INVESTIGATION OF THE OXYGEN UPTAKE OF DRIED, REMOISTENED SOILS

by

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INTRODUCTION

The past few years have seen a considerable resurgence of interest in the rather parochial field concerned with the physiological reactions of soils. The phenomenon which has received particular attention is the high rate of respiration which results immediately after dried soil has been remoistened. This activity seems to be correlated with a marked improvement in soil fertility and therefore has elements of practical application.

Outside of this consideration, there has been a desire to arrive at a basic understanding of the phenomenon. There is an apparent anomaly involved. The increase in respiration occurs in spite of the fact that the population has been greatly reduced.

A portion of the present experimentation was aimed at isolating some specific factor which might prove predominant. A more fruitful approach proved to lie in the characterization of the earlier phases of the activity after remoistening.

REVIEW OF LITERATURE

The attention of the prominent bacterial physiologist, Otto Rahn (1907), was drawn to this respiratory effect in soils, and he published the results of an intensive investigation of the effect of drying on the physiological activity of soil. He noted that soils which had been dried and remoistened showed a greater amount of bacterial activity than soils which had been kept moist. He also observed that a rich garden soil showed this effect to a greater degree than a sandy soil. He was at a loss to explain this since

the bacterial numbers were greatly decreased. Tests with plants showed that the general fertility of the soil was greatly improved. In this work Rahn measured the bacteriological activity by means of carbon dioxide and acid production in sugar solutions, and ammonia production in peptone solutions.

Soil chemists had earlier made use of soil analysis techniques and discovered chemical changes due to drying treatment (Richter, 1896). The production of carbon dioxide was also used as an index of activity and assayed by titration. A more direct method is, of course, the direct measurement of the volume of oxygen taken up. It is interesting to find that as early as 1905, Russell (1905) made use of a manometric method for measuring the physiological activity of soil directly by its uptake of oxygen. He devised an apparatus identical in principle to the Warburg flask and manometer. Unfortunately, he did not investigate the effects of drying. He was interested in the correlation between fertility and respiratory activity. Russell felt that the assumption that the evolution of carbon dioxide was directly proportional in all cases to the uptake of oxygen was unwarranted.

The soil microbiologists of this period were inspired by hopes that their work would have directly profitable applications in agriculture. The well established observation that drying of soils had a definite stimulatory effect on plant growth continued to encourage considerable experimentation to determine the causes.

Howard and Howard (1910), as well as Russell (1910), published papers calling attention to what they termed the fertilizing effect

of sunlight. Howard and Howard thought that it was probably due to partial sterilization of the protozoan population. Russell felt that a factor which limited productiveness had been removed. Greig-Smith (1911) held that the bacteriotoxins are destroyed by the sun-drying and permitted enlargement of the population.

Fischer (1912) commented on the conclusions drawn by Rahn and others that the activity was biological. He noted that oxidation must be the principal factor, since nitrates are increased on drying. But he considered the fact that nitrifying organisms are killed made it likely that colloids and surface tension must play an important part in this oxidation. He remarked on the low respiratory rate of bacteria in the soil after they had reached maximum growth, although they were still able to develop on a plate.

Klein (1915) did considerable experimental work with dried soils at Cornell University. He measured the carbon dioxide production from dried soils and confirmed that it was greatly increased. The rate subsided to normal in about 35 days. His work included an excellent review of the early literature on the subject of the physiology of dried soils.

Waksman and Starkey (1923) did extensive work on this phenomenon. They also were struck by the fact that carbon dioxide evolution reached a peak in the first 24 hours and then fell faster than the population counts. Mere mixing, they found, did not result in more than a slight stimulation. They were quite sure that the results were due to changes in condition of the soil and its organic matter, and to some degree a shift of population groups, rather

than removal of any one organism. They proposed that a rapid germination of spores and mycelial growth took place when the soil was remoistened, and that this was of major importance in the production of carbon dioxide.

They reported that longer drying periods, even up to 519 days, gave progressively more activity. In an effort to compare these results with other partial sterilization methods they heated soils to 65°C for one hour. This resulted in an immediate decrease in population followed by a rapid increase. Treatment with volatile disinfectants had a similar effect. It was clear from these results that three treatments: (1) drying, (2) mild heating and (3) application of volatile disinfectants gave similar results.

It is of interest to read (Waksman, 1927) p. 749, that treatment of soil with carbon bisulfide for the purpose of eliminating insects and harmful fungi had resulted in an increase in fertility of the soil. This work dates back to 1870.

Khalil (1929) repeated the observation that drying considerably reduced the bacterial content of the soil, but that subsequent moistening and incubation of dried soils gave higher numbers than permanently moist soils. He again called attention to the increase in nitrifying ability and the more ready decomposition of soil organic matter. He demonstrated that no evidence could be obtained to support the idea that the surviving microflora were more efficient. Tests for the possible presence of toxins by use of filtrates from moist, untreated soils did not decrease the nitrifying ability.

In recent years this area of study has made increasing use of the Warburg apparatus. Rovira (1953) suggested its applicability to the study of respiration in soils in periods of less than 24 hours. His preliminary experimentation showed that dried, rewetted soils had extremely short lag periods and high activity. This seemed to suggest the existence of a population in a relatively resting or inhibited state which was able to respond without significant multiplication or adaptation. Following this, Bunt and Rovira (1955) used the apparatus to investigate the effect of incubation temperature and heat treatment on soil metabolism. A high correlation was found between organic matter content and oxygen uptake. Experimentation directed at finding if the activity was due to living microbes, residual enzymes from killed organisms, or possibly chemical oxidation, was carried out.

They concluded that it was of a biological nature, including free enzymes in the soil. In their view, the hypothesis that enzymes may exist in soil in cells which were incapable of multiplication in the medium used in plate counts was made attractive by the fact that a low correlation exists between plate count and respiration, while a high correlation exists between total count and respiration as reported by Jensen (1936).

In order to see if organic matter could be exposed to attack by means other than drying, Rovira and Greacen (1957) devised equipment for extreme tillage and aggregation disruption of soils. These tests showed that such laboratory tillage caused an increase in the oxygen uptake of soil microorganisms. Unfortunately their

report did not give any quantitative information. It is therefore not entirely conclusive that this treatment can cause respiratory rates of the same quantity and character as found in dried soils. Addition of sugar to tilled and untilled soil gave the same uptake of oxygen, so aeration was probably not an important factor. That the substrate was the limiting factor was, they felt, indicated by the fact that addition of oxygen caused no increase in untilled soil. Rovira and Greacen concluded that the most important mechanism was exposure of organic matter from previously inaccessible pores. Biological inhibitors such as sodium azide, sodium chloride, and mercuric chloride had a strong depressive effect. This gave support to the idea that biological forces were at work.

Stevenson (1956) tested the early phases of respiration of remoistened air-dried soils in an attempt to clarify the period of adjustment. His assumption was that soluble nutrients were present and were allowing the high oxidation rates. By paper chromotography he demonstrated the presence in extracts of air-dried soils amino acid fractions which were not found in the extracts from fresh soil. He felt that this was representative of the increased nutrients in dried soils. Stevenson worked with four soils and recorded the hourly respiration rates. Soils with considerable organic matter showed a peak of activity in five or six hours. Less active samples showed highest activity the first hour. Air-drying for 48 hours decreased the numbers of organisms to about 25 per cent in highly organic soils and to about 30 per cent in others. He remarked, as have others, upon the high respiratory activity

present which appeared in spite of a lag of three hours during which the population numbers remained constant. Seemingly, the bacteria remaining after drying passed into a period of physiological youth upon remoistening and showed a rapid increase in mass but not in numbers. The extreme rapidity with which the oxygen uptake appeared led him to experimentally check the possibility that, when soils are dried, gases were adsorbed which were later released. The results were negative and Stevenson concluded that non-biological activity was probably insignificant.

Soluble organic matter resulting from drying was responsible for the respiratory burst, in the opinion of Birch (1959). Charcoal, when added to the soil with the idea of absorbing the soluble material did indeed inhibit the flush of activity. Working with soil extracts, he also found that decomposition of soluble material occurred when added to air dry soil and was proportional to the amount added. Birch reaffirmed that the longer the soils are dried the greater is the subsequent decomposition and the nitrate formation when remoistened. Since no more moisture was lost after the third day, the drying must be related to a factor in the dry state which increases with time. The increase in nitrate formation can be expressed as a function, at least for some highly organic soils, of the log of time that the soil dries. No appreciable differences were observed between the effects of air and vacuum drying.

In making respiration studies Birch made use of a respirometer in which the oxygen supply was automatically replenished as

it was used (Birch and Friend, 1956). The results were reported in terms of daily rates and no data were presented on the hourly activity immediately after remoistening.

Birch (1959) also found ether vapor to cause decomposition patterns similar to those resulting from drying, although this was probably not related to solubility of soil nutrients. Charcoal treatment here did not have any effect of decreasing the rate.

In a later paper, Birch (1960) reported that the flush of decomposition still occurred after 105 treatments. The magnitude of the flush declined with succeeding treatments. The decline, he observed, could be expressed mathematically and projection would indicate that about 250 such cycles were possible. He calculated that 73 per cent of the organic carbon originally in the soil would then be mineralized and the remainder was microbially unusable. It appeared to him to be reasonably clear that some mechanism existed which protected the organic fraction of the soil from constant attack. The indications were that it was not inhibition of the population by a toxic environment as much as a limitation of the substrate available for use.

Birch (1959) drew some interesting conclusions to this end. In his work he noted that the drying effect on the humus fraction was little affected by the soil with which the humus was associated. Even a sand and humus mixture gave similar results. In his opinion this would tend to show that drying must involve the organic fraction, probably in colloidal form. He speculated that treatment led to opening of gel pores which led to a greater area

of contact with added water. Reversibility would involve swelling with closing of the spaces, returning the colloidal gel to a condition characteristic of moist gels. The fact that grinding soil, moist, in a mortar after the flush was over did not renew the activity indicated that the organic material was involved as he conjectured.

The addition of galatin (Birch and Friend, 1956) led to flushes in successive treatments which were larger than those without galatin. They concluded that a mechanism such as they hypothesized was operating.

Birch stated that (1959) on the other hand, an argument against this concept was the fact that heating without drying caused a similar effect, but would not cause the postulated changes in the colloidal gels. The treatments with volatile antiseptics were also not explained. This latter problem came to the attention much earlier of Greig-Smith (Waksman, 1932) p. 760, who had proposed that organic matter was protected from attack by a fatty material from plant residues. He argued that the volatile antiseptics were acting as fat solvents and thus exposing material to attack.

Birch (1959) concluded that the factor common to all treatments causing the increase in activity was that of elimination of a large portion of the population. Part of the increase in activity, he said, could be attributed to the cell material of the dead population now available to the survivors.

The literature, in general, considers that some mechanism

serves to protect the organic matter in the soil from attack. Otherwise, it would be quickly depleted by the soil microflora. Much speculation has revolved about methods by which drying could serve to expose additional material for the soil microflora. A great deal of difficulty has been encountered in formulating a concept which would adequately explain the fact that a number of treatments of widely differing character all cause an increase in respiratory activity in the soil.

MATERIALS AND METHODS

For use in these experiments an assortment of five soils of differing characteristics were chosen. All samples were taken from the upper eight inches of soil, placed in plastic bags and stored in a refrigerator at 8°C.

The soils selected were:

Soil A Geary Silt Loam, field soil, intensive cultivation.

Soil B Sarpy Fine Sandy Loam, relatively high organic matter.

Soil C Sarpy Fine Sandy Loam, relatively low organic matter.

Soil D Alluvial Deposit, lowland pasture, highly organic.

Soil E Alluvial Deposit, field soil, cultivated.

Of these soils, the most use was made of soil D since its moisture content was at 60 per cent of total moisture-holding capacity. This represented the figure chosen as most desirable for

maximum activity, and made the addition of water to moist heated soils unnecessary. An additional variable was thus eliminated in some experiments. Soil D also exhibited high levels of activity when treated.

Table 1. Results of soil analysis test.

Soil	Organic Matter %	pH	Available Phosphorous lbs/acre	Exchangeable Potassium lbs/acre
A	1.9	5.8	30	550
B	1.4	7.0	30	502
C	0.3	7.4	22	450
D	3.0	7.5	28	550
E	2.6	7.5	55	512

Soil B, a sandy soil with relatively high organic content, was also much used, particularly in experiments involving substrate addition.

Oxygen uptake was measured with a Warburg respirometer. To prepare soils for use in the respirometer they were sieved through a No. 20 sieve with a nominal opening of .84 millimeters. Four grams of soil were placed in each manometer flask. In order to avoid having soil particles fall into the center well of the flask a cardboard cone glued to the end of an applicator stick was placed over the well as the soil was added. Addition of water and substrates was done by distributing them as evenly as possible from a pipette. The side arms of the flasks were not used for addition

of water or substrates because this resulted in poor liquid distribution. For absorption of carbon dioxide the center well contained a cylinder of filter paper and 0.2 milliliters of 20 per cent KOH. Temperature of the water in the Warburg apparatus was maintained at 30°C.

Manometric experiments were normally done in duplicate. The flasks were allowed to equilibrate for one-half hour before the stopcocks were closed, except as noted for some experiments. For daily activity rates the figure used is that of the hourly average of a five hour daily run.

By the term "fresh soil" is meant soils kept under refrigeration at 8°C. Experiments separated by several months and involving similar treatments of the same soil gave results indicating that no significant changes in activity resulted from this refrigeration.

"Air-dried" soils were spread on paper in the air stream of a fan at room temperature.

"Desiccator-dried" soils had been sieved and placed in a Warburg flask. They were then enclosed in a desiccator containing calcium chloride and subjected to a vacuum. The purpose of using this technique rather than air-drying was to achieve more uniform drying conditions.

"Oven-dried" means that the sample had been dried in a hot air oven at 80°C.

"Moist heat" treatments were done with the use of a water bath at 80°C for a period of 30 minutes. This temperature was

selected as a result of a series of experiments involving a succession of temperatures. The water bath used consisted of a two liter beaker of water set on a tripod. Heat was supplied by a Meker burner. The temperature was controlled by observing a thermometer in the bath and adjusting the gas supply screw on the burner. No difficulty was found in keeping the temperature within ±1°C. The samples were immersed in the water up to the necks of the flasks and held by burette clamps. Aluminum foil was used to cap the openings. When removed from the bath the flasks were placed in the freezing compartment of a refrigerator for five minutes in order to drop the temperature quickly. At this point they were either placed in the Warburg apparatus or stored for the moment in the refrigerator.

The length of time involved in the various treatments discussed above varies frequently with the individual experiment and is noted in the discussion of the particular experiments.

The moisture holding capacity of the soil was determined by first oven-drying the soil and noting the moisture loss. The second step was to determine the amount of water which could be added to the fresh soil. For this a known quantity of soil was placed in a funnel into which had been fitted a cone of moist filter paper. Water was then added slowly and the point of maximum capacity noted when the first drop of excess moisture appeared at the bottom of the funnel. The sum of the moisture lost by fresh soil and the amount of moisture it was possible to add to fresh soil represented the total moisture holding capacity. The figure of

60 per cent moisture-holding capacity was selected for all experiments. This is within the optimum range for activity of soil microflora.

In several experiments artificial substrates or soil extracts were added. These included one per cent peptone (Difco), and one per cent glucose solutions. Soil extracts were prepared from both dried and fresh soils for comparison. These extracts were prepared from 50 grams of soil to which had been added 50 milliliters of distilled water. This was then placed on a shaking machine for two hours. The supernatant was then centrifuged for 20 minutes, four times, in a Sorvall angle head centrifuge. After each spinning the supernatant was transferred with a sterile pipette and precipitated material discarded. It was desired to keep the number of soil microorganisms to a minimum in the extract without resorting to heating, which might denature material in the extract.

Some soils were lyophilized; standard procedures were used for this. Lyophilization, in one experiment, was preceded by agitation in a Waring blender. The soils were introduced into the blender with a minimum of distilled water needed to make a good slurry and blended for about one minute.

Soil counts were carried out by standard dilution plate count methods. Five gram samples of soils were used. When incubation was required the samples were placed in 50 milliliter Erlenmeyer flasks for treatments. Incubation then took place in the same water bath at the same time as the samples of the soils, which had undergone the same treatment, were being tested for respiratory

activity. Growth was on nutrient agar (Difco) and plates were done in triplicate. For enumeration of fungi, Rose Bengal agar was used as a selective medium. This was prepared by the procedure outlined in Allen (1959) p. 7.

EXPERIMENTAL

In the first series of experiments it was hoped that a treatment would be found which would give a higher respiration rate than that of the dried soil, and which could be clearly attributed to increased exposure of organic matter. Data were recorded as the average hourly uptake of a daily run of five hours. For purposes of comparison the oxygen uptake of fresh, untreated soils and the uptake of soil which had been dried at room temperature overnight and then sieved was measured. It was found, as expected, that the dried soil exhibited markedly higher respiratory rates than the fresh soil. In the soils with high organic content the effect was considerably magnified (Plate I).

Samples of the same soils were air-dried overnight and subsequently powdered in a mortar before remoistening. This treatment might reasonably be expected to increase the amount of organic material exposed. As can be seen in Plate I, no improvement was found.

To further pursue this line of inquiry samples of soil were agitated in a Waring blender as a slurry. After this treatment the sample was immediately lyophilized. Samples were also lyophilized without the blender treatment. From Plate II it can be

EXPLANATION OF PLATE I

Comparison of respiration rates as a result of soil treatments. Dried overnight, dried in mortar, fresh.

Soil C, sandy, 0.3% organic matter; soil D, from bottom land pasture, 3.0% organic matter; soil E, from cultivated field, 2.6% organic matter.

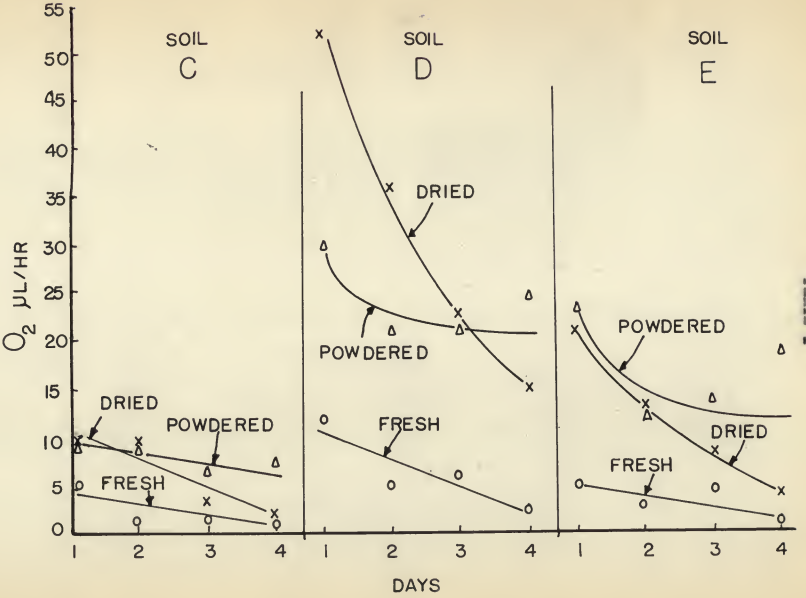

SOIL
C
SOIL
D
SOIL
E
O_2 µL/HR
DRIED
POWDERED
FRESH
DAYS
0
5
10
15
20
25
30
35
40
45
50
55
1
2
3
4

PLATE I

seen that the results obtained were very similar to those resulting from the simple powdering of the dried soil. In no case was there any indication that the additional treatments made organic material more available for attack and thus increased the stimulatory effect.

The work of Birch (1959), in particular, suggested that high rates could be obtained by use of oven-drying of soils. This would effectively eliminate, at the temperatures used, practically all activity except that by heat resistant spore-formers. This suggested that pure culture work might be carried out making use of sterile soils inoculated with a typical aerobic spore-former, such as Bacillus cereus.

It was thought that work with relatively pure cultures might make it easier to separate the effects of treatment on the soil, as opposed to effects on the population. Two samples of soil D were prepared, both had been autoclaved for sterility and one was air-dried overnight, while the other was not dried. An inoculum of approximately 8.5×10^6 cells of B. cereus was made to see if any difference could be determined between the dried and undried soil as a growth medium. As can be seen in Plate III, no difference was found.

No information was available regarding the effect of added substrates to treated soils for comparison with similarly treated soil without such addition. Therefore, a series of experiments making use of the addition of peptone to soils was set up. Four duplicate samples of soil D were air-dried overnight and then

EXPLANATION OF PLATE II

Comparison of respiration rates as a result of soil treatments. Dried overnight, lyophilized, Waring blender and lyophilized.

Soil C, sandy, 0.3% organic matter; soil D from bottom land pasture, 3.0% organic matter; soil E from cultivated field, 2.6% organic matter.

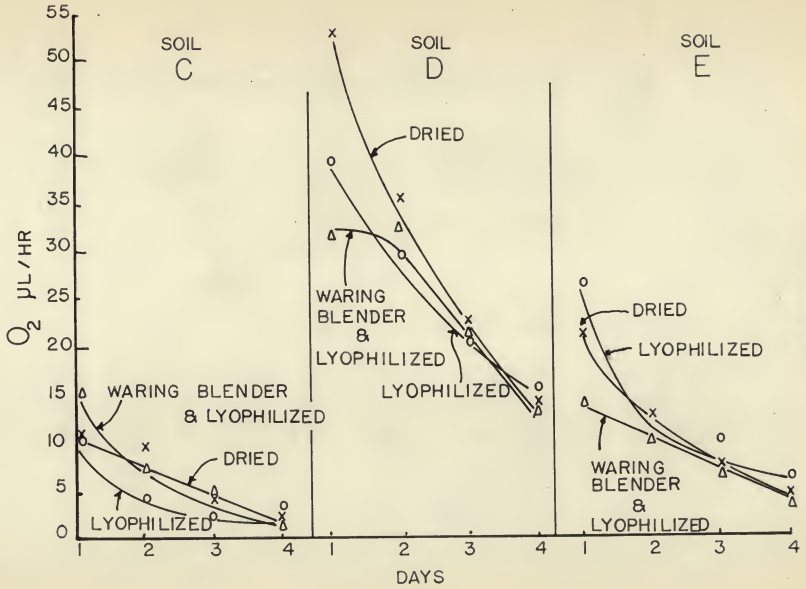

SOIL C
SOIL D
SOIL E
O_2 µL/HR
DRIED
WARING BLENDER & LYOPHILIZED
LYOPHILIZED
DAYS

PLATE II

EXPLANATION OF PLATE III

Soil sterilized in autoclave. One sample dried, other untreated. Both samples inoculated with B. cereus.

PLATE III

remoistened. Their activity over a four day period was observed to be essentially the same. The soils were then subjected to a variety of treatments. One of the soils was frozen at -20°C for 24 hours. This was intended to determine the effect that this treatment might have. Since it did not result in any change from normal activity this sample was considered as an untreated soil for the balance of the experiment.

The different treatments of the four soils may be itemized:

1. Frozen at -20°C, 24 hours.

2. Untreated, 0.15 milliliters of one per cent peptone, water to 60 per cent moisture-holding capacity.

3. Oven-dried, four hours, 80°C, water to 60 per cent moisture-holding capacity.

4. Oven-dried, four hours, 80°C, 0.15 milliliters of one per cent peptone, water to 60 per cent moisture-holding capacity.

Plate IV shows the respiratory behavior of these soil samples. The oven-dried samples show the expected high rate of respiration, a rate which remains higher than that of the untreated samples after five days. The addition of peptone seems to have had an additive effect which was particularly noticeable the first day but which continued for the duration of the experiment.

When all of the samples were oven-dried four hours it was found that the previously unheated soils now had the highest rate.

In the course of recording the progress of the soils respiration after the second heat shock it was noted that the curves,

EXPLANATION OF PLATE IV

Test of addition of substrate to fresh and oven-dried soils for comparison and allowed to run five days.

Both sets of samples oven-dried after being allowed to equilibrate.

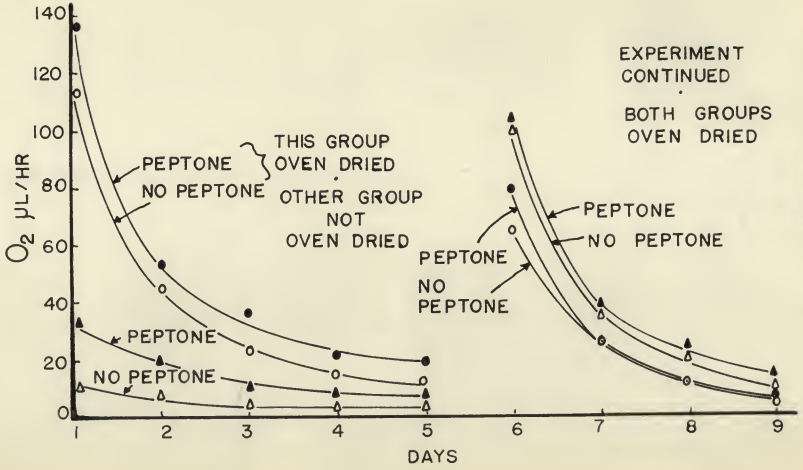

140
120
100
80
60
40
20
0
O_2 µL/HR
PEPTONE
NO PEPTONE
THIS GROUP OVEN DRIED
OTHER GROUP NOT OVEN DRIED
PEPTONE
NO PEPTONE
1
2
3
4
5
DAYS
EXPERIMENT CONTINUED
BOTH GROUPS OVEN DRIED
PEPTONE
NO PEPTONE
PEPTONE
NO PEPTONE
6
7
8
9

PLATE IV

as plotted hourly, differed widely in character. The samples, as can be seen in Plate V, which had not been previously oven-dried started with very low rates but rose rapidly. These samples reached a peak at five and six hours, and then dropped quickly to a new plateau which was considerably higher than before. On the other hand, the previously oven-dried samples started with respiration rates almost four times higher than that at which they had been found before this latest heat treatment. But they rose only briefly before going into a fairly steep decline.

Air-dried soils in previous experiments had shown hourly respiration rates which remained quite steady. The different characteristics of the curves resulting from oven-drying for four hours suggested that, although both treatments give flushes of activity, that different mechanisms might be at work. Tests were therefore made to observe the effect of moist heat on soil to separate the effect of heat from that of drying.

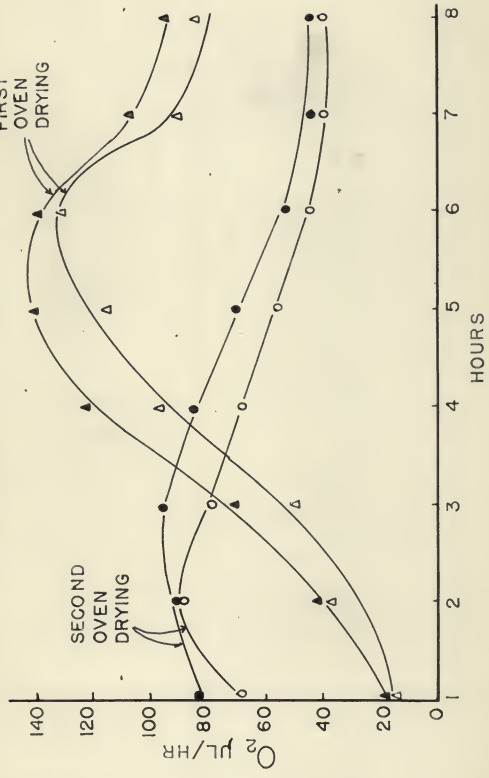
Soil D was selected for these experiments and tests were run at different temperatures in the water bath arrangement described in Materials and Methods. The results are graphically illustrated in Plate VI. There is a greater increase in activity at 70°C and 80°C as compared to lower temperatures. It was found that 100°C resulted in almost total loss of activity. The temperature of 80°C was selected as optimum for investigation of this phenomenon.

A comparison of the curves obtained with oven-drying and moist heat at the same temperature showed a close correlation (Plate V and Plate VII). In both cases the initial respiration

EXPLANATION OF PLATE V

Oxygen uptake, recorded on hourly basis, of soil samples which had been, respectively, oven-dried and untreated. Both had been allowed to equilibriate and then both oven-dried.

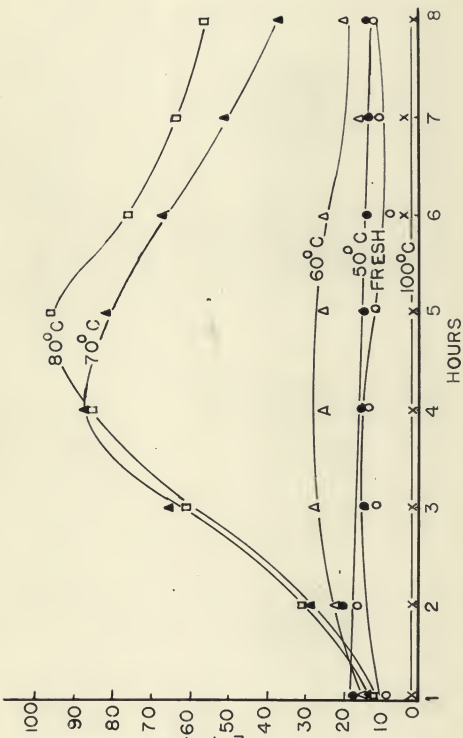
PLATE V


FIRST
OVEN
DRYING
SECOND
OVEN
DRYING
O_2 µL/HR
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
HOURS

EXPLANATION OF PLATE VI

Oxygen uptake resulting from moist heat treatments at different temperatures.

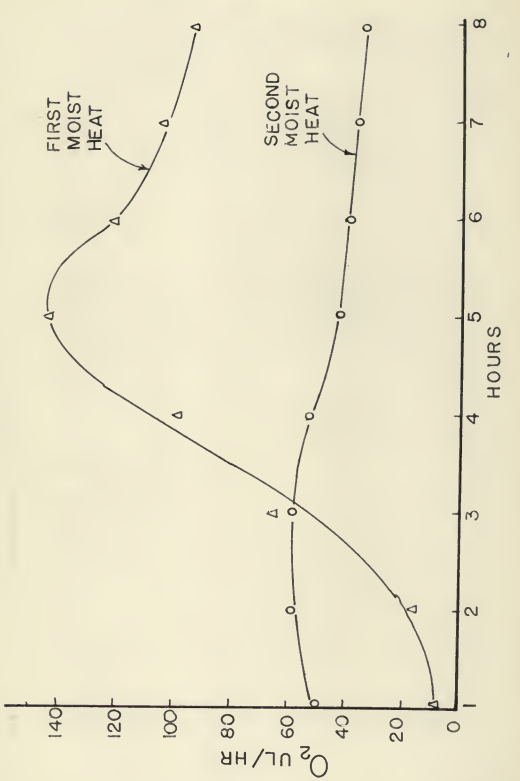
PLATE VI



EXPLANATION OF PLATE VII

Oxygen uptake recorded on hourly basis after moist heat treatment. Compare with similar curves obtained by successive oven-drying on Plate V.

PLATE VII


FIRST
MOIST
HEAT
SECOND
MOIST
HEAT
O_2 UL/HR
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
HOURS

was low but rose to a peak in five or six hours, after which it dropped to a plateau. A second treatment showed a much higher initial rate which declined after a brief rise. It would seem logical to conclude that in the case of oven-drying at 80°C that the heat was more involved in the increase in respiration than was the drying.

Although it is not entirely pertinent to the question it is interesting to find that soil which had been moist heated to 80°C, placed in a freezer at -20°C, and then thawed, promptly demonstrated the usual activity due to moist heat in undiminished form.

There are several possibilities which might explain this effect of heat. Among them are: that some inhibitory substance was destroyed, that the population had been drastically cut and the survivors propagated rapidly, that some material had been released that was being rapidly oxidized by the surviving bacteria, or more likely, that the heat treatment caused the simultaneous germination of spores.

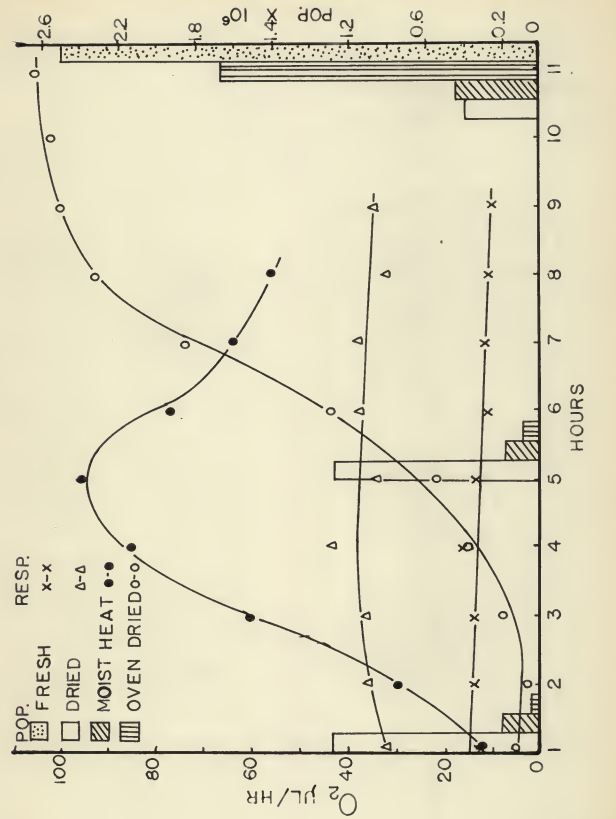
It can be seen that the data corresponds extremely well with that of several workers (Tarr, 1930; Levinson and Hyatt, 1956; Evans and Curran, 1943) who have tested the effect of heat on the germination and the oxidative properties of spores. Examination of the population after this treatment demonstrated that it consisted of typical aerobic spore-formers. Experimentation, Plate VIII, showed that the rise in oxidation was not matched by an increase in population.

If it can be accepted that the moist heating of soils re-

EXPLANATION OF PLATE VIII

Correlation of oxygen uptake with population counts after different soil treatments.

PLATE VIII


POP.
FRESH
DRIED
MOIST HEAT
OVEN DRIED
RESP.
x-x
Δ-Δ
•-•
o-o
O_2 µL/HR
POP x 10^6
HOURS

sults in a curve representing the synchronized germination of spores, it suggests a method of determining the relative numbers of ungerminated spores remaining in a soil.

With this in mind, a series of experiments with soil D were carried out. It is a logical supposition that a contributing factor to the activity of remoistened air-dried soils is the germination of surviving spores as was mentioned by Waksman (1926). An effort was made to resolve this by means of the technique of moist heating.

In Plate IX are shown the results when the soil was initially heat-shocked, incubated at 30°C, and then heat-shocked a second time. This procedure was carried out with several samples and the period of incubation was varied in length. It can be seen that after an incubation of only one-half hour the second heat shock had much less effect. After an hour the response was almost negligible. This is consistent with the idea that the initial heat shock had germinated the spores and that they had become heat labile.

In Plate X the results are shown of a similar test in which the soil sample had been desiccator-dried for 20 hours and then heated. Incubation times were again varied in length. The results showed that, even after an interval of four hours, the effect of the heat shock was largely unimpaired. This seemed to indicate that the germination of spores contributed very little to the high oxidation rates observed in dried soils, at least under the conditions of this experiment.

EXPLANATION OF PLATE IX

Test of effect of second moist heat treatment, with different incubation periods, following first heat shock. Magnitude of response assumed to be proportional to spores germinating.

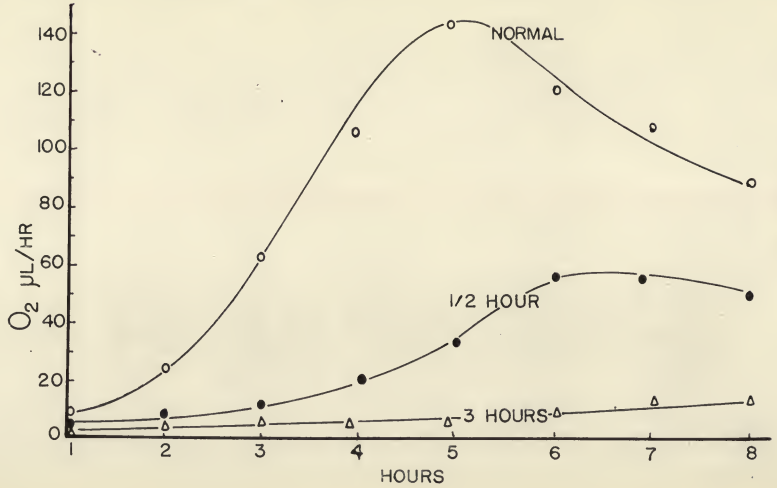

140
120
100
80
60
40
20
0
O_2 µL/HR
NORMAL
1/2 HOUR
3 HOURS
1
2
3
4
5
6
7
8
HOURS

PLATE IX

EXPLANATION OF PLATE X

Test of effect of moist heat treatment, with different incubation times, following drying.

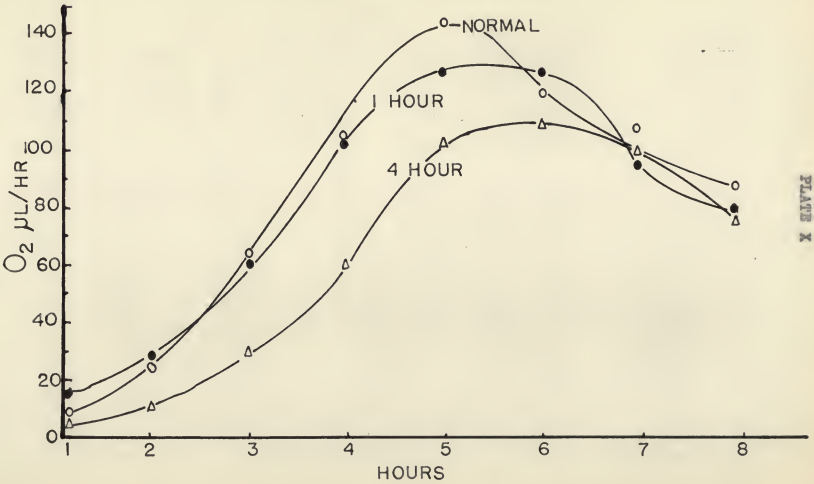

140
120
100
80
60
40
20
0
O_2 µL/HR
o—NORMAL
1 HOUR
4 HOUR
1
2
3
4
5
6
7
8
HOURS

PLATE X

Similar experiments were carried out with the same soil, but with no treatment (Plate XI). The results seemed to indicate that the spores were germinating in fresh soil after refrigeration, but that the respiratory activity of the soil was nonetheless very low.

Plate counts on selective agar showed that mold spores were not present after moist heat treatment at these temperatures. Therefore, the possibility that the germination of mold spores contribute to the respiration of dried soils has not been eliminated.

Further investigation was made of the differences between the activities of dried and moist heated soils. A series of experiments was conducted making use of soil B. This soil was selected because it was relatively low in moisture content and thus permitted addition of substrate even to fresh soil.

In Plate XII is shown the reaction of this soil to different treatments. We see that moist heat-treated soil shows the usual peak of activity in about five hours. The activity is much less than in the highly organic soil D. Desiccator-drying overnight resulted in a higher level of activity than found in fresh soil, but no peak was observed as in heating.

The addition of 0.5 milliliters of one per cent peptone (Plate XIII) resulted in a very high level of respiration. The curve was remarkably similar with all treatments. Inspection of the curves seemed to indicate that a period of adaptation was necessary before high oxidation rates appeared.

EXPLANATION OF PLATE XI

Test of effect of moist heat treatment, with different incubation periods, following removal of untreated soil from refrigerator.

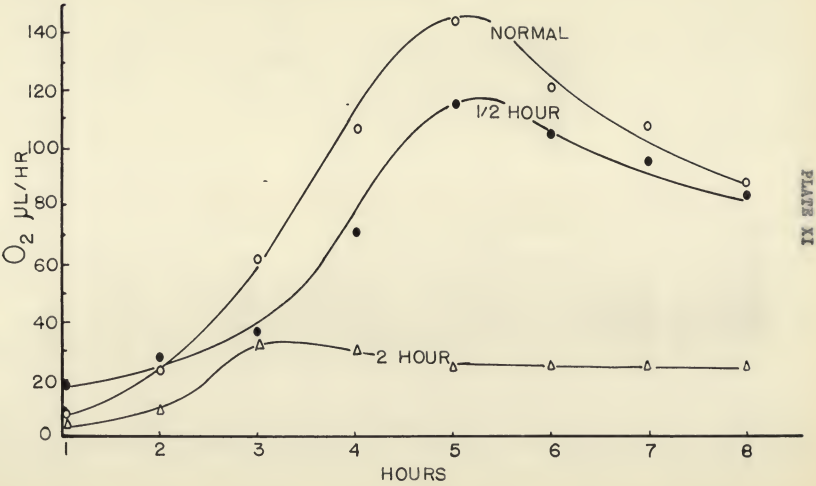
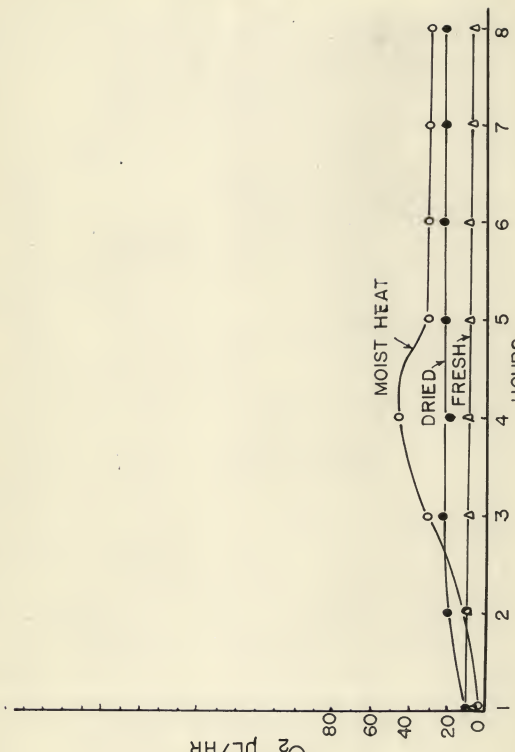

140
120
100
80
60
40
20
0
O_2 µL/HR
NORMAL
1/2 HOUR
2 HOUR
1
2
3
4
5
6
7
8
HOURS

PLATE XI

EXPLANATION OF PLATE XII

Effect of various treatments on sandy soil. No substrates added.

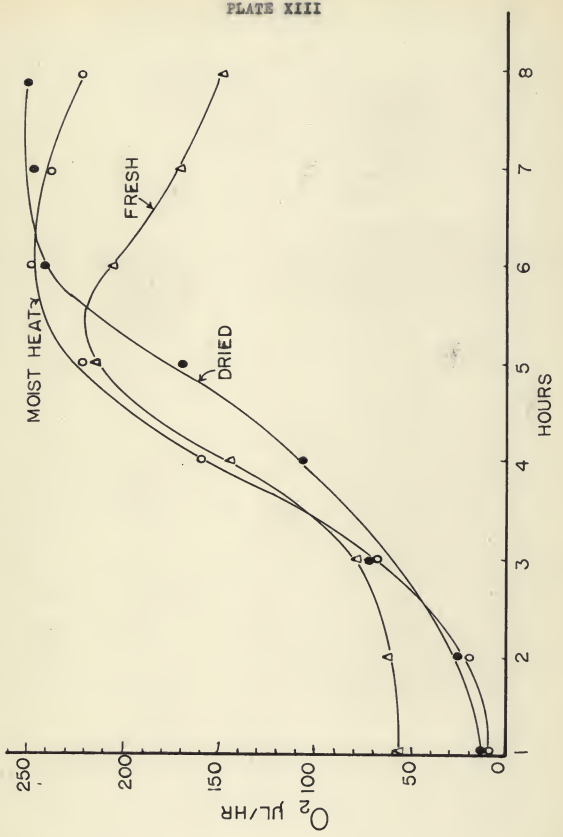
PLATE XII



EXPLANATION OF PLATE XIII

Effect of addition of peptone to sandy soil after various treatments.

PLATE XIII


MOIST HEAT
FRESH
DRIED
O_2 µL/HR
250
200
150
100
50
0
1
2
3
4
5
6
7
8
HOURS

When 0.5 milliliters of one per cent glucose was placed in a fresh soil, as shown in Plate XIV, it was oxidized at a linear rate and showed no peak, differing in this respect from peptone. When added to the dried soil it was noted that the ability of the soil to use glucose was much impaired. The rate then increased slowly and after about eight hours began to decline.

Extracts were prepared from the highly organic soil D. One extract was made from untreated soil and another from soil which had been air-dried overnight. Somewhat greater activity was found in the extract from the dried soil.

When such a dried soil extract, made from soil D, was added to this same soil D the results shown in Plate XV appeared. No difference was found in either fresh soil or moist heated soil between the effect of water or this extract.

A correlation was made between population counts and respiratory activity. Treatments varied and included with fresh soil: overnight desiccator-drying, moist heat, and a more extended oven--drying of 18 hours. These results are reported in Plate VIII. It was very clearly shown in these data that the moist heat respiratory peak was in no way connected with an increase in population. The initial respiration for the first hour was observed to be about that of fresh soil, although the population was only about eight per cent of fresh soil. At five hours the respiration was enormously increased to 640 per cent of fresh soil but the population had increased to only ten per cent of fresh soil.

The dried soil was found to have suffered a drop in population

EXPLANATION OF PLATE XIV

Effect of addition of glucose to sandy soil following various treatments.

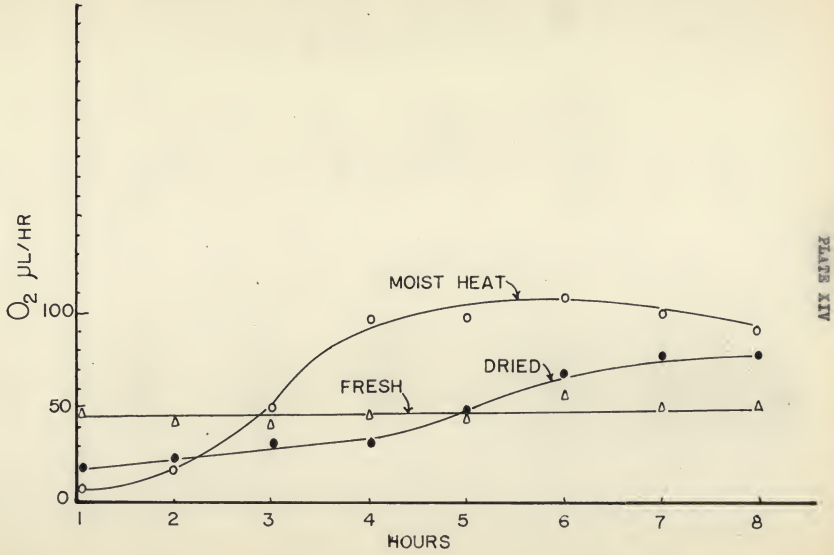


PLATE XIV

EXPLANATION OF PLATE XV

Effect of addition of extract from soil D back to soil D, both when moist heated and untreated.

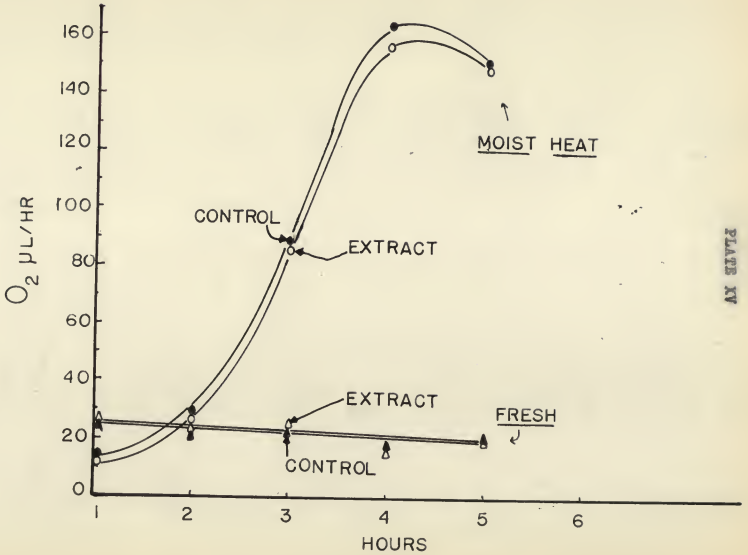

160
140
120
100
80
60
40
20
0
O_2 µL/HR
1
2
3
4
5
6
HOURS
MOIST HEAT
CONTROL
EXTRACT
EXTRACT
FRESH
CONTROL

PLATE XV

to about four per cent of fresh soil. The respiration was at the same time about ten times greater and continued at this high rate. The population did not increase and indeed appeared to decline after about five hours.

The more severe treatment of oven-drying for an extended time resulted in an initial depression of population to a very small fraction of fresh soil, less than two per cent. The respiration and population increased in general proportion to each other. At ten hours the population and respiration had both increased greatly and a substantial portion of the original population loss had been replaced.

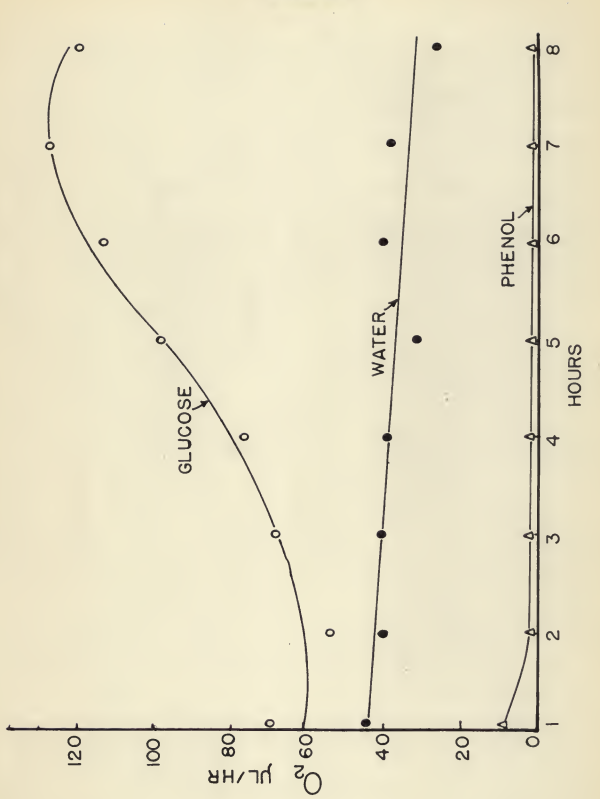
A particularly striking phenomenon in these investigations was the extreme rapidity with which dried soils reached the high level of respiration after remoistening. Although there is substantial evidence in the literature that the phenomenon is biological, an experimental check was made. Samples of soil D were remoistened with water, with glucose, and with five per cent phenol solution respectively. A 15 minute equilibration time was used. Results were as might be expected for a biological system (Plate XVI). The phenol showed negligible activity. The little activity shown for the first hour probably reflected the fact that the short equilibration time did not permit the solution to penetrate to all parts of the soil. The glucose gave an initial rate much higher than the soil which had been only remoistened, which was compatible with a biological origin.

These hourly readings showed that the rates had already

EXPLANATION OF PLATE XVI

Addition of phenol and glucose to dried soil. Responses indicate biological activity.

PLATE XVI


120
100
80
60
40
20
0
Q_{O_2} µL/HR
GLUCOSE
WATER
PHENOL
1
2
3
4
5
6
7
8
HOURS

reached maximum in dried soils during the first hour. Experiments were conducted making use of shorter time intervals in order to determine more exactly how early these rates made their appearance. The need for flask equilibration and the fact that moisture and substrates do not distribute through the soil instantly made it difficult to get an exact picture. But it can be said with some confidence that such errors would probably tend in the direction of less, rather than more, activity being shown.

A test was made measuring the uptake of oxygen for each ten minute period by soil D. An equilibration period of only ten minutes was used after addition of the moisture or substrate. The temperatures were brought as close to bath temperature as possible in advance of the addition of the material. Two samples of soil D were used; one consisted of some soil which had been dried for several weeks at room temperature and another which had been desiccator-dried for 24 hours.

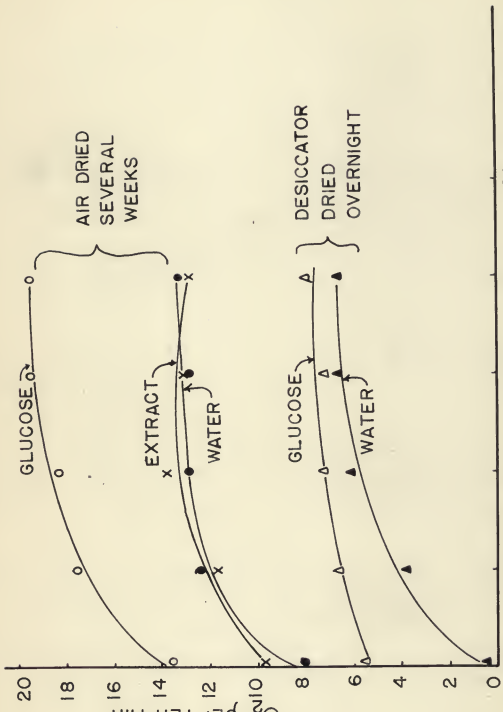
The results are illustrated in Plate XVII. It was noted that the samples which had been dried for an extended time showed higher activity in general. Addition of glucose to both soils gave an immediate response considerably higher than the water-moistened controls in the respective cases. An addition of extract from dried soil D showed no change from that of water-moistened soil when it was applied to the air-dried sample.

A final experiment was made to investigate the possibility that soluble enzymes might be free in soil extracts and contribute to this activity. Extracts from dried and fresh soil D were

EXPLANATION OF PLATE XVII

Oxygen uptake of dried soils for ten minute intervals. Soil with highest activity had been air-dried several weeks. Other soil had been desiccator-dried overnight.

PLATE XVII


AIR DRIED SEVERAL WEEKS
DESICCATOR DRIED OVERNIGHT
GLUCOSE
EXTRACT
WATER
GLUCOSE
WATER
O_2 µL/TEN MIN
MINUTES
0
2
4
6
8
10
12
14
16
18
20
10
20
30
40
50
60

again used. One milliliter of extract was placed in a Warburg flask and 0.5 milliliters of substrate was placed in the sidearm. After equilibration they were mixed to enable them to react. No reaction was observed with glucose, peptone, or in the controls.

DISCUSSION

When confronted with a biological situation which shows a rapid increase in oxidation the logical supposition is that the biological system has been presented with a new supply of material to oxidize. An increase in water soluble organic matter has been noted as a result of drying of soils. The amounts seem proportional to the length of drying. The activity of the soil also seems proportional to the length of drying. Therefore, there has been a general assumption that this is at least a contributing factor to the burst of respiratory activity observed in remoistened, air-dried soil.

This present investigation may be divided into three general areas:

1. The effect of drying on the soil as a nutrient medium.

2. The effect of drying on the population, that is, if the drying per se stimulates the population into activity without regard to nutrients available.

3. The characterization of the early phases of the respiratory activity after treatment, with the view in mind of differentiating between the effects of various treatments and the

mechanisms by which they increase respiration in the soil.

In regard to the first area, in the past several experimenters have made attempts to produce a burst of activity similar to that of dried soil by mechanical disruption of the soil to expose more organic material. One investigator, Rovira, reported some success with this and ascribed it to exposure of new organic material. In the present experiments this approach failed to yield any increase. It was found that soil which had been respectively; powdered in the dry state, lyophilized, and subjected to the shearing action of a Waring blender, failed to show any advantage in the treatment over simple air drying.

Considerable use was made of soil extracts, particularly in order to see if extracts from dried soils were superior as a nutrient to those from fresh soils. No analysis was made of such extracts, but extracts from dried soils were noticeably different, darker in color, than extracts from fresh soils. One investigator also reported the presence of amino acid fractions in the dried extracts, something he did not find in fresh soil extracts. Another reported an additive effect when extracts were used as substrates.

Birch, in his work, was able to show that addition of charcoal to dried soil inhibited the flush of activity. He ascribed this to absorption of the soluble nutrients. On the other hand, treatment of soil with volatile antiseptics caused an effect similar to drying, but was not inhibited by charcoal.

The use of soil extracts in the present work did not result

in any definitive information. These extracts were made from a highly organic soil. When these extracts were added to a sandy soil with somewhat lower organic content than the soil from which the extracts were made, there seemed to be a significantly higher degree of activity caused by the dried soil extract than the fresh soil extract.

This same dried soil extract had no noticeable effect when added back to the highly organic soil from which it was originally extracted. This observation was made several times with respect to both dried and fresh soil. What significance, if any, should be attached to this is not known.

An investigation into the possibility that soluble enzymes might be present in such extracts was made. The results were negative. Dried, remoistened soils were capable of immediate oxidation of peptone and glucose. Extracts from dried soil did not show any activity with these substrates. If such enzymes are present they must be insoluble in water, centrifuged out under conditions of the experiment, adsorbed to soil material and not in solution, or otherwise not active in the extract.

A test with pure cultures added to sterile soil was made to see if such a sterile soil, which had first been dried, would comprise a better medium for growth than one that had not been dried. No difference was found, but it must be admitted that autoclaving for sterility introduced an unnatural factor.

The evidence for presence of an improved supply of nutrients in the dried soil was considered inconclusive on the basis of

these experiments. At least the demonstration of soluble organic matter as a clearly defined factor was not accomplished.

An attractive hypothesis to explain the phenomenon of the activity stimulated in soil by drying, is that the surviving organisms managed to so survive because they were spore-forming or otherwise resistant, and that, when the soil was remoistened, they promptly germinated. This would result in a rapid increase in protoplasm with an accompanying high rate of respiration. The lag in population increase behind the increase in respiration would thus be handily explained.

The technique of moist heat treatments at 80°C appeared to show quite clearly that it was possible to obtain a measure of the relative number of bacterial spores which had germinated in a soil as a result of treatment. No evidence was found that the observed activity in dried soil was caused by spore germination. On the contrary, under the conditions of the experiment, something seemed to be preventing them from germinating, at least for the four hours of incubation in the experiment.

Fresh soil removed from the refrigerator showed fairly rapid germination of spores, but without the accompanying high respiratory rate. It was not possible to say if this lack of activity was due to a deficiency of available nutrients, or inhibition by the high population already present.

Since the moist heat treatment eliminated the mold spores in the population, there is no evidence one way or the other regarding the effect that these might have on the dried soil.

That the activity of the dried soil was biological in origin, was apparently confirmed. The quick oxidation of added substrates, and the complete inhibition by phenol are substantial evidence for this.

Much interesting information was derived from the characteristics of the early respiration in response to different treatments. A good deal of the conclusions to be drawn from such work are speculative. However, the fact that it is possible to clearly differentiate between the effects of different treatments, even though they all resulted in higher activity in the soil, is a useful advance.

This is particularly obvious when population counts were done in correlation with respiratory activity after different treatments. By means of such methods it was easily seen that moist heat treatment of the soil was capable of stimulating a high activity in a short time, but that the population increase lagged behind.

Prolonged oven-drying resulted in probably the greatest stimulation of the soil. It differed in important respects from air or desiccator-drying. The population in oven-dried soil was more drastically reduced and the initial oxidizing ability was also decreased. In this it resembled the effect of moist heat. The population recovery in oven-dried soil was associated with the increase of population, and rapidly approached that in fresh soil.

The reaction of dried soil differed from both of these. The population was not so drastically reduced, but its activity was

much higher than that observed in fresh soil with a much higher population. Heat treatments resulted in an initial rate lower than fresh soil. The population in dried soil did not show any early signs of increase and the respiratory rate remained steady. It would seem that if the drying in this experiment released considerable amounts of nutrients that the population would have recovered more quickly. Perhaps longer drying periods would accomplish this.

The high rate, and its quick appearance, led to the thought that the smaller population was able to respire at a higher rate, but lacked nutrients to increase sharply in numbers. The observation that spores seemed to be germinating in fresh soil without contributing much to the general activity seemed to indicate an inhibiting effect of high populations.

The extreme rapidity with which the high rate of oxidation appeared after remoistening was emphasized in these experiments. More experimentation is indicated to determine to what extent this is inversely proportional to the length of time of drying and the surviving population. It would be interesting to determine if a break in this relationship, that is, a sudden drop in this initial activity could be correlated with a selection of the population. It would seem that the rather lower initial rates after heat treatment was due to such a selection.

As has been observed in the literature, the high rate of respiration, as recorded on a daily basis, may be caused by several treatments. A hypothesis which would serve to account for

the release of oxidizable material is difficult to contrive for all of these cases.

It is apparent that the common factor in all of these treatments is a reduction in population. The possibility exists that such a drop in population may account for a major part of the observed increase in physiological activity, without regard to the supply of nutrients, although this is quite likely also involved. The observation of the early phases of such respiratory activity can be of undoubted value in determining the answer to these questions.

SUMMARY

When soils which have been allowed to dry are remoistened, they exhibit a burst of respiratory activity. This is an apparent anomaly since the population is greatly reduced by this treatment. Considerable interest has been attracted to this phenomenon, because of the increased fertility which is also associated with these soils.

Efforts were made in the present work to isolate a factor, such as an increase in soluble organic matter, to which could be clearly attributed the high level of oxygen uptake. The results of this work were inconclusive.

Experiments have shown that a similar increase in oxidative activity also occurs when soils are treated with mild heat or volatile antiseptics. Studies which emphasized the characteristics of the early phases of this burst of activity, and correlation

with population counts, served to clarify the fact that different mechanisms were involved in the response to different treatments.

Experimental evidence seems to indicate that the germination of spores is not responsible for the phenomenon.

It was determined that the rate of oxygen uptake is almost immediate in appearance.

The biological nature of the activity was confirmed, and it is suggested that the lowered population pressure permits an immediate high respiratory level in the surviving population when the soil is remoistened.

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INVESTIGATION OF THE OXYGEN UPTAKE OF DRIED, REMOISTENED SOILS

by

BERDELL ROBERT FUNKE

B.S., Kansas State University, 1959

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1961

When soils are allowed to dry and subsequently remoistened they exhibit a marked flush of physiological activity. Since the population has been drastically reduced by the drying, this phenomenon represents an interesting anomaly and has stimulated a good deal of experimentation to determine its causes. The fact that this treatment is also associated with an increase in soil fertility which may be due to the stimulation of the soil microflora has been an additional reason for continuing attention.

Regarding the factors responsible, exposure of a new supply of organic material to microbial attack by the action of drying has been usually considered as the principal contributor. Attempts were made in the present work to see if treatments of the soil designed to expose more organic matter would result in an increase in the oxidation rate. These experiments were inconclusive. Also, no evidence was found to show that the soil, after drying, was a better growth medium for bacteria.

Drying of soil is not the only treatment which results in a burst of respiratory activity by the microflora. Mild heat and volatile antiseptics cause a similar response. There has been considerable difficulty encountered in formulating a single hypothesis which will account for a release of organic material under all of these conditions. It is probably significant in this connection that the only common factor involved is a sharp decrease in the population.

The present investigations have shown that observation of the oxygen uptake in the early hours, immediately after the soil

has been remoistened, shows respiration responses of differing character following different treatments. Correlation of these respiratory curves with population counts is particularly informative. There is a clear cut indication that different mechanisms are at work in these cases.

The hypothesis that the germination of surviving spores in the remoistened soil is a major contributor to the oxidation rate was tested. This was eliminated as a possibility under the conditions of these experiments.

The lack of a lag period before the activity is proceeding at a high rate is of particular interest. This activity, at its maximum rate, was observed to be present within the first ten minutes after rewetting.

In the case of drying, it is considered probable that the immediacy of uptake is due to the drop in population pressure in the soil, which allows the surviving cells to begin immediate respiratory activity without the necessity of a lag period. The possibility that free enzymes present in the soluble material might be responsible for the quickness of response was explored. No evidence was found in favor of this hypothesis.