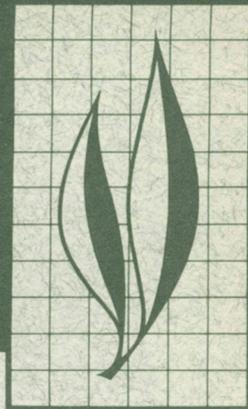


HILGARDIA

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Measurement of Oxygen Diffusion Rates with the Platinum Microelectrode

I. Theory and Equipment

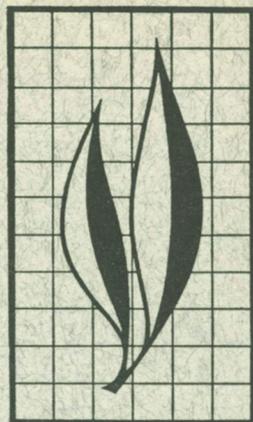
J. Letey and L. H. Stolzy

II. Factors Influencing the Measurement

D. E. Birkle, J. Letey, L. H. Stolzy,
and T. E. Szuszkiewicz

III. Correlation of Plant Response to Soil Oxygen Diffusion Rates

L. H. Stolzy and J. Letey



In 1952, E. R. Lemon and A. E. Erickson introduced a method for measuring the rate of oxygen diffusion to a small platinum wire electrode inserted into the soil. This was considered analogous to measuring the oxygen movement to a plant root that would be in the same position as the wire electrode.

In spite of certain limitations, the platinum microelectrode technique appears still to be the best method available at present for providing a measurement of oxygen conditions in soil which can be interpreted with respect to biological behavior.

The first two papers of this series discuss theory, equipment, and the factors that can influence measurements. The third paper reviews the literature reporting research on correlation of oxygen diffusion rates to biological response.

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II. Factors Influencing the Measurement¹

INTRODUCTION

IN MAKING the measurements, certain decisions about procedures could influence the results. It is important that all investigators follow the same procedures so that results will be comparable. Such factors as time to allow for steady-state conditions to be established, and choice of electrical potential were investigated.

Several soil factors influence the oxygen diffusion rate (O.D.R.) and may influence the results of measurements

obtained with the platinum microelectrode differently from the way in which they influence true diffusion rates. For reliable interpretation of data, it is necessary to know which factors affect the measured values to give erroneous results and which do not. Results of studies on measurement procedures and on soil factors are presented in this paper, together with recommended procedures for future experiments.

ESTABLISHING STEADY STATE

Immediately after the electrodes are inserted and a potential is applied, a high current occurs, which decreases rather rapidly and approaches a constant value. The reason for this behavior is that initially, oxygen surrounds the electrode; as soon as the potential is applied, that oxygen is reduced. Consequently, more oxygen must diffuse in from the surrounding medium. The electrical current is proportional to the amount of oxygen reduced. As oxygen diffuses from greater distances, it arrives at the electrode more slowly, thus creating a lower current. Eventually, the oxygen diffusion approaches steady state—in other words, a constant rate

resulting in a constant current. In measuring soil oxygen diffusion rates, a measurement during a steady state is desired.

Typical relationships between current and time are shown in figure 1. The decrease in current with time becomes very small after four or five minutes. In a system in which potential is applied simultaneously to 10 electrodes, approximately 45 seconds will be required to measure and record all values. If at least three minutes (preferably four) are allowed before reading the first value, very little difference will occur between the first and tenth because of difference in time of measurement.

CHOICE OF POTENTIAL

Oxygen begins to reduce at a potential of about -0.2 volt. As the potential

is increased, the current increases. (Increase is used in the sense of the numer-

¹ Submitted for publication October 2, 1963.

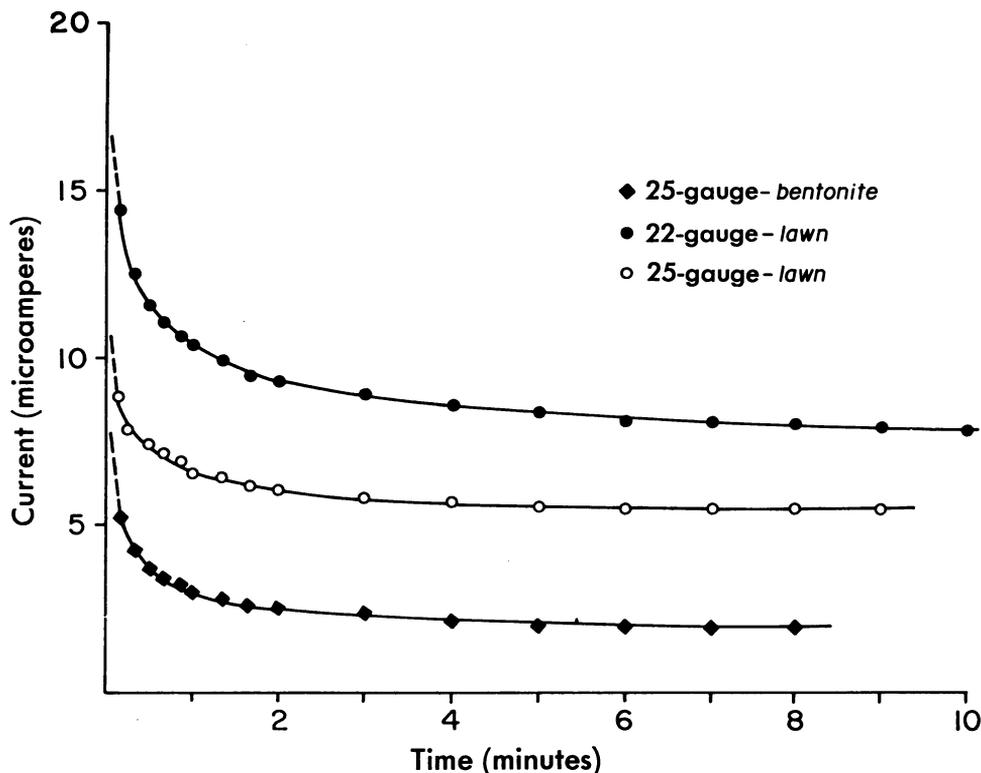


Fig. 1. Current as a function of time, after application of the potential for the electrodes, and under conditions indicated.

ical magnitude, neglecting the minus sign; i.e., -0.8 is considered to be higher than -0.2 .)

Lemon and Erickson (1955)² and Poel (1960) observed a low slope in the current-potential curve in the range of -0.4 to -0.8 volt when measurement was made in a soil-water suspension.

We studied the current-potential relation in unsaturated soil. The electrodes were inserted in a lawn; a potential was applied; and the current was observed, to determine when a steady-state condition (constant current) resulted. After steady state was achieved, the potential was changed and the current checked. Generally, the potential was increased in steps of 0.05 volt and then decreased in equal steps. The measurements were made in the range of -0.5 and -0.9 volt. In one case, electrodes were inserted and

a given potential was applied. After steady state, the current was recorded. The electrodes were removed and reinserted, and a different potential was applied. The procedure was repeated over the desired range of potentials.

The results of measurements with 25-gauge wire are presented in figure 2. A straight-line relationship between diffusion rate and potential occurred until the potential reached approximately -0.8 volt after which the diffusion rate increased more rapidly with increased potential. The diffusion rate, which is proportional to the current, is plotted, rather than the current, for purposes of comparison.

Diffusion rates in saturated soil are often less than 10. A curve with a slope of 10 would correspond to conditions under which Lemon and Erickson

² See "Literature Cited" for citations referred to in the text by author and date.

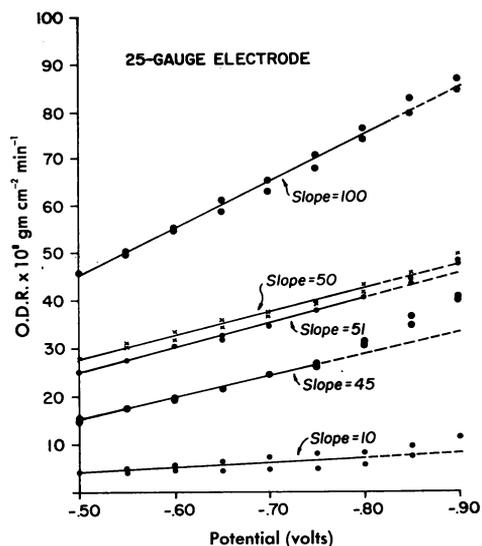


Fig. 2. O.D.R. as a function of applied potential, as measured with 25-gauge platinum wire electrode. Each curve represents measurements made in a particular environment. The slope values are $\times 10^8$ gm cm⁻² min⁻¹ volt⁻¹.

(1955) and Poel (1960) made their measurements. Under conditions of higher diffusion rates, the slope of the curve increases, and the "plateau" would tend to disappear. Lemon and Erickson actually demonstrated this when they equilibrated their soil-water suspension with high oxygen concentrations.

Since the curve has a finite slope even

at low oxygen diffusion rates, the increase in slope might be expected to be proportional to the increase in diffusion rate. If this were the case, the ratio of diffusion rate to the slope of the curve would be a constant for each curve. The ratios are presented in table 1. Although they are not identical for each curve, they fall within a relatively narrow range. Similar data for 22-gauge electrodes are given in figure 3 and table 2. The line with a slope of 6.8 in figure 3 was determined both by leaving the electrodes in place and varying the potential and by removing the electrodes and readjusting the potential before each insertion and measurement. Identical results were achieved.

From the data in figures 2 and 3, it is evident that the diffusion rate measured will depend upon the potential applied. If results of various investigators are to be comparable, all must use the same potential. A value measured under one potential may either be estimated or corrected to that of another potential by using the K values (O.D.R./slope) given in tables 1 and 2. However, K is not constant, and an error in O.D.R. as high as 15 per cent can result in making an adjustment between potentials. This being the case, it would obviously be preferable for all investigators to use one potential in measuring O.D.R.

TABLE 1
RATIOS OF DIFFUSION RATE TO SLOPE OF DIFFUSION RATE-POTENTIAL CURVE AT FOUR POTENTIALS SHOWN IN FIGURE 2

Slope	Potential							
	-0.65V		-0.70V		-0.75V		-0.80V	
	O.D.R.	K*	O.D.R.	K*	O.D.R.	K*	O.D.R.	K*
100.....	60	.60	65	.65	70	.70	75	.75
51.....	33	.65	36	.70	38	.74	40	.79
50.....	35	.70	38	.75	40	.80	42	.85
45.....	22	.49	24	.53	26	.59	29	.65
10.....	5.5	.55	6	.60	6.5	.65	7	.70
Average.....		.60		.65		.70		.75

* K = O.D.R./slope.

Any potential between -0.55 and -0.75 volt could be used. However, in several instances, particularly with the 22-gauge electrode (figs. 2 and 3), the O.D.R. potential departs from linearity at a potential of -0.8 volt, and -0.75 might be too close to that point of departure. To measure as high a current as possible is advisable for a given soil oxygen status, particularly if the currents involved are already low. However, with low currents, the increase in current with increased potential is also low.

Many investigators have published results of measurements made at -0.65 volt (see third paper in this series). Since no compelling reasons exist for using other potentials, we recommend -0.65 volt for measuring O.D.R.

Failure to observe a plateau in the O.D.R.-potential curve raises the question of whether the current is diffusion controlled. Recent preliminary investigations indicate that the current is diffusion controlled under conditions of low O.D.R. but not under conditions of high O.D.R. These studies are being continued.

If it should be necessary to convert a value from one potential to another, the method is as follows: Assume that a 22-

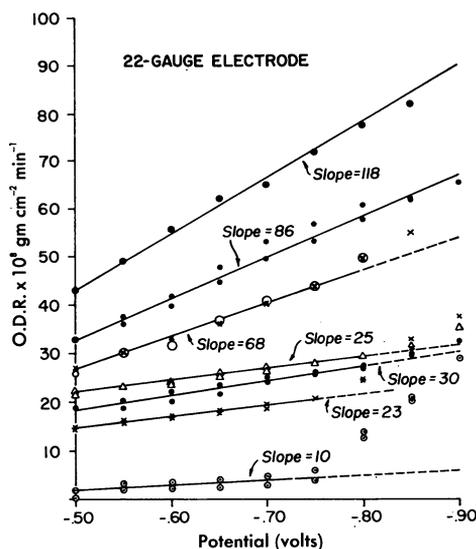


Fig. 3. O.D.R. as a function of applied potential, as measured with 22-gauge platinum wire electrode. Each curve represents measurements made in a particular environment. The slope values are $\times 10^8 \text{ gm cm}^{-2} \text{ min}^{-1} \text{ volt}^{-1}$.

gauge electrode is used and an O.D.R. of 60×10^{-8} is calculated when a potential of -0.75 volt is applied. It is desired to determine a comparable O.D.R. if -0.65 volt were used. From table 2, the K value (O.D.R./slope) for -0.75 volt is .75. The slope of the O.D.R.-potential line is calculated by dividing the O.D.R.

TABLE 2
RATIOS OF DIFFUSION RATE TO SLOPE OF DIFFUSION RATE-POTENTIAL CURVE AT FOUR POTENTIALS SHOWN IN FIGURE 3

Slope	Potential							
	-0.65V		-0.70V		-0.75V		-0.80V	
	O.D.R.	K*	O.D.R.	K*	O.D.R.	K*	O.D.R.	K*
118.....	61	.52	66	.56	72	.71	78	.66
86.....	46	.54	50	.58	54	.63	58	.68
68.....	37	.54	40	.59	44	.65	47	.69
30.....	23	.77	24	.80	26	.87	27	.90
25.....	26	1.04	27	1.08	28	1.12	29	1.16
23.....	18	.78	19	.83	20	.89	†
10.....	3.2	.32	3.8	.38	4.1	.41	†
Average.....		.64		.69		.75		.82†

* K = O.D.R./slope.

† Values were on portion of curve that was not linear.

‡ For O.D.R. greater than 25.

by K. For the example stated, the slope would be 60×10^{-8} divided by .75, equal to 80×10^{-8} gm cm⁻² min⁻¹ volt⁻¹. A de-

crease of 0.1 volt would therefore result in a decrease in O.D.R. of 8×10^{-8} . The O.D.R. at -0.65 should equal 52×10^{-8} .

INSTALLATION OF ELECTRODES

The porous cup is used to complete the salt bridge, and the electrodes are placed in the soil. A hole slightly smaller than the cup may be made for easy insertion. Good contact between cup and soil is essential. For measurements at shallow depths, the electrodes may simply be pushed into the soil. At greater depths, the problem of electrode installation becomes more complex. Whatever insertion techniques are designed, it is imperative that the method not alter the soil-oxygen status at the point of measurement. If a hole is made by an auger or a rod, force the electrode as far as possible beyond the bottom of the hole, because atmospheric oxygen will now have been introduced there rather than at the soil surface. Take measurements as soon as possible to lessen the probability of oxygen change at the point of interest as a result of opening the access hole. Chances of modification of oxygen at measuring point are lower in wet rather than relatively dry soil.

Wiersum (1960) constructed a rugged platinum electrode for insertion to great depth. It overcomes the difficulties described in electrode installation at depths greater than a few centimeters, but its geometry is such that Wiersum's results are not comparable with those obtained by most other investigators.

Measurements at great depths could become simpler and more meaningful if the electrodes could be buried and left in place throughout the course of an investigation. However, because of electrode "poisoning" (p. 560), the electrode cannot be left in the soil for an extended period of time.

To determine whether the distance between the porous cup and the electrode

affects the results, a study was conducted, on a lawn, with 22-gauge, epoxy-sealed electrodes. The porous cup was placed approximately 10 cm from electrodes numbered 1 through 5, and 2.5 m

TABLE 3
EFFECT OF DISTANCE BETWEEN ELECTRODE AND POROUS CUP ON CURRENT PER UNIT ELECTRODE LENGTH

Electrode number	Distance between cup and electrode		
	0.1 m	1.2 m	2.5 m
	<i>current per unit electrode length*</i>		
1.....	12.4	11.4	11.9
2.....	12.4	11.9	10.6
3.....	15.0	13.8	14.4
4.....	9.5	13.4	12.1
5.....	14.4	16.7	13.0
6.....	13.2	12.9	16.1
7.....	7.5	14.2	10.8
8.....	12.4	7.8	8.1
9.....	13.8	13.5	14.2
Average†.....	12.4	12.8	12.5

* Each value is an average of four measurements.
† No significant difference at 5 per cent.

from electrodes 6 through 10. The O.D.R. was measured, and the electrodes were moved slightly for four successive measurements. The cup was then placed near electrodes 6 through 10, and 2.5 m from electrodes 1 through 5, for four more measurements. Finally, the cup was placed equidistant between the two sets of electrodes, and the procedure was repeated four times. Results are shown in table 3. (One electrode was found to be faulty so the results of only nine are presented.) No significant difference occurred in current per unit electrode length at various distances. The electrodes may therefore be placed at least 2.5 m from the cup without affecting the results.

POISONING

The term "poisoning" denotes a change in the platinum surface—probably a chemical deposit—that changes the electrode reaction. To investigate poisoning, we buried thirty 25-gauge and ten 22-gauge electrodes in the soil for five weeks. Equal numbers of electrodes were removed just before meas-

At the third week, the mean values for all treatments were nearly equal; however, the variance at that time was higher than it was at any time throughout the experiment, and no statistical test could be made.

The very low O.D.R. at three and one-half weeks resulted from the very high

TABLE 4
OXYGEN DIFFUSION RATES SHOWING "POISONING" OF ELECTRODES

Time (weeks)	22-gauge electrode			25-gauge electrode		
	O.D.R. removed	O.D.R. stationary	F _{1,18} †	O.D.R. removed	O.D.R. stationary	F _{1,18} †
0.....	30.1	37.1	1.50	47.3	43.5	< 1
1.....	46.0	45.5	< 1	54.3	51.6	< 1
2.....	52.7	42.0	1.73	66.7	43.1	18.08***
3.....	51.6	50.2	< 1	54.9	52.1	< 1
3½.....	14.8	3.7	32.18***	15.2	11.4	4.32*
4.....	46.6	28.5	31.57***	51.0	39.8	4.28*
5.....	48.0	26.8	16.28**	47.6	39.1	3.19
Moved and reinserted.....	45.0	37.8	1.59	46.8	41.6	1.20
Polished.....	42.2	37.5	1.74	51.1	50.0	< 1

* **, ***, indicate significance at .05, .01, and .001, respectively.
† F values. Subscripts indicate degrees of freedom.

urements were taken, and then reinserted. Readings were taken at one-week intervals, under fairly constant moisture conditions. One reading, at three and one-half weeks, was made at a high soil moisture level.

Table 4 indicates that even after electrodes had been left in the soil for only two weeks, a significant difference showed in the readings. This continued until the fifth week, when all electrodes were removed, reinserted, and new measurements were made. Following this, all electrodes were polished with steel wool, and reinserted for the final reading. It can be seen (table 4) that both these treatments tended to bring the electrodes to a common O.D.R. regardless of previous treatment.

moisture content of the soil, but the difference was still significant.

The data indicate that poisoning could occur on buried platinum electrodes, but it can be prevented merely by removing the electrodes from the soil and reinserting them. The abrasive action of the soil apparently removes whatever deposit is causing the decrease.

Possibly the geometry of the soil-water system around the electrode is modified by insertion, thus resulting in differences in oxygen diffusion to the electrode, and the accompanying unequal readings. If this is true, the electrode should be left in the soil, because it would sample a more nearly true environment.

SOIL FACTORS INFLUENCING MEASUREMENT

Soil Moisture

Theoretical data (see first paper in this series) show that the O.D.R. is expected to increase as the soil moisture decreases. Measurements taken as a function of moisture (Lemon and Erickson, 1955; Lemon and Kristensen,

balance with a tensiometer inserted (fig. 4), thus allowing moisture-release curves to be obtained simultaneously with the oxygen diffusion rates. Soils used were < .5-mm mixed sand and a Ramona sandy loam screened through a

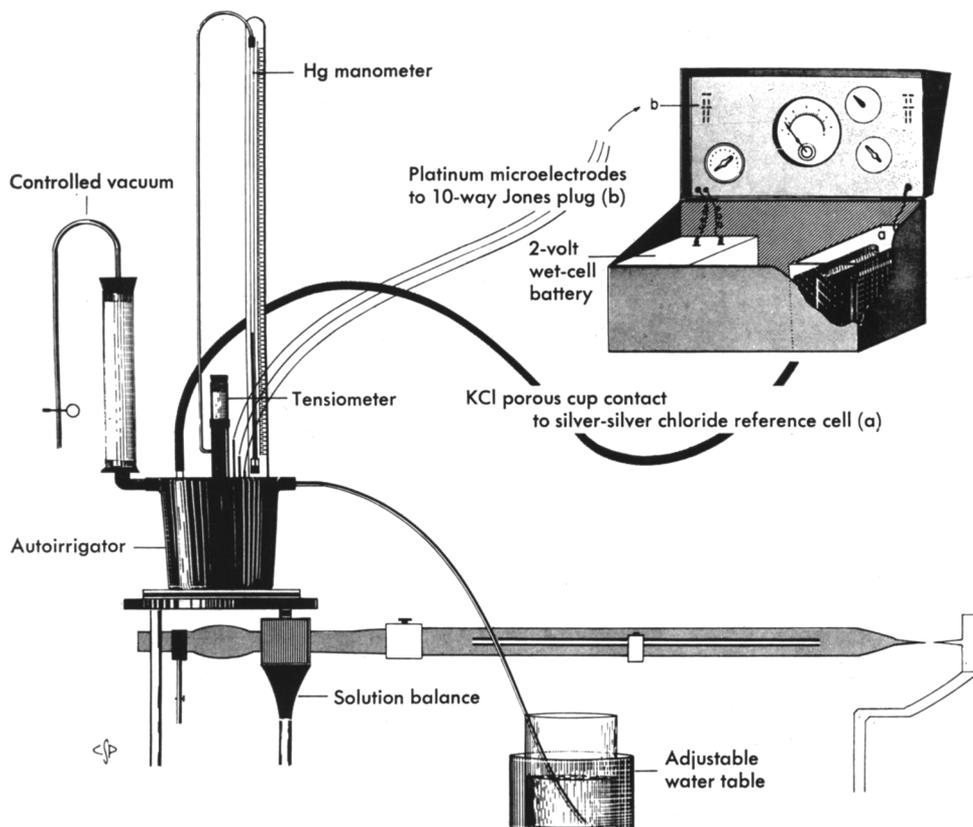


Fig. 4. Apparatus for studying O.D.R. as a function of moisture.

1960), however, showed this to be true to a point, after which the O.D.R. decreased with further lessening of the moisture content. The effect of electrode size on this behavior has not been previously investigated.

To study this problem, a ceramic double-walled pot was used for moisture control. Water was held in the pot between a porous inner wall and a glazed outer wall. The pot was mounted on a

5-mm sieve, divided in half, and one half being washed with 0.1*N* HCl until a pH 3 was obtained. All materials were wet from the bottom to exclude as much air as possible.

Ten electrodes of two sizes (22- and 25-gauge) were used. Current was read four minutes after the applied potential of -0.65 volt.

Curves presented in figure 5, for the sand, show the general type of relation-

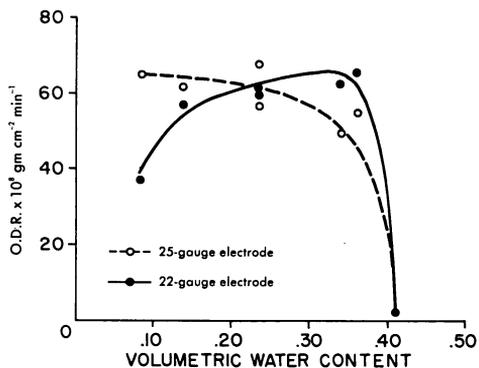
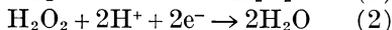
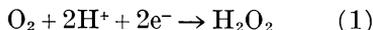


Fig. 5. Oxygen diffusion rates at various moisture contents, for two different-sized platinum microelectrodes in sand.

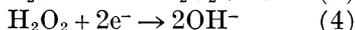
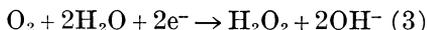
ship between moisture content and the measured diffusion rate. It can be seen that, at low moisture contents, the curves level off and begin to drop. Furthermore, the curve of the larger (22-gauge) electrodes tends to decrease at a higher moisture content than does that of the 25-gauge, in sand. Results for the other soils are shown in figure 6.

The decrease in O.D.R. at low soil-water content has been explained as a disruption of the moisture film around the platinum electrode (Lemon and Erickson, 1955; Lemon and Kristensen, 1960) and as the slow diffusion, from the electrode, of OH^- ions formed by the reaction (Lemon and Kristensen, 1960). A build-up of OH^- would tend to cause the electrode reaction for a neutral or alkaline soil (see below) to shift from right to left in a neutral or alkaline medium.

Acid medium



Neutral or alkaline medium



Since the decrease in O.D.R. is noted on the acid soil, accumulation of OH^- presumably is not the cause. Furthermore, the moisture film would be expected to rupture on the larger-sized

wire first. The data indicate that incomplete wetting of the electrode causes the decrease in O.D.R. Further investigation, varying the OH^- concentration in an inert medium, indicated the same conclusion.

The lower O.D.R. in the acid-treated soil is the result of changes in structure and soil packing as a result of the acid treatment.

The possible OH^- effect was studied by taking diffusion rates in silica sands saturated with NaOH at various concentrations. These data, presented in figure 7, show that the O.D.R. drops off slowly with increasing OH^- concentration until about 0.6N concentration is reached. Then the diffusion rates drop to a very low value and remain constant. The question is, do OH^- concentrations of this magnitude develop at the electrode surface while the O.D.R. measurement is being made?

Since the extent of a chemical reaction can be related to current by application of Faraday's law, the number of OH^- ions produced by the reduction of oxygen at the platinum electrode can easily be calculated. Faraday's law states: 96,500 coulombs of electricity produce a chemical change of 1 gram equivalent. For oxygen reduction

$$96,500 \text{ amp-sec} = 17 \text{ gm OH}^- \quad (5)$$

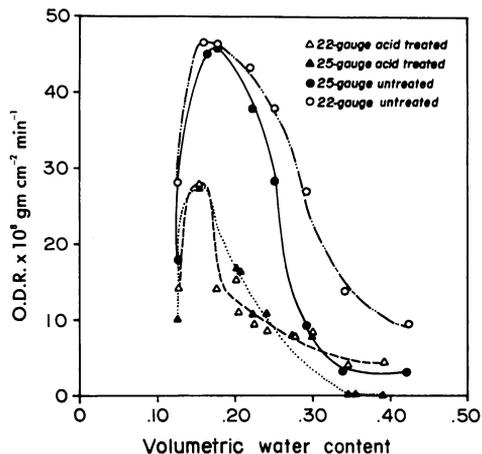


Fig. 6. Oxygen diffusion rates in Ramona sandy loam, showing effect of electrode size and acid treatment.

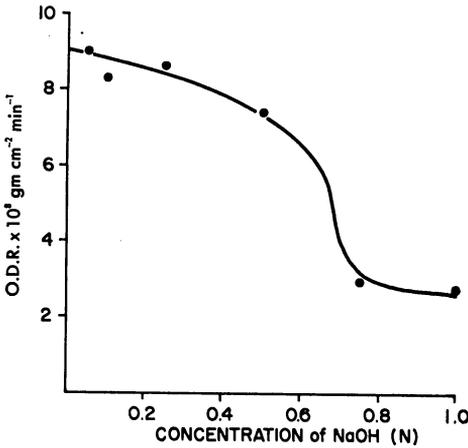


Fig. 7. Oxygen diffusion rates in sands saturated with various solutions of sodium hydroxide.

Therefore, dividing by the Faraday constant

$$1 \text{ amp} = 1.76 \times 10^{-4} \text{ gm OH}^- \text{ per second} \quad (6)$$

since we are dealing only with microamperes, the practical relationship for our problem becomes

$$1 \text{ microamp} = 1.76 \times 10^{-10} \text{ gm OH}^- \text{ per second} \quad (7)$$

This amount of OH⁻ can be compared with concentrations studied in the sand if we know the volume in which the electrode exists and the amount and time that the current flows. Using the volumes and theoretical O.D.R. presented in the first paper of this series, we obtain the curves shown in figure 8.

Those curves show that the hydroxide-ion concentration does not reach the general order of magnitude at which it could significantly reduce the current until extremely high diffusion rates are reached. This also indicates that an accumulation of OH⁻ concentration does not cause the reduction in current at low moisture contents.

Thus, soil moisture levels are important in determining the oxygen diffusion rate. With the high moisture con-

tents, the electrode is affected in the same manner as is the plant root in obtaining oxygen, and therefore gives a true indication of the oxygen availability in soils. At lower moisture contents, where plant symptoms due to low oxygen are not usually noted, the diffusion rate drops as a result of incomplete electrode wetting. Accumulation of OH⁻ concentration is not considered to cause this drop—rather, the decrease in O.D.R. is a mere artifact associated with the measurement and in no way indicates that oxygen is a limiting factor to plant growth at low moisture contents.

Salt Content of the Soil

The conductivity of the soil may be important in measuring diffusion rates since the reaction is electrochemical. Oxygen diffusion rates were taken in sands saturated with various concentrations of KCl and K₂SO₄ solutions. Figure 9 shows the data taken from the KCl system. In order to effectively reduce the current at low concentrations, triple-distilled, deionized water was needed. When plain deionized water was used, the diffusion rate was back to a level comparable with those taken in salts at higher concentrations. Figure 9

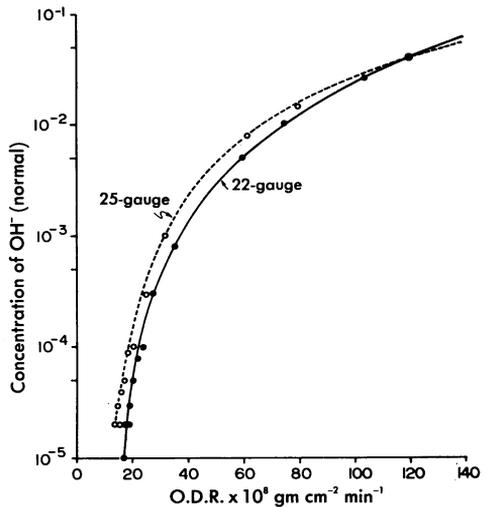


Fig. 8. Theoretical hydroxide ion build-up as a function of oxygen diffusion rates.

Oxygen diffusion rate vs. KCl concentration
in a saturated sand

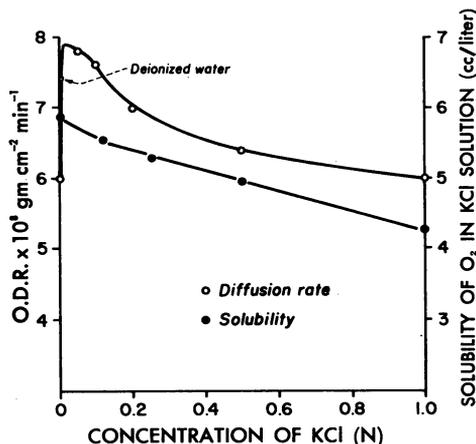


Fig. 9. Oxygen diffusion rate and solubility of oxygen for various KCl concentrations. Diffusion rates were taken in saturated sands with -0.65 volt applied for five minutes.

shows that a maximum is reached at about $0.05N$ solution, and the rate then drops off. It is assumed that at this point the conductivity of the solution is high and the solubility of the oxygen is not yet limiting.

On figure 9, solubility of oxygen is plotted as a function of KCl concentration. At higher concentrations, the diffusion rate shows the same type of linear decline as that of the solubility curve, indicating further that the factor being measured in the soil is the oxygen availability.

Similar data for K_2SO_4 (fig. 10) show that the oxygen solubility is the only important factor in determining the diffusion rate in this salt. It will be noted that the oxygen diffusion rate in the K_2SO_4 is lower than that of the KCl in every case. Also, the curves tend to

separate as the concentration of the salts increases. Table 5 shows that the solubility of oxygen in KCl and K_2SO_4 tends to follow the same type of relationship as that of the O.D.R. in the

TABLE 5
SOLUBILITY OF OXYGEN IN KCl AND
 K_2SO_4 SOLUTIONS AT 25°C

Concentration of salt	Concentration of O ₂ in KCl	Concentration of O ₂ in K ₂ SO ₄
<i>N</i>	cc/l	cc/l
.125	5.52	5.11
.250	5.30	4.66
.500	4.98	3.89
1.000	4.26

SOURCE OF DATA: Seidell, A. Solubilities of inorganic and organic compounds. New York: D. Van Nostrand Co. Vol. 2, p. 471. 1919.

two solutions. The O.D.R. measuring technique may involve some factor that keeps the relationship between O.D.R. and oxygen solubility from being directly linear; however, the correlation is close enough to be considered the main factor. We conclude that enough salt is found in soils to provide conductivity for obtaining maximum diffusion rates.

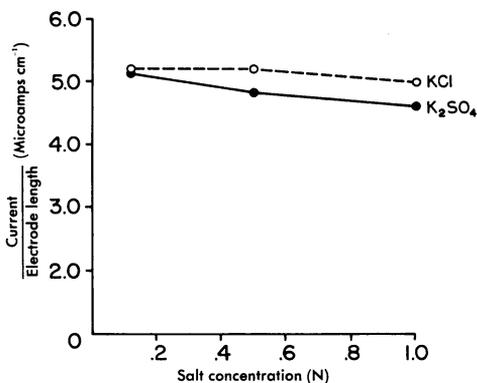


Fig. 10. Oxygen diffusion rates in KCl and K_2SO_4 at various concentrations.

OXYGEN CONCENTRATION

Lemon and Erickson (1955) studied the current as a function of the oxygen concentration. They found a linear relationship with a slope that was de-

pendent upon the time the electrode was allowed to equilibrate with the soil suspension. Their data, however, indicated a residual current when the oxygen con-

centration was zero. According to their description, gas flow and stirring were stopped completely one minute before the measurement was taken. This would allow time for some diffusion of oxygen back into the system, and hence give rise to a current at zero concentration.

To determine if this was the case, we used a lucite container with holes to accommodate the porous cup, 10 electrodes, a stirrer, and a tube for bubbling gas through the bentonite suspension. The holes were sealed as well as possible with black plastic tape, and gas was allowed to bubble through the stirred suspension at a rapid rate. Just before measurements were taken, the bubbler and stirrer were raised to a position just above the suspension, and the gas was allowed to continue to sweep out the chamber. Mixtures of nitrogen, oxygen, and air were used to change the oxygen levels. Pure nitrogen was used to obtain zero oxygen. The electrodes were lowered and the measurements taken after 10 minutes of applying -0.65 volt. The results in figure 11 show a straight-line curve very similar to that produced earlier (Lemon and Erickson, 1955);

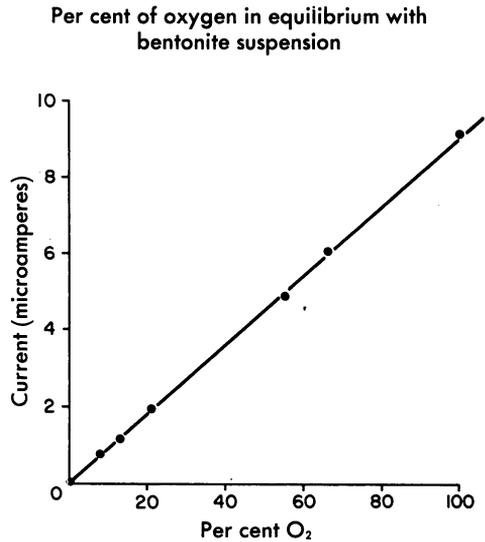


Fig. 11. Current produced by reduction of oxygen at the platinum microelectrode surface as a function of oxygen concentration in a bentonite suspension.

however, instead of an intercept on the current axis, we obtained zero current with zero oxygen. As shown by Lemon and Erickson, this linear relationship indicates that no substance other than oxygen is reduced.

TEMPERATURE

The measurement of oxygen diffusion rate depends upon such temperature-controlled factors as concentration, diffusion coefficient, and rate of reaction. In experiments with cotton and sunflowers, higher oxygen diffusion rates were obtained in containers in which the daytime temperature was 31°C than in containers at 23°C (Letey *et al.*, 1961). Since it was not the purpose of the experiment to obtain any relationship of temperature to oxygen diffusion rate, these data were not conclusive. In another experiment, sunflowers were grown in a controlled environment at

three air temperatures and two soil temperatures (Letey *et al.*, 1962). Night and day temperatures did not change, and a more nearly accurate measurement was taken as a function of the temperature.

These results indicate a 1.8 per cent increase in the oxygen diffusion rate per degree centigrade. This agrees with theoretical calculations based upon change in the diffusion coefficient of oxygen in water and the concentration of oxygen as a function of temperature in this range.

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