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COMPARISON OF AERATED AND NON AERATED CULTURES FOR NITROGEN FIXATION STUDIES BY SOILS

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In any good system of soil management the farmer has two natural ways of maintaining the nitrogen supply in the soil; (1) by the proper growth and use of inoculated legumes; and (2) by encouraging the development of the non-symbiotic nitrogen fixing microorganisms in the soil. Altho the first method is probably the more important especially in those regions where legumes are commonly grown, undoubtedly many farmers have unconsciously practised the second method. It has been definitely shown that even where legumes are not grown the nitrogen balance in the soil is automatically cared for, to some extent at least, by microorganisms which are capable of fixing large amounts of nitrogen from the air without the aid of a host plant. The exact relation of this process of non-symbiotic nitrogen fixation to soil fertility is an interesting problem of both practical and scientific importance. It has attracted the attention of many soil bacteriologists, but in spite of much accumulated information on the subject, there are many questions still to be answered before it will be known just how much nitrogen is fixed annually per acre of soil by this process.

Possibly one reason why more rapid progress in this field of research has not been made is due to the lack of suitable and accurate methods for studying the activities of these microorganisms in their natural environment. It has been necessary to use very abnormal conditions in the laboratory, and unfortunately results obtained in this manner can not always be carried to the practical application stage. Therefore, if progress is made in the future, it necessarily follows that, either improvement in our present methods is essential, or new methods must be introduced.

For certain phases of the study of nitrogen fixation by field soils, the ordinary solution method has usually given the best results. This consists of adding from 1 to 10 grams of fresh soil to 100 c.c. of a sterile standard mannite or dextrose solution. After incubating at room temperature or 28° C. for 7 to 28 days the increase in total nitrogen over that in sterilized control cultures is taken to be the amount fixed from the atmosphere. A number of investigators have shown that aeration greatly increases the

rate of nitrogen fixation by pure cultures of Azotobacter which are the large aerobic non-symbiotic nitrogen fixing bacteria. We have taken advantage of this idea of increasing the rate of fixation by mixed soil cultures in a dextrose solution in an attempt to make an improvement in the solution method. The object of this paper is to present the results of some studies on the comparison of aerated and non-aerated solution cultures where soils of widely different origin and treatment have been used.

Two of the soils No. 1 and No. 2, were Carrington loam. Soil No. 1 was collected July 29, 1924 from one of the no treatment plots of the continuous corn series on the Agronomy Farm of the Iowa Agricultural Experiment Station. It is definitely known that this plot has not had any fertilizer treatment since 1914, but it has shown a rather high nitrogen fixing power. Soil No. 2 was collected the last part of October 1925 from the plot receiving 4 tons of manure annually in the humus series of plots on the Iowa State College campus. The sample of soil from Colorado was obtained the early part of November 1925 from Dr. W. G. Sackett and Professor J. C. Ward. Professor P. L. Gainey sent us the sample of soil from Kansas about the first of November 1925. With the exception of sample No. 1 all of the soils were kept moist in the laboratory until the experiments were completed.

The standard dextrose solution used in the experiments was made as follows: Distilled water 1000 c.c.; K_2HPO_4, 0.2 gm.; $MgSO_4$, 0.2 gm.; Ca Cl_2, 0.02 gm.; Dextrose, 10 gms.; and $FeCl_3$ (10% solution), 2 drops. The reaction was adjusted to pH 6.8.

One hundred c.c. portions of this solution were transferred by means of a pipette to 500 c.c. Erlenmeyer flasks. One-half of the flasks were plugged with cotton, sterilized in the autoclave, and used for the non-aerated cultures. The other one-half of the flasks were fitted with two-hole rubber stoppers containing two pieces of bent glass tubing to provide for a means of aeration. One of these tubes reached to the bottom of the flask, while the other was cut off about one-half an inch below the stopper. On the outer ends of these tubes were attached short pieces of thick-walled rubber tubing which permitted the cultures to be connected in series. Into one of these pieces of rubber tubing a short glass tube filled with cotton was inserted to serve as a trap for the prevention of any possible contamination from one flask to the other. These flasks with rubber stoppers and fittings were sterilized in the autoclave and used for the aerated cultures. After sterilization either 10 or 15 flasks were connected in a series to one suction pump. Aeration was accomplished by bubbling air

vigorously thru the series. The non-aerated culture was placed on the table beside its corresponding areated culture.

In the experiment where the Iowa soil sample No. 1 was used the solutions were inoculated with one gram of the soil immediately after it was brought in from the field. Duplicate cultures, both aerated and non- aerated, were incubated for 2, 3, 4, 6 and 10 days. Control cultures were sterilized immediately after adding the soil. Total nitrogen determinations were made on all of these cultures at the end of the incubation periods, and the results are shown in Table 1.

In the next experiment twenty aerated and twenty non-aerated cultures were used for each of the three samples of soil including the Iowa soil sample No. 2, the Colorado soil and the Kansas soil. Duplicate cultures for each soil were sterilized immediately after the soil was added to serve as controls. The solutions were inoculated with one gram of the moist soil, and duplicate cultures both aerated and non-aerated were incubated for 1, 2, 3, 4, 5, 6, 7, 10, 15 and 28 days. When this experiment was completed it was repeated in every detail with the same soils except that instead of incubating for 28 days only 20 days incubation was used for the longest period. Since these two experiments were almost identical and the results obtained were so very similar, they will be treated as one experiment in the discussion. The results given in Table 1 for these three soils, therefore, represent the averages of four determinations in all cases except where the cultures were incubated for 20 and 28 days. The figures shown for these cultures are averages of duplicates only.

Discussion of Results

By comparing the results for the aerated cultures with those for the non-aerated cultures made from the Iowa soil sample No. 1, it is readily seen that aeration greatly increased the rate of nitrogen fixation. At the end of two days, four times as much nitrogen had been fixed in the aerated cultures as in the non-aerated. The third day the amount of nitrogen fixed in the aerated cultures was 9.93 mg. as compared with 3.07 mg. for the non-aerated cultures. The maximum fixation of nitrogen was reached on the fourth day for the aerated cultures. Continued aeration for six and ten days showed a decided loss in the amount of fixed nitrogen. This point will be discussed later in this paper. In the case of the non-aerated cultures, the amount of nitrogen fixed gradually increased, but even on the tenth day the amount of fixation was considerably behind the maximum fixation for the aerated cultures.

These results were so evident in pointing out the advantage of aerating cultures for nitrogen fixation studies in solutions that it was decided to plan a more detailed experiment and use soils from different sources. The results of this experiment, which was duplicated as explained previously do not show such a striking advantage for the aeration method over the non-aerated method. We believe that this discrepancy may be explained by the fact that fresh field soils were not used in these experiments as was the case with the first experiment. By keeping these soils moist in the laboratory it is entirely possible that their nitrogen fixing power was greatly decreased, which would account for the apparently poor results.

The results given in Table 1 for the Iowa soil sample No. 2, the Colorado soil and the Kansas soil do show, however, that aeration increased the rate of nitrogen fixation by all of the soils. This was especially true for the first four or five days of incubation. In the case of the Colorado soil the maximum amount of fixation was nearly reached on the fourth day. The length of time necessary for maximum fixation varied with each soil, and consequently no definite time limit for incubation could be set for all soils if we are to use these results. These results emphasize the need of further studies on fresh field soils to determine primarily the length of time necessary to accomplish maximum fixation for different soils, and soils under different systems of cropping and fertilizer treatment.

An interesting observation which is clearly brought out in the data presented in Table 1 is the fact that after the maximum fixation was reached there was always a decrease in the amount of fixed nitrogen if the cultures were incubated beyond this point. This may be accounted for by assuming that after the nitrogen fixing microorganisms had reached their maximum development, they began to die off, and other organisms attacked their dead bodies and protein compounds and liberated free ammonia. This ammonia was readily lost to the atmosphere either when the cultures were aerated or not aerated.

It seems safe to conclude from the results presented in this paper that aeration of solution cultures for nitrogen fixation studies increased the rate of nitrogen fixation and this was especially noticeable if fresh soil was used for the inoculum. In agricultural practices these results may be taken to indicate the importance of recommending those soil treatments which tend to maintain sufficient soil aeration for the maximum fixation of atmospheric nitrogen by the non-symbiotic nitrogen fixing microorganisms.

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TABLE I

NITROGEN FIXATION IN DEXTROSE SOLUTION BY FOUR SOILS SHOWING COMPARISON OF AERATED AND NON-AERATED CULTURES

Number Days Incubated	Iowa Soil				Colorado Soil		Kansas Soil	
	Nitrogen Fixed in Cultures				Nitrogen Fixed in Cultures		Nitrogen Fixed in Cultures	
	Sample No. 1		Sample No. 2					
	Aerated	Not Aerated	Aerated	Not Aerated	Aerated	Not Aerated	Aerated	Not Aerated
	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
1	.....	.....	.25	.27	.54	.09	.09	.01
2	4.53	1.13	.22	.15	1.12	.09	1.86	.35
3	9.93	3.07	.77	.00	5.50	.48	4.99	1.41
4	13.58	4.09	1.70	.95	7.30	2.44	4.54	5.18
5	.....	.....	2.79	1.82	6.95	4.79	5.21	4.37
6	12.27	7.96	2.49	2.47	6.07	6.92	6.02	4.21
7	.....	.....	2.99	3.63	7.74	7.14	5.95	5.44
10	8.40	9.40	4.56	3.54	7.51	8.27	7.08	5.24
15	.....	.....	4.59	4.06	8.00	6.41	5.57	4.89
20	.....	.....	4.12	3.99	6.11	7.77	6.44	5.66
28	.....	.....	4.25	3.15	5.79	3.99	5.79	4.76