was divided in half, and from the two halves two 1:5 soil-water suspensions were prepared. These were then placed in a constant temperature bath at 30° C, and the potentials read continuously until the readings became constant, which usually took about twenty minutes. After that time there was usually a negative drift in potential value.

**Results of Incubation Studies**

The results of these experiments are shown in Figs. 11 to 19. Inspection of the graphs shows that the organic
Palos Verdes Sandy Loam
+
STARCH (0.5%)

UNPUDDLED

PUDDLED STERILE

UNPUDDLED STERILE

pH AFTER INCUBATION — 7.60

FIG. 11
INCUBATION TIME
FIG. 12 INCUBATION TIME

Palos Verdes Sandy Loam
+ Gum Arabic (0.5%)  

pH after incubation — 8.05
FIG. 13

**Palos Verdes Sandy Loam**

+ Casein (0.5%)

pH after incubation = 8.60

- Puddled Sterile
- Unpuddled Sterile
- Unpuddled
- Puddled

Incubation time graph showing changes in pH over 18 days.
Palos Verdes Sandy Loam

+ Cystine (0.5%)

pH after incubation — 7.90

FIG. 14

Incubation Time

0 2 4 6 8 10 12 14 16 18 Days
MV.

<table>
<thead>
<tr>
<th>600</th>
<th>UNPUDDLED</th>
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<tr>
<td>500</td>
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<td>400</td>
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<td>100</td>
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FIG. 15

Palos Verdes Sandy Loam + Succinic Acid (0.5%)

pH AFTER INCUBATION — 4.00
Palos Verdes Sandy Loam

+ Benzoic Acid (0.5%)

pH after incubation — 4.30

FIG. 16

Incubation time
Palos Verdes Sandy Loam

+ UREA (0.5%)
UNPUDDLED
PUDDLED STERILE
UNPUDDLED STERILE
PUDDLED

AFTER INCUBATION 18 DAYS

INCUBATION TIME

Palos Verdes Sandy Loam
+ cellulose (0.5%)

pH after incubation — 7.30
UNPUDDLED

UNPUDDLED STERILE

PUDDLED STERILE

PUDDLED

Palos Verdes Sandy Loam

NO ORGANIC MATTER

pH AFTER INCUBATION — 7.20
compounds can be grouped together on the basis of the similarity of the Eh curves. Figs. 11, 12, 13, show that the starch, gum arabic, and casein curves are very much alike. In these three graphs the Eh curve for the puddled and unpuddled sterile samples are almost identical. Under these conditions, therefore, all three compounds have the same effect on the redox potential. In each case where the soil is puddled but not sterilized, the Eh-curve drops rather rapidly at first and then gradually levels off to an equilibrium value some 300 millivolts lower than that of the original soil suspension. In the unpuddled non-sterile samples, the effect of the three compounds are also similar. However, the gum arabic curve tends to level off at a value approximately 125 millivolts higher than the original suspension while casein reaches an equilibrium value some 50 millivolts lower. The starch sample gave essentially constant Eh values. It should be noted here that these values for casein are different from those secured by Burrows and Gordon (8), who found that the Eh rises with time.

The Eh curve for cystine is quite different from those secured for the other organic materials. The redox potentials of the puddled and unpuddled samples, which had been sterilized, showed no change with time, but are about 100 millivolts lower than is shown by the same curves for the starch, casein, and gum arabic. The
curve for the non-sterile sample is similar to that for starch, etc., but the curve for the unpuddled non-sterile sample drops rather sharply at first, reaching a value of about 250 millivolts lower than that of the original suspension in about four days. It then gradually rises to approximately the original value. The reason for this is not exactly known. Since the cystine of the cystine-cysteine system is already in the oxidized form, it is surprising that a strongly reducing potential is developed under aerobic conditions. It is possible that the sulfides such as hydrogen sulfide and ethylene sulfide which are formed from cystine account for this low potential. These sulfides would tend to accumulate because their formation by the numerous soil heterotrophs is more rapid than their oxidation to free sulphur and sulphates by the sulfifying bacteria. Hydrogen sulfide could be strongly detected in these aerated samples when they were in the lowest state of reduction.

The succinic acid and benzoic acid curves are also very similar. Here again no potential change occurred in the sterile sample, and the non-sterile puddled and unpuddled curves were practically super-imposable. Even though the minimum Eh value of the curve for the puddled sample was some 75 millivolts lower than that for the unpuddled samples, it was nevertheless greater than the value for the original sample for both compounds. The
pH value of these soil-acid samples was about four. This low pH would favor mold growth, which leads to dissimilation products different from those which are formed by the bacteria and actino-mycetes which predominate at the higher pH values.

The Eh curves for the cellulose, urea, and untreated samples were almost the same. It is assumed that the initial drop which occurred in the puddled non-sterile sample was due to substances already present in the soil. After seven days, however, the Eh value had again risen to its former value. This may have been due to the decreased activity of the microorganisms as the available food supply decreased. The cellulose curve is essentially the same as that for the untreated sample. Cellulose decomposes so slowly that little loss had occurred even after twenty days. This effect is due chiefly to the low nitrogen content.

In the unsterile urea sample, puddling had no effect after seven days. Since the decomposition products of urea are ammonia and carbon dioxide, the products formed under aerobic and anaerobic conditions should be essentially the same. Therefore, the two decomposition curves should be similar, which is shown by the close similarity of the curves to be the case.

It is recognized that the above explanations are rather brief. But in view of the fact that so little
work has been done on this particular phase of the redox potential problem, we do not feel that we yet have sufficient information for further explanations. The Eh curves of all of the possible dissimilation products of these various compounds must be determined before the phenomenon can be completely explained.

It should be pointed out here that in every case the sterile soil gave almost the same Eh curve whether the soil was puddled or unpuddled, and that the Eh value was constant with time. This is rather an important point, for if the only potential measured in the soil was an oxygen potential, then the Eh value of the sterile, unpuddled sample should have dropped to a lower value after a certain length of time. This is more obvious when it is noted that the conductivity water used in making up the soil suspension contained a constant amount of dissolved oxygen. It is safe to assume that the Eh measurements were completed before all of the dissolved oxygen could be used; nevertheless, the Eh values of the sterile, unpuddled samples steadily dropped. Therefore, it can be assumed that the values obtained under puddled non-sterile conditions are the potential changes caused by the microorganisms, and that the potential changes so measured represent more nearly the true potential of the soil suspension.
SUMMARY

1. A study has been made of the conditions affecting the method of measuring the redox potentials of soils in water suspension and the method applied to soil samples which had been treated with various pure organic compounds.

2. Because of polarization of the electrodes in poorly poised soil suspensions a vacuum tube circuit was constructed to eliminate this difficulty. The construction and operation of such a circuit is described.

3. Studies made on the type of platinum electrode best suited for redox potential measurements indicated that a platinum foil electrode was the most satisfactory. A method of cleaning such electrodes is described.

4. The effect of stirring on the redox potential was determined. The results obtained lead to the recommendation that all soil suspensions be vigorously stirred while the potential is being measured.

5. The effect of oxygen, nitrogen and air on the potential of the platinum electrode in various buffer solutions and soil suspensions was determined. These results indicate that the measurement of the potential under an atmosphere of nitrogen is not justified. Consequently it is recommended that the potential of the
6. The effect of the decomposition of pure organic compounds on the redox potential of the soil were determined. The compounds used were starch, gum arabic, cystine, casein, urea, succinic acid, benzoic acid and cellulose. It was found that these compounds could be grouped together on the basis of the similarity of the Eh curves. In all cases the Eh values obtained in the puddled and unpuddled samples which were sterilized were found to be constant. Therefore, it can be assumed that the values obtained under puddled non-sterile conditions are the potential changes caused by the microorganisms and that the potential changes so measured represent more nearly the true potential of the soil suspension.
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