The occurrence of Azotobacter in Iowa soils and factors affecting their distribution

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The Occurrence of Azotobacter in Iowa Soils and Factors Affecting Their Distribution

By William P. Martin, R. H. Walker and P. E. Brown

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS
R. E. Buchanan, Director

SOILS SUBSECTION
AGRONOMY SECTION

AMES, IOWA
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SUMMARY AND CONCLUSIONS

1. Two hundred eighty-seven Iowa soils representing 52 soil types and 37 soil series and distributed in the five soil areas of the state—the Wisconsin drift, the Iowan drift, the Missouri loess, the Southern Iowa loess and the Mississippi loess—were examined for the presence of Azotobacter.

2. The presence of the Azotobacter was determined in these soils by the Winogradsky soil plaque and the selective culture agar-plate methods using mannitol as the energy source.

3. Of the 287 samples collected, 101 or 35.2 percent contained Azotobacter.

4. The virgin, high-lime Knox soils of the Missouri loess soil area, the Webster soils of the Wisconsin drift soil area, the Clyde-Floyd soils of the Iowan drift soil area and the bottomland soils were generally positive with 100, 91.7, 71.4 and 76.4 percent, respectively, of the samples of these soils containing the bacteria.

5. The Clarion samples of the Wisconsin drift soil area and the terrace soils contained Azotobacter in only about one-third of the samples; the loessial soils, with the exception of the Knox, and the remaining drift samples only occasionally showed any growth of the organisms.

6. The samples were classified according to their topographic position which showed that the more depressed the position, the greater the percentage of samples which contained Azotobacter.

7. An analysis of the samples for pH, content of available phosphate, total nitrogen and calcium carbonate and a calculation of the ratio of carbonate to phosphate was made in order to determine whether or not any relation existed between the presence of the Azotobacter and any one or all of these soil constituents.

8. A multiple correlation of the data arbitrarily summarized on the basis of pH showed that the presence of the Azotobacter in Iowa soils was closely associated with the pH and available phosphate content of the samples and associated very little with the total nitrogen content, the calcium carbonate content or the carbonate-phosphate ratio. The association with pH was closer than that with available phosphate.

9. A study of the limiting pH and available phosphate content values for Azotobacter in Iowa soils led to the conclusion that soils with pH values from about pH 5.42, the limiting value obtained by an extrapolation of the simple regression equation, to pH 6.0, below which only one sample contained the bacteria, and available phosphate contents less than 35 pounds per acre would probably not contain the bacteria.

10. A method recently developed by Fisher (12) was used to determine whether or not the pH, available phosphate content and total nitrogen content of the soils would serve to differentiate be-
tween the samples which contained Azotobacter and those which did not contain the organisms. It was found that these variables did significantly differentiate the two groups. The pH had the greatest influence in this respect, the available phosphate content the next greatest and the total nitrogen content had the least influence as a differentiating factor. These conclusions are in agreement with those obtained by the multiple correlation method.

11. The presence or absence of the Azotobacter in the principal soil series of Iowa may be largely explained on the basis of the characteristic pH and the content of available phosphate of the samples in each series.

12. In addition to the determination of factors which limited the occurrence of the Azotobacter in Iowa soils, a study was made of the factors which influenced the amount of growth which the Azotobacter would make in the soil. For this study only those samples were considered which contained the bacteria.

13. The available phosphate content of the samples which contained Azotobacter did not appear to be associated with the amount of Azotobacter growth.

14. The results for pH showed that an increase in the pH of the sample did improve conditions so that the Azotobacter could make a better growth.

15. The results for total nitrogen indicated that this variable exerted the greatest influence upon the amount of growth which the Azotobacter would make in the soil, when other factors were not unfavorable.

16. Some experimental plots were sampled to determine whether or not soils which did not originally contain the organisms could be treated with lime and rock phosphate to correct the acidity and deficiency in available phosphate and thus be put into condition to support a flora of Azotobacter. The results showed that the addition of lime to these soils had improved conditions so that the organisms were detected. Where rock phosphate was added in addition to the lime, a better growth of the organisms was not obtained.

17. A group of experimental plots on high-lime soils which contained large amounts of organic matter, as indicated by total nitrogen determinations, contained a vigorous growth of the Azotobacter as expected.
The Occurrence of Azotobacter in Iowa Soils and Factors Affecting Their Distribution

BY WILLIAM P. MARTIN, R. H. WALKER AND P. E. BROWN

The discovery of the nitrogen-fixing bacteria of the genus Azotobacter by Beijerinck in 1901 has led to many researches dealing with their distribution in soils and their economic importance in the maintenance of soil fertility. It has been estimated that an active flora of these organisms may fix from 15 to 40 pounds of nitrogen per acre per year in the soil and may act, therefore, as an economically important aid in keeping up the nitrogen content of the soil. It is of considerable interest, therefore, to determine the distribution of the Azotobacter in Iowa soils.

The Azotobacter are widely distributed over the earth's surface, having been found in soils from practically every nation in the world. They have been detected in very humid and very dry regions; in soils in hot and cold climates; at high as well as at low elevations; in both fertile and infertile soil; in surface soil and subsoil; in virgin, cultivated and forest soils; and in newly-formed peat and volcanic soils; yet, approximately half of all soils examined have failed to show their presence. There must, therefore, be some factor or factors limiting the growth and hence the distribution of these organisms in soils.

The work of many investigators has indicated that the Azotobacter are sensitive to acidity, and it has been claimed that they ordinarily do not occur in soils having a reaction more acid than pH 6.0, whereas, soils with a higher pH usually contain the organisms. This conclusion was arrived at largely from studies in which liquid culture media were used. Inasmuch as the Azotobacter are strict aerobes, they do not grow well in liquid media which provide partly anaerobic conditions, and the results of tests thus obtained certainly are not representative of those which would be obtained under the natural conditions existing in the soil. In the film which is formed upon the surface of the medium by these organisms, there is competition with other bacteria for the mannitol available, and in addition, amoebae flourish in the solution and may devour many Azotobacter cells. Furthermore, the liquid medium frequently becomes charged with carbonic and butyric acids which may limit Azotobacter development so that the brown film characteristic of these organisms is formed only when the soil tested contains a relatively large number of the specific organisms. It is possible, therefore, for a soil to contain large numbers of the organisms, and yet they may escape detection by the liquid culture test.

1Project 221 of the Soils Subsection, Iowa Agricultural Experiment Station.
2The authors are indebted to Prof. George W. Snedecor and Miss Gertrude M. Cox of the Statistical Laboratory for the suggestions and criticisms offered.
In a search for a more accurate method of detecting the presence of the Azotobacter under an environment which more nearly duplicates that existing in the soil, Winogradsky developed two methods: the soil plaque method and the silica gel plate method. These two methods, with some modifications, were used in this investigation. Frequently soils which show no Azotobacter when tested in a liquid culture may be found well supplied with these organisms when tested by one or both of the above methods.

Using either the silica gel or the soil plaque method, various investigators have recently been able to demonstrate the presence of Azotobacter in certain soils which were more acid than pH 6.0. By the use of the silica gel plates, Vandecaveye and Anderson (29) showed that the organisms were present in small numbers in western Washington soils which were more acid than pH 5.5. Wilson and Wilson (32) using the soil plaque method found Azotobacter in a large majority of virgin peat soils tested, many of which were distinctly acid in reaction. These investigators came to the conclusion that the carbonate-phosphate ratio was more important than the soil reaction in controlling the growth of the organisms.

Burk, Lineweaver and Horner (8), using the manometric technique developed by Warburg, showed that the Azotobacter existed in a medium well below a pH of 6.0 and even as low as pH 4.5 or less as long as fixed nitrogen was present in the medium. Winogradsky (36), however, found that the effect of nitrogen upon the growth of Azotobacter in the soil was to depress and often to entirely eliminate them. He attributed this to the inability of the Azotobacter to compete either for the nitrogen available or for the carbonaceous energy-yielding material which was rapidly utilized by the other organisms in the presence of the larger amounts of nitrogen.

The work of various investigators has also indicated that the organisms are sensitive to a lack of an available supply of phosphate. The phosphorus content of Azotobacter cells has been recorded as varying from 2.51 to 4.93 percent of the dry material, and this certainly indicates a striking need for phosphorus during the growth of the cells.

With these facts in mind, an investigation was planned to study the presence of Azotobacter in some soil types in the more important soil series of Iowa in order to determine the distribution of the organisms in the state. Analysis of the samples of the soils collected for pH, content of available phosphate, of calcium carbonate and of total nitrogen and a calculation of the ratio of carbonate to phosphate were made in order to determine whether or not any of these factors were correlated with the occurrence of Azotobacter. Finally a study was made of the presence of Azotobacter in the soils of some experimental plots located upon various soil types to determine the influence of applications of lime and other fertilizers upon the bacteria. The results secured in this work are presented in this bulletin.
PLAN OF THE EXPERIMENT

Two hundred eighty-seven samples of Iowa soils were collected and examined for the presence of Azotobacter. They were selected at random under a variety of field conditions and were taken in various parts of the state. They represented 52 soil types of 33 soil series, and they were collected in the five soil areas of the state—the Wisconsin drift, the Iowan drift, the Missouri loess, the Southern Iowa loess and the Mississippi loess. The locations from which the samples were taken are shown in fig. 1.

The soil survey reports for the individual counties (6, 26) give the description of each soil type.

Each sample taken was a composite of 6 to 12 subsamples taken to a depth of from 4 to 6 inches. The samples were collected in a new “Titelok” waxed, quart container and precautions were taken to prevent contamination from one sample to the next. A portion of the well mixed, damp soil was taken for a determination of the presence of Azotobacter, and the remainder was air-dried, passed through a 20-mesh screen and sampled later for the chemical determinations. All determinations were made in duplicate or triplicate, and the data presented are averages of all the determinations on individual samples.

The presence of Azotobacter was determined in the first soils tested by two methods: (a) The Winogradsky soil plaque method (33, 34, 35) and (b) the selective culture agar-plate method (10). The two methods were found to give agreeing results in the studies on the first 169 samples. Thereafter, therefore, the selective culture agar-plate method was used on all the samples and the soil plaque method only upon those samples having a pH value greater than 6.0.

Fig. 1. Distribution of Azotobacter in Iowa Soils
The procedure in the Winogradsky soil plaque tests consisted of the thorough mixing of 0.75 gm. of mannitol, 200 mgm. of calcium carbonate and 300 mgm. of potassium hydrogen phosphate with 50 gm. of soil, and the addition of enough water so that a moist, pasty mixture was obtained. The material to be added, when soluble, was dissolved in the water and thus incorporated. The mixture was then placed in a 5-centimeter petri dish and the surface made smooth with a moist spatula. These plaques were then incubated in a moist chamber at 28°C. for 48 to 96 hours. If a suitable environment was provided and Azotobacter were present in the soil, macroscopic colonies were readily seen on the surface of the soil. The appearance of such colonies demonstrated the presence of the Azotobacter in the soil.

In the agar-plate method a nitrogen-free nutrient agar medium was prepared. This was poured into large petri dishes and allowed to solidify. The soil to be tested was dried sufficiently to pass a 40-mesh screen. The sieved soil was spread evenly over the surface of the petri dish. After 4 days incubation at 28°C. and if Azotobacter were present in the soil, large, limpid, partially opaque, raised colonies appeared on the surface of the plate. These colonies turned a dark brown or almost black upon standing. Stained mounts showed the typical Azotobacter cells. The nitrogen-free nutrient agar medium used in this method consisted of 15 gm. of agar, 20 gm. of mannite and 2 gm. of the following stock salt mixture for each liter of medium:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-potassium phosphate</td>
<td>100</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>60</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>60</td>
</tr>
<tr>
<td>Ferric sulfate</td>
<td>1</td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>1</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>178</td>
</tr>
</tbody>
</table>

The pH of all samples taken was determined electrometrically by the quinhydrone method as described by Biilmann and Jensen (3). The calcium carbonate content was determined volumetrically by the Schollenberger (25) method. Truog's (28) 0.002 N sulfuric acid extraction method was used to determine the available phosphate content of the samples. Total nitrogen determinations were made according to the recommendations of the Association of Official Agricultural Chemists (1). The ratio of carbonate to phosphate was calculated from the content of these constituents.

In addition to the 287 soil samples described above, samples were taken from experimental plots and analyzed for Azotobacter and for pH, available phosphate content and total nitrogen content. These plots had been treated with varying amounts of lime and different fertilizers. The fertilizer treatments will be given in connection with the results of the analyses. Three types of experimental fields were sampled: (a) A series of plots at the Agronomy Farm located at Ames, (b) certain plots on cooperative experimental fields located throughout the state and upon a variety of soil types and (c) some
plots on experimental fields on high-lime soils located in the Wisconsin drift soil area.

As an aid to the interpretation of the data on pH, available phosphate, total nitrogen and the presence of Azotobacter presented in this bulletin, correlation coefficients and regression equations were calculated by the methods described by Wallace and Snedecor (30). The data were also analyzed by the method of Fisher (12).

RESULTS

DISTRIBUTION OF AZOTOBACTER IN IOWA SOILS

Figure 1 shows the locations from which samples were taken and also which samples contained Azotobacter. Most of the samples taken in the Wisconsin drift soil area showed Azotobacter. A greater percentage of Wisconsin drift samples contained the organisms than samples from any of the other soil areas. The outstanding fact appearing in fig. 1, however, is the large number of samples which did not contain the organisms. Of the 287 samples, only 101 or 35.2 percent showed Azotobacter present.

The occurrence of Azotobacter in Iowa soils is shown more specifically in table 1 in which the samples are grouped by the soil series. Ninety-two percent of the Webster samples from the Wisconsin drift soil area contained the organisms. The next highest result obtained among the drift soils was with the Clyde-Floyd soils of the Iowan drift soil area; about 70 percent of these samples contained the bacteria. Both the Webster and the Clyde-Floyd series are upland soils occurring in the flat to depressed areas, and the growth of the Azotobacter in the samples which contained them was good as indicated by the three pluses in the "growth type" column.

About 30 percent of the Clarion samples showed the presence of the organisms while in the case of the related soil of the Iowan drift, the Carrington, only 19 percent contained any. These two series occur on the more rolling uplands surrounding the Webster and the Clyde-Floyd soils in the two drift areas and contained a less active Azotobacter flora as indicated by the two pluses in the "growth type" column.

The remaining Iowan drift and Wisconsin drift soils were not sampled so extensively since they are relatively unimportant in acre-age in the state, but of the five samples taken, none contained the bacteria.

The Lindley and Shelby soils were practically all lacking in Azotobacter; only one sample out of 11 contained the organisms. These soils were developed from the drift material of the Kansan glaciation upon which the loessial material of the southern part of the state was deposited. In many places this loessial surface soil has been washed away, leaving the old Kansan till exposed. On these exposures the Lindley and Shelby soils have developed.

With the exception of the Knox samples, few of the loessial soils contained Azotobacter; only 13 samples out of 101 showed the presence of any of the organisms. In addition, where they do occur
TABLE 1. THE OCCURRENCE OF AZOTOBACTER IN THE PRINCIPAL SOIL SERIES OF IOWA TOGETHER WITH THE ESTIMATED ACREAGE OF EACH SERIES IN 81 SURVEYED COUNTIES.

<table>
<thead>
<tr>
<th>Series grouping</th>
<th>Soil* area</th>
<th>Number of samples</th>
<th>Number of samples which contained Azotobacter</th>
<th>Estimated acreage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Growth type</td>
</tr>
<tr>
<td>Drift Soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarion</td>
<td>W.D.</td>
<td>23</td>
<td>7</td>
<td>30.4</td>
</tr>
<tr>
<td>Carrington</td>
<td>I.D.</td>
<td>21</td>
<td>4</td>
<td>19.0</td>
</tr>
<tr>
<td>Webster</td>
<td>W.D.</td>
<td>24</td>
<td>22</td>
<td>91.7</td>
</tr>
<tr>
<td>Clyde-Floyd</td>
<td>I.D.</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
</tr>
<tr>
<td>Lindley</td>
<td>L.</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Shelby</td>
<td>L.</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Conover</td>
<td>W.D.</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Dickinson</td>
<td>I.D.</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Dodgeville</td>
<td>I.D.</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loess Soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinton</td>
<td>M.L. &amp; S.L.L.</td>
<td>19</td>
<td>4</td>
<td>21.0</td>
</tr>
<tr>
<td>Grundy</td>
<td>S.L.L.</td>
<td>26</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>Knox</td>
<td>M.L.</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>Marshall</td>
<td>M.L.</td>
<td>27</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>Tama</td>
<td>M.L.</td>
<td>28</td>
<td>4</td>
<td>14.3</td>
</tr>
<tr>
<td>Fayette</td>
<td>M.L.</td>
<td>5</td>
<td>1</td>
<td>20.0</td>
</tr>
<tr>
<td>Muscatine</td>
<td>M.L.</td>
<td>9</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Marion-Putnam</td>
<td>S.L.L.</td>
<td>6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Terrace Soils</td>
<td></td>
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<tr>
<td>Benoit</td>
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<tr>
<td>Bremer</td>
<td></td>
<td></td>
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<tr>
<td>Buckner</td>
<td></td>
<td></td>
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<tr>
<td>Chariton</td>
<td></td>
<td></td>
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<tr>
<td>Fargo</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hancock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Judson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O’Neill</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sioux</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waukesha</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottomland Soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larnoure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarpy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wabash</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>26</td>
<td>76.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>**W.D.—Wisconsin Drift Area</td>
<td>287,101,804</td>
<td>35.2</td>
<td>28,720,894</td>
<td></td>
</tr>
<tr>
<td>L.—Mississippi Loess Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.—Mississippi Loess Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.—Southern Iowa Loess Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo.—Missouri Loess Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All.—Found in all the soil areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

they are much less active as is indicated by the single plus in the “growth type” column.

The Knox silt loam reacted very peculiarly in this investigation. It contains a high percentage of lime (the average pH of the samples was 8.39). On the agar plates at the end of an incubation period of 3 days, the colonies were very small, compact and opaque, whereas the colonies on the other plates were large, raised, limpid and less opaque. After a longer incubation period, however, the colonies developed normally and upon longer standing turned dark brown in the characteristic manner. Under the microscope the cells in the colonies appeared to be typical Azotobacter. The explanation of
this slow development is not known. Perhaps the natural environment was not favorable for an active growth of the organisms, and they were present in a so-called resting state in which case the lag phase, after the organisms are placed in a suitable environment, may be longer and thus account for the results obtained.

The 10 terrace soil series were sampled inadequately by types, and for this reason the data were assembled and considered as a unit. The soils of two of these series, the O'Neill and the Waukesha, are worthy of special consideration because of the large acreage they cover in the state. Forty-four percent of the O'Neill samples contained Azotobacter. With the Waukesha soils, on the other hand, the organisms were present in only 1 sample out of 10. Considering the terrace soils as a whole, however, the results showed a fairly good growth, since 40 percent of the soils collected contained Azotobacter and the two pluses in the "growth type" column indicate a medium growth of the organisms in those soils which contained them.

As in the case of the terrace soils, the swamp and bottomland soils were considered as a unit. Twenty-six out of the 34 samples of these soils showed a very good growth of the bacteria. Two of the six series sampled contained the organisms in 100 percent of the cases, three of them in more than 90 percent of the cases, but the most important bottomland series from the standpoint of total acreage covered in the state, the Wabash, contained the organisms in only 60 percent of the samples. In percentage of samples which contained Azotobacter and in the activity of the organisms in those samples in which they did occur, the bottomland soils would rank with the Webster and the Clyde-Floyd soils of the Wisconsin and Iowan drift soil areas.

These relationships can best be visualized by reference to fig. 2 which places the soils by series in the order of decreasing percentage of samples which contained Azotobacter.

Estimates of the total acreage in the 81 surveyed counties of Iowa covered by each of the soil series studied were obtained. These estimates are given in table 1. The total estimated acreage in the 81 surveyed counties was 29,087,475 and the acreage covered by the soils of the series studied in this investigation was 28,720,804 or about 98.75 percent of the total. The soils of the series examined in this investigation, therefore, cover the bulk of the estimated acreage in the surveyed counties of Iowa.

Some interesting relationships between the topographic position of the soils and the percentage of samples which contained Azotobacter have been noted. In order to make these relationships clearer, the samples have been classified according to the topographic position of the soils, and this grouping is shown in table 2. This table shows that the upland loessial soils contained few Azotobacter. This situation was much the same in the case of the upland drift soils which occur on the higher, more rolling land, although more of these soils did contain the Azotobacter than the soils of the more eroded loessial areas.
The Azotobacter were more commonly present in the terrace soils, over a third of the samples of these soils containing them. The bottomland soils and the upland drift soils which occur in depressions, however, showed the best growth of the organisms; over 75 percent of the samples contained them. From these data it appears that the more depressed the topographic position of the soil, with resulting poor drainage, the greater is the percentage of samples which contain Azotobacter, provided, of course, that the soil is drained sufficiently to prevent anaerobic conditions from developing.

The influence of topographic position upon the growth of Azotobacter is further emphasized by a comparison of related soils in the Wisconsin and Iowan drift soil areas (see table 1). The soils in the Wisconsin drift are much younger geologically than the soils in the Iowan drift, and hence they are less eroded and leached. This is indicated by the higher pH and greater amount of plant nutrients in the various surface soils. It might be expected, therefore, that

**TABLE 2. DISTRIBUTION OF AZOTOBACTER IN IOWA SOILS IN VARIOUS TOPOGRAPHICAL POSITIONS.**

<table>
<thead>
<tr>
<th>Topographical position</th>
<th>Number of samples</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upland soils Drift</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressions</td>
<td>31</td>
<td>27</td>
<td>87.1</td>
</tr>
<tr>
<td>Elevations</td>
<td>60</td>
<td>12</td>
<td>20.0</td>
</tr>
<tr>
<td>Loess</td>
<td>130</td>
<td>23</td>
<td>17.7</td>
</tr>
<tr>
<td>Terrace Soils</td>
<td>32</td>
<td>13</td>
<td>40.6</td>
</tr>
<tr>
<td>Bottomland Soils</td>
<td>34</td>
<td>28</td>
<td>76.4</td>
</tr>
</tbody>
</table>
the Wisconsin drift soils would contain more Azotobacter than those in the Iowan drift. This was the case in these results as 92 percent of the Webster soils contained the Azotobacter, while only 71 percent of the Clyde-Floyd soils in the Iowan drift showed any organisms present. With the Clarion soils of the Wisconsin drift, 30 percent of the samples contained the organisms, and with the related Carrington soils of the Iowan drift, only 19 percent showed their presence.

CORRELATION BETWEEN THE OCCURRENCE OF AZOTOBACTER IN IOWA SOILS AND THE CHEMICAL COMPOSITION OF THE SOILS SAMPLED

The pH of the individual samples ranged from 4.99 to 8.58, the total nitrogen content from 1,400 pounds to 15,600 pounds per acre, the amount of readily available phosphate from 15.6 to 520 pounds per acre, the content of calcium carbonate from 202 to 227,860 pounds per acre and the ratio of carbonate to phosphate from 1.2 to 3,646.

In order to discover any relationships that might exist between the occurrence of Azotobacter in these soils and the chemical composition given in such a mass of data as those obtained from the individual sample analyses, it was necessary to choose one of the chemical factors as a base and to summarize the rest of the data on that basis. Since the pH scale provided a convenient set of units, it was chosen for this purpose. The data, therefore, have been summarized and classified according to pH in table 3.

A multiple correlation was computed from the data in table 3. In this correlation the percentage of samples in the different pH classes which contained Azotobacter was taken as the dependent variable. The pH measurements used were the mid-points of the pH classes. Those used for total nitrogen were coded values giving the milligrams of nitrogen per 10 grams of soil.

Since the carbonate-phosphate ratio was calculated from the amount of calcium carbonate and of available phosphate present in

<table>
<thead>
<tr>
<th>pH Class</th>
<th>Number of samples</th>
<th>Samples containing Azotobacter</th>
<th>Average calcium carbonate (Lbs./acre)</th>
<th>Average available phosphate content (Lbs./acre)</th>
<th>Average carbonate to phosphate ratio</th>
<th>Average available phosphate (Lbs./acre)</th>
<th>Total nitrogen (Lbs./acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00-5.29</td>
<td>6</td>
<td>0</td>
<td>.0</td>
<td>862</td>
<td>11.8</td>
<td>84.6</td>
<td>3,500</td>
</tr>
<tr>
<td>5.30-5.59</td>
<td>33</td>
<td>0</td>
<td>.0</td>
<td>815</td>
<td>24.2</td>
<td>42.9</td>
<td>4,130</td>
</tr>
<tr>
<td>5.60-5.89</td>
<td>38</td>
<td>0</td>
<td>1.6</td>
<td>814</td>
<td>21.6</td>
<td>42.8</td>
<td>4,240</td>
</tr>
<tr>
<td>5.90-6.19</td>
<td>34</td>
<td>1</td>
<td>21.0</td>
<td>821</td>
<td>19.9</td>
<td>57.9</td>
<td>4,680</td>
</tr>
<tr>
<td>6.20-6.49</td>
<td>34</td>
<td>1</td>
<td>14.7</td>
<td>1,193</td>
<td>21.5</td>
<td>70.0</td>
<td>4,660</td>
</tr>
<tr>
<td>6.50-6.79</td>
<td>27</td>
<td>12</td>
<td>44.4</td>
<td>1,769</td>
<td>37.7</td>
<td>78.3</td>
<td>4,600</td>
</tr>
<tr>
<td>6.80-7.09</td>
<td>20</td>
<td>16</td>
<td>80.0</td>
<td>3,539</td>
<td>60.6</td>
<td>141.0</td>
<td>5,960</td>
</tr>
<tr>
<td>7.10-7.39</td>
<td>18</td>
<td>10</td>
<td>62.5</td>
<td>2,121</td>
<td>23.0</td>
<td>122.1</td>
<td>4,600</td>
</tr>
<tr>
<td>7.40-7.69</td>
<td>8</td>
<td>4</td>
<td>80.0</td>
<td>11,569</td>
<td>74.6</td>
<td>112.8</td>
<td>4,700</td>
</tr>
<tr>
<td>7.70-7.99</td>
<td>14</td>
<td>11</td>
<td>84.6</td>
<td>38,912</td>
<td>491.0</td>
<td>124.0</td>
<td>7,400</td>
</tr>
<tr>
<td>8.00-8.29</td>
<td>16</td>
<td>16</td>
<td>100.0</td>
<td>45,913</td>
<td>303.0</td>
<td>172.1</td>
<td>7,120</td>
</tr>
<tr>
<td>8.30-8.59</td>
<td>18</td>
<td>18</td>
<td>100.0</td>
<td>87,518</td>
<td>975.8</td>
<td>129.5</td>
<td>5,540</td>
</tr>
</tbody>
</table>
TABLE 4. REGRESSION STATISTICS FOR PERCENTAGE OF SAMPLES WHICH CONTAINED AZOTOBACTER (X) UPON AVERAGE pH, TOTAL NITROGEN CONTENT AND CARBONATE-PHOSPHATE RATIO OF THE SAMPLES FALLING IN 12 pH CLASSES.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>pH (A)</th>
<th>Total nitrogen (E)</th>
<th>Carbonate-phosphate ratio (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order correlation coefficients, r.</td>
<td>0.961**</td>
<td>0.788**</td>
<td>0.642*</td>
</tr>
<tr>
<td>Standard partial regression coefficients, ( \beta )</td>
<td>0.968*</td>
<td>0.084</td>
<td>-0.100</td>
</tr>
</tbody>
</table>

Multiple correlation coefficient, \( R \). \( R^2 = 0.932; \ R = 0.965** 

*—significant. **—highly significant.

The standard partial regression coefficients show, after the associations of the independent variables with each other had been taken into consideration, that the total nitrogen and carbonate-phosphate ratio were associated very little with the percentage of samples which contained the Azotobacter. The percentage weight of each of these variables was 7.2 percent and 8.6 percent, respectively, whereas 85.2 percent of the accounted-for variation in percentage of samples which contained Azotobacter may be based on the average pH of the samples. In addition, it may be observed that the correlation was increased very little over that which existed between the percentage of samples which contained Azotobacter and the pH by the consideration of the average total nitrogen content of the samples and the carbonate-phosphate ratio.

Eliminating these variables from the discussion, therefore, the average pH, calcium carbonate and available phosphate content of the samples were considered as the second group. The regression statistics for this group are given in table 5.

The standard regression coefficients show that about 54 percent of the accounted-for variation in percentage of samples which contained Azotobacter may be based on pH, about 45 percent on available phosphate and only about 1 percent on the content of calcium carbonate. Little association seemed to exist between the calcium carbonate and available phosphate content. Therefore, the average pH, available phosphate and calcium carbonate content of the samples were considered as the second group. The regression statistics for this group are given in table 5.

TABLE 5. REGRESSION STATISTICS FOR PERCENTAGE OF SAMPLES WHICH CONTAINED AZOTOBACTER (X) UPON AVERAGE pH, AVAILABLE PHOSPHATE AND CALCIUM CARBONATE CONTENT OF THE SAMPLES FALLING IN 12 pH CLASSES.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>pH (A)</th>
<th>Calcium carbonate (B)</th>
<th>Available phosphate (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order correlation coefficients, r.</td>
<td>0.961**</td>
<td>0.702*</td>
<td>0.955**</td>
</tr>
<tr>
<td>Standard partial regression coefficients, ( \beta )</td>
<td>0.552*</td>
<td>-0.010</td>
<td>0.455*</td>
</tr>
</tbody>
</table>

Multiple correlation coefficient, \( R \). \( R^2 = 0.958; \ R = 0.979** 

*—significant. **—highly significant.
carbonate content of the samples and the percentage of samples which contained Azotobacter outside that explained by its correlation with available phosphate. On the other hand, the available phosphate content of the samples was closely correlated with the percentage which contained Azotobacter as was the pH. The multiple correlation coefficient $R = 0.979$ shows, in addition, that the introduction of the closely associated variable, available phosphate, improved the correlation materially over either $r_{AX} = 0.961$ or $r_{DX} = 0.955$.

From the results given in tables 4 and 5, therefore, it may be concluded that in the samples of Iowa soils considered, the percentage of samples in the different pH classes which contained Azotobacter was closely associated with the average pH and available phosphate content of the samples and associated very little with the average total nitrogen content, calcium carbonate content and the carbonate-phosphate ratio. The association with pH appeared to be closer than that with available phosphate.

For the purpose of obtaining a regression equation based only upon pH and available phosphate, the calculations given in table 6 were made. It may be noted that the elimination of calcium carbonate from the calculations in table 5 did not decrease the multiple correlation coefficient which was $R = 0.979$ in both tables 5 and 6. The regression equation shows that in the samples of Iowa soils under consideration with an average available phosphate content of 80.9 pounds per acre and an average pH of 6.43, 33.4 percent of the samples might be expected to contain Azotobacter. Actually 35.2 percent of them did contain the organisms, the error of estimate in this case being very small. This regression equation will be used for estimation purposes in the section on series relationships.

**LIMITING pH VALUE AND AVAILABLE PHOSPHATE CONTENT FOR AZOTOBACTER IN IOWA SOILS**

The fact that two variables are closely associated does not necessarily mean that one of the variables is controlling the other, although it may be. In the correlations that have been calculated, the percentage of samples which contained Azotobacter obviously did not control the soil content of available phosphate nor its pH. Logically,
however, there is every reason to believe that the content of available phosphate and the pH may influence the presence of the Azotobacter. In this analysis, therefore, there is some justification for interpreting the degree of association of these variables with that of the percentage of samples which contained Azotobacter in terms of cause and effect. On this basis, the soil pH seems to be the most important factor controlling the presence of the Azotobacter in Iowa soils, and the content of available phosphate is also of importance in this respect.

It is of interest to determine the limiting pH value and available phosphate content for Iowa soils. It is recognized that the limiting value probably varies from soil to soil, as it is influenced by a variety of soil characteristics. The multiple regression equation in table 6 shows, for example, that the limiting pH for Azotobacter growth would probably be about pH 5.856 if the samples contained 20 pounds per acre of available phosphate, whereas the limiting value would drop to about pH 5.640 if the samples contained 30 pounds per acre of available phosphate. For Iowa soils, therefore, the best estimate of the limiting value for both pH and available phosphorus content, recognizing its limitations, can probably be obtained from the simple regression equations calculated from the data of table 3.

The regression equation for percentage of samples which contained Azotobacter \((X)\) upon the pH \((A)\) is:

\[ \bar{X} = 35.7 A - 193.8, \]

from which it is estimated, when \(\bar{X}\) is assigned a value of 0, that the limiting value for pH is about pH 5.42. Actually no samples contained the Azotobacter which had pH values less than 5.84 which emphasizes the need for caution in the interpretation of extrapolations of this type.

The regression equation for percentage of samples which contained Azotobacter \((X)\) upon the content of available phosphate \((D)\) is:

\[ \bar{X} = 0.84 D - 29.86, \]

from which it is found, when \(\bar{X}\) is assigned a value of 0, that the limiting content of available phosphate is about 35.4 pounds per acre. Actually two samples, each with an available phosphate content of 27 pounds per acre, contained the organisms.

Since these limiting values are obtained from extrapolations of the regression line in each case, caution should be used in their application. It would probably be safe to conclude that Iowa soils with pH values approaching pH 5.42 or less and available phosphate contents less than 35 pounds per acre would probably not contain Azotobacter, whereas soils with values higher than these may contain the organisms; the higher the pH value and content of available phosphate, the greater the probability that they would be found.
DISTINGUISHING pH, AVAILABLE PHOSPHATE AND TOTAL NITROGEN DIFFERENCES BETWEEN THE SAMPLES WHICH CONTAINED AZOTOBACTER AND THOSE WHICH DID NOT

The results just discussed were obtained from an analysis of the data of a table arbitrarily summarized on the basis of pH, hence, there may be some question regarding them. As supporting evidence, therefore, Fisher’s method (12) for the differentiation of two or more populations which have been sampled in several characters was applied to the data as a whole. For this purpose the data were divided into two groups on the basis of Azotobacter presence; those samples in the first group showed the Azotobacter present while those in the second did not. The characters which were measured were pH, available phosphate content and total nitrogen content.

| TABLE 7.—ANALYSIS OF VARIANCE OF THE CRUDE COMPOUND X. BETWEEN AND WITHIN GROUPS. |
|---|---|---|
| Degrees of freedom | Sum of squares | Mean square |
| Between groups | 3 | 0.0307564 | 0.0102518** |
| Within groups | 282 | 0.0217522 | 0.0000771 |
| Total | 285 | 0.0525076 | **—highly significant. |

The results of the analysis are given in Table 7. The mean square between groups was very highly significant, which shows that the pH, content of available phosphate and of total nitrogen did serve to differentiate between those samples of soil which contained Azotobacter and those which did not. The higher pH and larger amounts of available phosphate and total nitrogen were associated with the samples which contained the Azotobacter.

The relative influence of each variable apparently may be obtained from the formula:

\[ III. \ X = 0.1184 \frac{X_1}{\sqrt{Sx_1^2}} + 0.05412 \frac{X_2}{\sqrt{Sx_2^2}} + 0.03360 \frac{X_3}{\sqrt{Sx_3^2}}, \]

in which \( X \) = the chosen compound maximized, \( x_1 \) = the mean pH, \( x_2 \) = the mean available phosphate content and \( x_3 \) = the mean total nitrogen content; and where \( Sx_1^2, Sx_2^2, Sx_3^2 \), are the pooled sum of squares of deviations from the means.

Formula III shows that the relative influence of pH in the differentiation of the two groups was roughly twice that of the available phosphate content and three and a half times that of total nitrogen. The available phosphate content was roughly twice as effective as total nitrogen.

These results agree with those of the previous analyses by indicating that the presence of Azotobacter in Iowa soils may be most closely associated with the pH, closely associated with the available phosphate content and least associated with the total nitrogen content.
SERIES RELATIONSHIPS

In view of the results which have been presented, it would not only be interesting but would also serve as contributory evidence to determine whether or not the percentage of samples in the more important soil series of Iowa which contained Azotobacter may be explained on the basis of the average pH and available phosphate content of the samples falling within the different groupings. For this purpose, therefore, the regression equation derived in table 6 was used to obtain an estimate of the percentage of samples in the different series or series groupings which might have been expected to contain the bacteria. The results are given in table 8, together with the pH and available phosphate data which were used as the basis of the estimation.

The relationship between the percentage of samples in the different series and series groupings which contained Azotobacter and the percentages estimated to contain them is shown in fig. 3. A highly significant correlation existed between the two. This indicates that

### Table 8. The Percentage of Samples in the Principal Soil Series of Iowa Which Contained Azotobacter, the Percentage Estimated to Contain Them, the Average pH and Average Available Phosphate Content of the Samples.

<table>
<thead>
<tr>
<th>Series Grouping</th>
<th>Number of Samples</th>
<th>Percentage of Samples Which Contained Azotobacter</th>
<th>Percentage* Estimated to Contain Azotobacter</th>
<th>pH</th>
<th>Available Phosphate (Lbs. / acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drift Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarion</td>
<td>23</td>
<td>30.4</td>
<td>18.1</td>
<td>6.27</td>
<td>44.0</td>
</tr>
<tr>
<td>Carrington</td>
<td>21</td>
<td>19.0</td>
<td>10.2</td>
<td>5.98</td>
<td>41.4</td>
</tr>
<tr>
<td>Webster</td>
<td>24</td>
<td>91.7</td>
<td>70.4</td>
<td>7.61</td>
<td>106.5</td>
</tr>
<tr>
<td>Clyde-Floyd</td>
<td>7</td>
<td>71.4</td>
<td>38.0</td>
<td>6.45</td>
<td>83.8</td>
</tr>
<tr>
<td>Lindley</td>
<td>11</td>
<td>9.1</td>
<td>8.0</td>
<td>5.91</td>
<td>36.8</td>
</tr>
<tr>
<td>Shelby</td>
<td>5</td>
<td>0.0</td>
<td>16.7</td>
<td>6.15</td>
<td>46.5</td>
</tr>
<tr>
<td><strong>Loess Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinton</td>
<td>19</td>
<td>21.0</td>
<td>19.5</td>
<td>6.32</td>
<td>45.0</td>
</tr>
<tr>
<td>Grundy</td>
<td>26</td>
<td>3.8</td>
<td>11.4</td>
<td>5.79</td>
<td>51.2</td>
</tr>
<tr>
<td>Knox</td>
<td>10</td>
<td>100.0</td>
<td>92.7</td>
<td>8.39</td>
<td>123.1</td>
</tr>
<tr>
<td>Marshall</td>
<td>27</td>
<td>7.4</td>
<td>13.8</td>
<td>6.22</td>
<td>36.0</td>
</tr>
<tr>
<td>Tama</td>
<td>28</td>
<td>14.3</td>
<td>18.1</td>
<td>6.07</td>
<td>53.9</td>
</tr>
<tr>
<td>Fayette</td>
<td>5</td>
<td>20.0</td>
<td>33.1</td>
<td>6.54</td>
<td>50.0</td>
</tr>
<tr>
<td>Muscatine</td>
<td>9</td>
<td>11.1</td>
<td>14.8</td>
<td>5.98</td>
<td>50.2</td>
</tr>
<tr>
<td>Marion-Putnam</td>
<td>6</td>
<td>0.0</td>
<td>-0.5</td>
<td>5.69</td>
<td>26.9</td>
</tr>
<tr>
<td><strong>Terrace Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benoit</td>
<td>32</td>
<td>40.6</td>
<td>59.2</td>
<td>6.78</td>
<td>119.5</td>
</tr>
<tr>
<td>Deuel</td>
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<tr>
<td>Buckner</td>
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<tr>
<td>Charlton</td>
<td></td>
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<td></td>
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<tr>
<td>Fargo</td>
<td></td>
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<tr>
<td>Hancock</td>
<td></td>
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<td></td>
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<tr>
<td>Judson</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Niel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sioux</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waukesha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bottomland Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>34</td>
<td>76.4</td>
<td>88.7</td>
<td>7.12</td>
<td>175.0</td>
</tr>
<tr>
<td>Larcouer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarpy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wabash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>287</td>
<td>35.2</td>
<td>33.4</td>
<td>6.43</td>
<td>80.9</td>
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*Estimated from the equation: \( X = 19.9A + 0.41D - 124.74 \) from table 6; the average pH and average available phosphate content of each series were substituted for A and D respectively.
the presence or absence of the Azotobacter in the principal soil series of Iowa may be largely explained on the basis of the characteristic pH and content of available phosphate of the samples in each series.

CORRELATION BETWEEN THE AMOUNT OF AZOTOBACTER GROWTH AND THE pH, AVAILABLE PHOSPHATE CONTENT AND TOTAL NITROGEN CONTENT OF THE SAMPLES WHICH CONTAINED THE ORGANISMS

In the previous pages an attempt was made to correlate the presence of the Azotobacter with certain chemical characteristics of the soils in order to determine what factors affected the occurrence of the bacteria in Iowa soils. An attempt will be made here to throw additional light on this subject and in addition to determine which of the chemical soil characteristics studied influence the amount of Azotobacter growth in the soil. For this study, therefore, only those samples of soil, 101 in all, which contained the Azotobacter were considered.

The results of the individual sample analyses are given in table 9. This table shows that the amount of Azotobacter growth ranged

Fig. 3. Scatter diagram of the percentage of samples in the different series groupings which contained Azotobacter (A) against the percentage estimated to contain them (X) together with the regression line.
TABLE 9. AZOTOBACTER GROWTH OBTAINED FROM 101 IOWA SOILS TOGETHER WITH LOCATION, SOIL SERIES, pH, AVAILABLE PHOSPHATE CONTENT AND TOTAL NITROGEN CONTENT OF THE SAMPLES.

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<td>298</td>
<td>6,400</td>
</tr>
<tr>
<td>&quot;</td>
<td>82</td>
<td>Carroll</td>
<td>2</td>
<td>7.05</td>
<td>223</td>
<td>3,600</td>
</tr>
<tr>
<td>&quot;</td>
<td>100</td>
<td>Guthrie</td>
<td>1</td>
<td>7.14</td>
<td>123</td>
<td>4,800</td>
</tr>
<tr>
<td>&quot;</td>
<td>107</td>
<td>Dallas</td>
<td>1</td>
<td>6.47</td>
<td>65</td>
<td>4,600</td>
</tr>
<tr>
<td>&quot;</td>
<td>54</td>
<td>Palo Alto</td>
<td>2</td>
<td>6.88</td>
<td>140</td>
<td>10,200</td>
</tr>
</tbody>
</table>

*1—A scant but noticeable colony growth.
*2—An average growth with colonies distinguishable.
*3—A heavy, vigorous growth in which the individual colonies are distinguishable with difficulty.
*4—A very dense growth overgrowing the entire plate.

from 1 to 4 depending upon the quantity of growth which appeared upon the surface of the nitrogen-free agar plates; that the pH ranged from 5.84 to 8.58, the total nitrogen content from 1,400 pounds to 15,600 pounds per acre and the available phosphate content from 27 to 520 pounds per acre.

A multiple correlation was computed from the data in table 9. The dependent variable was the quantity of Azotobacter growth. The measurements used for total nitrogen were coded values giving the milligrams of nitrogen per 10 grams of soil. In the regression equation the nitrogen factor was not decoded. The regression statistics are given in table 10.

The standard regression coefficients show that about 60 percent of the accounted-for variation in quantity of Azotobacter growth may be based on total nitrogen content, about 30 percent on the pH and only about 10 percent on the content of available phosphate. It may be concluded, therefore, from both the zero order
TABLE 10. REGRESSION STATISTICS FOR THE QUANTITY OF AZOTOBACTER GROWTH (Y) UPON pH, AVAILABLE PHOSPHATE CONTENT AND TOTAL NITROGEN OF 101 SOIL SAMPLES WHICH CONTAINED AZOTOBACTER.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>pH (A)</th>
<th>Available phosphate (B)</th>
<th>Total nitrogen (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order correlation coefficients, r.</td>
<td>0.3165**</td>
<td>0.1146</td>
<td>0.5122**</td>
</tr>
<tr>
<td>Standard regression coefficients, β</td>
<td>0.2384*</td>
<td>0.0846</td>
<td>0.4827*</td>
</tr>
<tr>
<td>Multiple correlation coefficient, R</td>
<td>$R^2 = 0.3324$; $R = 0.5765**$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple regression equation. $\hat{y} = 0.3447 A + 0.0011 B + 0.0371 C - 1.746$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

The correlation coefficient and the standard regression coefficient that little association existed between the available phosphate content of the samples which contained Azotobacter and the amount of bacterial growth given by those samples. On the other hand, these statistics indicate, other factors not being unfavorable, that the pH and total nitrogen content of the sample may actually control the amount of Azotobacter growth in the sample, the total nitrogen content in this case exerting a greater influence than the pH.

The multiple regression coefficient $R = 0.5765$ was an improvement over either $r_{AX} = 0.3165$ or $r_{CX} = 0.5122$ which shows that a better estimation of the Azotobacter growth would be obtained from a combination of the two variables than from a consideration of each separately.

The results of this analysis compared to those previously made lead to some very interesting suppositions. In the earlier analyses, it was found that the presence of the Azotobacter was associated with the pH and content of available phosphate but not with the content of total nitrogen, the associations in this case being interpreted in terms of factors which limit the presence of the Azotobacter in Iowa soils. Nitrogen, therefore, did not seem to be a factor which would limit the presence of the Azotobacter in Iowa soils, at least through the range in which it was found. The pH and content of available phosphate, on the other hand, may logically become limiting factors.

In the analysis given here, those samples have been eliminated which contained an environment unsuitable for the Azotobacter so that the results of this analysis may not be interpreted in terms of factors which limit the presence of the Azotobacter but only in terms of factors which favor the amount of growth which the organisms will make. The results for phosphate indicate that while an available supply of phosphate is recognized as necessary for the presence of the organisms, as shown previously, when those soil samples were eliminated in which it might have been a limiting factor for the growth of the organisms, a larger supply of phosphate would not necessarily bring about an increased growth. Even though the pH, on the other hand, seemed to be the prime limiting factor for Azotobacter in Iowa soils, when it was not a limiting factor, as in the samples considered here, an increase in the pH of the
sample did improve conditions so that the Azotobacter could make a better growth. The most interesting feature of these analyses, however, is the close relationship that existed between the quantity of Azotobacter growth and the total nitrogen content of the samples. The earlier analyses indicated that the nitrogen content of the sample may not, at least in Iowa soils, be a limiting factor for Azotobacter. When other conditions are favorable for the growth of the organisms, however, the quantity of growth obtained is most closely associated with the total nitrogen content of the sample.

GROWTH OF AZOTOBACTER ON EXPERIMENTAL PLOT SOILS

RESULTS ON AGRONOMY FARM AND COOPERATIVE SOIL EXPERIMENTAL FIELDS

The results which have been obtained have indicated that the most important factor controlling the presence of the Azotobacter in Iowa soils is the pH and that the available phosphate content is also of importance in this respect. It was thought desirable, therefore, to sample a group of experimental plots located upon soils which originally did not contain the bacteria to determine whether or not treatments with lime and rock phosphate had improved the environment of these soils so that the Azotobacter may grow.

For this purpose, two groups of experimental plots were sampled. Four groups of plots located on Clarion loam at the Agronomy Farm at Ames were sampled toward the end of May, 1935; and during June a series of plots from a number of cooperative experimental

TABLE 11. AZOTOBACTER GROWTH OBTAINED FROM SOILS OF EXPERIMENTAL PLOTS FROM THE AGRONOMY FARM TOGETHER WITH FIELD TREATMENT, pH, AVAILABLE PHOSPHATE CONTENT AND TOTAL NITROGEN CONTENT OF THE SAMPLES.

<table>
<thead>
<tr>
<th>Plot number</th>
<th>Field treatment</th>
<th>Amount of growth</th>
<th>pH</th>
<th>Available phosphate (Lbs./acre)</th>
<th>Total nitrogen (Lbs./acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>917</td>
<td>*CK</td>
<td>0</td>
<td>5.40</td>
<td>42</td>
<td>3,200</td>
</tr>
<tr>
<td>918</td>
<td>CR</td>
<td>0</td>
<td>5.43</td>
<td>29</td>
<td>4,000</td>
</tr>
<tr>
<td>919</td>
<td>CR+L</td>
<td>0</td>
<td>6.54</td>
<td>44</td>
<td>4,000</td>
</tr>
<tr>
<td>920</td>
<td>CR+L+RP</td>
<td>0</td>
<td>6.50</td>
<td>312</td>
<td>4,000</td>
</tr>
<tr>
<td>924</td>
<td>CK</td>
<td>0</td>
<td>5.75</td>
<td>24</td>
<td>3,000</td>
</tr>
<tr>
<td>925</td>
<td>M</td>
<td>0</td>
<td>5.75</td>
<td>27</td>
<td>3,400</td>
</tr>
<tr>
<td>926</td>
<td>M+L</td>
<td>0</td>
<td>6.56</td>
<td>46</td>
<td>3,400</td>
</tr>
<tr>
<td>927</td>
<td>M+L+RP</td>
<td>0</td>
<td>6.71</td>
<td>520</td>
<td>4,400</td>
</tr>
<tr>
<td>930</td>
<td>CR</td>
<td>0</td>
<td>6.29</td>
<td>60</td>
<td>7,800</td>
</tr>
<tr>
<td>931</td>
<td>CR+L</td>
<td>++</td>
<td>7.21</td>
<td>90</td>
<td>6,000</td>
</tr>
<tr>
<td>932</td>
<td>CR+L+RP</td>
<td>++</td>
<td>7.60</td>
<td>416</td>
<td>5,400</td>
</tr>
<tr>
<td>1024</td>
<td>CK</td>
<td>0</td>
<td>6.36</td>
<td>59</td>
<td>5,000</td>
</tr>
<tr>
<td>1025</td>
<td>M</td>
<td>++</td>
<td>6.44</td>
<td>62</td>
<td>5,600</td>
</tr>
<tr>
<td>1026</td>
<td>M+L</td>
<td>++</td>
<td>7.65</td>
<td>94</td>
<td>5,800</td>
</tr>
<tr>
<td>1027</td>
<td>M+L+RP</td>
<td>++</td>
<td>7.69</td>
<td>416</td>
<td>6,000</td>
</tr>
</tbody>
</table>

*CK—No treatment.
M—Farm manure 8 tons per acre once in 4 years.
L—Lime once in 4 years in an amount equal to that indicated by lime requirement tests.
CR—Crop residues, straw and stover were returned to the land and the second crop of clover was plowed under.
RP—Raw rock phosphate, 2,000 pounds per acre once in 4 years.

**No growth of Azotobacter.
++—A scant but noticeable growth.
+++—An average growth with colonies distinguishable.
fields, located upon a variety of soil types, were also sampled. These latter plots were located on typical fields of Tama, Marshall and Clinton silt loams, Grundy silty clay loam and Clarion loam. Table 1 shows that few of the samples of these soil types contained the organisms. Whether or not the samples contained Azotobacter, the pH, content of available phosphate and content of total nitrogen were determined. The results of the analyses and the fertilizer treatments are given in tables 11 and 12.

These results indicate that the original soil in each field probably did not contain the Azotobacter since none of the “no treatment” plots contained them. They also show that a treatment of lime in 6 of the 11 fields sampled improved conditions so that the Azotobacter came in. A treatment of rock phosphate in addition to the lime treatment did not induce a better growth of the bacteria than on the plots treated with lime alone. While these results are far from conclusive, they do lend support to the conclusion that the Azotobacter require soil with a high pH in order to grow and that many

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Field treatment</th>
<th>Amount of growth</th>
<th>pH</th>
<th>Available phosphate (Lbs./acre)</th>
<th>Total nitrogen (Lbs. / acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>216</td>
<td>*CK</td>
<td>*</td>
<td>6.15</td>
<td>65</td>
<td>4,600</td>
</tr>
<tr>
<td>217</td>
<td>M+L</td>
<td>+</td>
<td>6.65</td>
<td>52</td>
<td>4,200</td>
</tr>
<tr>
<td>218</td>
<td>M+L+RP</td>
<td>+</td>
<td>7.05</td>
<td>208</td>
<td>4,400</td>
</tr>
<tr>
<td>219</td>
<td>CK</td>
<td>0</td>
<td>5.61</td>
<td>40</td>
<td>4,600</td>
</tr>
<tr>
<td>220</td>
<td>M+L</td>
<td>0</td>
<td>6.50</td>
<td>57</td>
<td>4,800</td>
</tr>
<tr>
<td>221</td>
<td>M+L+RP</td>
<td>0</td>
<td>6.82</td>
<td>312</td>
<td>4,800</td>
</tr>
<tr>
<td>222</td>
<td>CK</td>
<td>0</td>
<td>6.23</td>
<td>62</td>
<td>3,400</td>
</tr>
<tr>
<td>223</td>
<td>M+L</td>
<td>0</td>
<td>6.89</td>
<td>87</td>
<td>3,200</td>
</tr>
<tr>
<td>224</td>
<td>M+L+RP</td>
<td>0</td>
<td>6.53</td>
<td>223</td>
<td>3,400</td>
</tr>
<tr>
<td>225</td>
<td>CK</td>
<td>0</td>
<td>6.48</td>
<td>125</td>
<td>5,600</td>
</tr>
<tr>
<td>226</td>
<td>M+L</td>
<td>+</td>
<td>6.40</td>
<td>104</td>
<td>6,200</td>
</tr>
<tr>
<td>227</td>
<td>M+L+RP</td>
<td>+</td>
<td>6.40</td>
<td>312</td>
<td>6,200</td>
</tr>
<tr>
<td>228</td>
<td>CK</td>
<td>0</td>
<td>5.98</td>
<td>30</td>
<td>2,800</td>
</tr>
<tr>
<td>229</td>
<td>M+L</td>
<td>0</td>
<td>6.60</td>
<td>40</td>
<td>3,200</td>
</tr>
<tr>
<td>230</td>
<td>M+L+RP</td>
<td>0</td>
<td>6.77</td>
<td>260</td>
<td>3,000</td>
</tr>
<tr>
<td>231</td>
<td>CK</td>
<td>0</td>
<td>6.22</td>
<td>36</td>
<td>5,200</td>
</tr>
<tr>
<td>232</td>
<td>M+L</td>
<td>+</td>
<td>7.22</td>
<td>48</td>
<td>4,800</td>
</tr>
<tr>
<td>233</td>
<td>M+L+RP</td>
<td>+</td>
<td>7.09</td>
<td>416</td>
<td>5,200</td>
</tr>
<tr>
<td>234</td>
<td>CK</td>
<td>0</td>
<td>6.67</td>
<td>35</td>
<td>5,200</td>
</tr>
<tr>
<td>235</td>
<td>M+L</td>
<td>++</td>
<td>7.14</td>
<td>49</td>
<td>5,200</td>
</tr>
<tr>
<td>236</td>
<td>M+L+RP</td>
<td>++</td>
<td>7.32</td>
<td>260</td>
<td>4,800</td>
</tr>
</tbody>
</table>

*CK—No treatment.
M—Farm manure, 8 tons per acre once in four years.
L—Lime once in 4 years in an amount equal to that indicated by lime requirement tests.
RP—Raw rock phosphate, 2,000 pounds per acre once in 4 years.
**No growth of Azotobacter.
++—A scant but noticeable growth.
++—An average growth with colonies distinguishable.
of the more important soil types of Iowa are characteristically too acid to contain them.

RESULTS ON EXPERIMENTAL FIELDS ON HIGH-LIME SOILS

In the Wisconsin drift of northern Iowa are many areas of high lime soils. The individual areas are not large, varying from a few square rods to a few acres, and usually they appear after a peat bog or a swampy area has been drained. In general, very poor yields of such crops as corn have been obtained. The reason for this unproductiveness has been attributed to the excessive accumulation of the calcium carbonate and bicarbonate which characterize these soils.

Regardless of the poor crop yields, it seemed that these soils should offer a good chance to test out the second conclusion derived from the results of the previous analyses, namely, that when other factors were favorable, the best growth of the Azotobacter would be obtained in those soils rich in nitrogen and with the higher pH values.

Five different experimental fields located upon four different soil types in three different counties were sampled. The results of the individual sample analyses are given in table 13. The Azotobacter

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Field treatment</th>
<th>Amount of growth</th>
<th>pH</th>
<th>Available phosphate (Lbs./acre)</th>
<th>Total nitrogen (Lbs./acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hancock County—Virgil Cook Field—Webster silt loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>*CK</td>
<td>++</td>
<td>8.30</td>
<td>57</td>
<td>12,000</td>
</tr>
<tr>
<td>35</td>
<td>SP</td>
<td>+++</td>
<td>8.34</td>
<td>83</td>
<td>11,600</td>
</tr>
<tr>
<td>36</td>
<td>KCL</td>
<td>+++</td>
<td>8.29</td>
<td>69</td>
<td>9,800</td>
</tr>
<tr>
<td>37</td>
<td>KCL+SP</td>
<td>+++</td>
<td>8.29</td>
<td>113</td>
<td>10,600</td>
</tr>
<tr>
<td>Palo Alto County—T. J. Reinders Field—O'Neill loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>CK</td>
<td>++</td>
<td>8.21</td>
<td>222</td>
<td>11,000</td>
</tr>
<tr>
<td>44</td>
<td>SP</td>
<td>+++</td>
<td>8.40</td>
<td>57</td>
<td>10,000</td>
</tr>
<tr>
<td>45</td>
<td>KCL</td>
<td>++</td>
<td>8.24</td>
<td>113</td>
<td>10,000</td>
</tr>
<tr>
<td>46</td>
<td>KCL+SP</td>
<td>+++</td>
<td>8.23</td>
<td>49</td>
<td>7,600</td>
</tr>
<tr>
<td>Palo Alto County—A. B. Chism Field—Cass silt loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>CK</td>
<td>+++</td>
<td>8.30</td>
<td>71</td>
<td>8,800</td>
</tr>
<tr>
<td>49</td>
<td>SP</td>
<td>++</td>
<td>8.15</td>
<td>117</td>
<td>8,400</td>
</tr>
<tr>
<td>50</td>
<td>KCL</td>
<td>+++</td>
<td>8.40</td>
<td>104</td>
<td>8,000</td>
</tr>
<tr>
<td>51</td>
<td>KCL+SP</td>
<td>+++</td>
<td>8.34</td>
<td>86</td>
<td>7,600</td>
</tr>
<tr>
<td>Kossuth County—John Origer Field—Webster silt loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>CK</td>
<td>+++</td>
<td>8.50</td>
<td>86</td>
<td>8,000</td>
</tr>
<tr>
<td>62</td>
<td>SP</td>
<td>+++</td>
<td>8.39</td>
<td>86</td>
<td>7,600</td>
</tr>
<tr>
<td>63</td>
<td>KCL</td>
<td>+++</td>
<td>8.29</td>
<td>62</td>
<td>7,000</td>
</tr>
<tr>
<td>64</td>
<td>KCL+SP</td>
<td>+++</td>
<td>8.26</td>
<td>66</td>
<td>9,400</td>
</tr>
<tr>
<td>Kossuth County—George Peterson Field—Webster loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>CK</td>
<td>+++</td>
<td>8.39</td>
<td>52</td>
<td>9,600</td>
</tr>
<tr>
<td>69</td>
<td>SP</td>
<td>+++</td>
<td>8.30</td>
<td>68</td>
<td>10,000</td>
</tr>
<tr>
<td>70</td>
<td>KCL</td>
<td>++</td>
<td>7.96</td>
<td>70</td>
<td>9,400</td>
</tr>
<tr>
<td>71</td>
<td>KCL+SP</td>
<td>+++</td>
<td>8.18</td>
<td>65</td>
<td>9,400</td>
</tr>
</tbody>
</table>

*CK—No treatment.
SP—Superphosphate, 200 pounds of 20 percent per acre.
KCL—Potash, 500 pounds per acre

**+++-An average Azotobacter growth with colonies distinguishable.
+++---A heavy vigorous growth where it is difficult to distinguish individual colonies.
++++-A very dense growth overgrowing the entire plate.
occurred in the soils of all the plots and, regardless of fertilizer treatments, were found growing very vigorously. It appears, therefore, that these high-lime, high-organic matter soils present an environment favorable for the growth of the bacteria.

It is interesting to point out that since these soils do not present an environment favorable for the production of crops, in this case at least, it would perhaps be unwise to judge the fertility of the soil by its Azotobacter flora.

**DISCUSSION**

This report summarizes the data collected during a 2 year's study of the occurrence and distribution of Azotobacter in Iowa soils. Many samples representing a wide range in soil characteristics were collected and examined for the presence of the Azotobacter by methods which were designed to imitate as closely as possible the conditions existing in the field: the Winogradsky soil plaque method and the selective culture agar-plate method.

Of the 287 samples collected from 33 of the soil series of Iowa, barely a third contained the organisms and many of these contained only a scant growth when tested by the methods used in this investigation. The Grundy and Tama soils of the Southern Iowa loess area, the Marshall soils of the Missouri loess area and the Lindley and Shelby soils of the southern part of the state were practically devoid of the bacteria. From the standpoint of total farming acreage covered, these soil series are among the most important in Iowa.

An attempt was then made to determine some of the factors responsible for the absence of the Azotobacter in such a large majority of Iowa soils. It was found that an important factor was the high acidity of many of these soils. The actual limiting pH value could not be placed exactly since it would be affected by other soil characteristics and would naturally vary from soil to soil. It probably ranged, however, from about pH 5.42, the limiting value obtained by an extrapolation of the simple regression equation, to pH 6.0 below which only one sample contained the bacteria. This is in agreement with the results of other investigators such as Gainey (14, 15) and Christensen (9) who placed the limiting pH value for Azotobacter for mineral field soils at about pH 6.0 or slightly below.

Some investigators, however, using the methods proposed by Winogradsky have found Azotobacter in soils with pH values far below 6.0. Thus Vandecaveye and Anderson (29) detected the presence of the organisms in small numbers in western Washington forest soils which were as acid as pH 5.5 and less. Wilson and Wilson (32) found Azotobacter in the large majority of a group of virgin peat soils, many of which were distinctly more acid in reaction than pH 6.0. It was concluded that a carbonate-phosphate ratio was the important factor controlling the growth of the organisms in these soils rather than the pH. An attempt was therefore made
in the present investigation to determine whether or not the carbonate-phosphate ratio influenced the presence and growth of Azotobacter in the mineral field soils of Iowa. No correlation could be found.

An explanation of the fact that mineral field soils below a pH 6.0 characteristically do not contain the Azotobacter while other soils may contain the organisms far below this reaction may be obtained from the pure culture work of Burk and his associates (7, 8). They found that the Azotobacter may exist in a medium well below a pH of 6.0 and even as low as pH 4.5 or less as long as the organisms have sufficient fixed nitrogen in the medium. The ability to fix nitrogen from the atmosphere, however, ceased at pH 6.0. They state that, “The limiting pH value 6.0 for fixation is a characteristic constant. No factors are known capable of altering this limit.”

The virgin peat soils and, to a lesser extent, the western Washington forest soils characteristically contain large amounts of organic matter with a subsequent high content of fixed nitrogen. It follows, therefore, that the environment in these soils may be such that the Azotobacter might live. At pH values below 6.0, however, the presence of the organisms may be of no economic importance if fixation of nitrogen may not proceed below this point.

In mineral field soils, however, the conditions are characteristically somewhat different from those found in peats or forest soils. The organic matter present or added to mineral field soils serves as the carbon or energy source for a great many microorganisms such as bacteria, fungi and actinomycetes. To break down the complex material which usually has a rather wide C:N ratio, the different organisms must utilize certain amounts of fixed nitrogen in order to maintain their normal C:N ratio. Upon the addition of organic matter to these soils, therefore, an increased number of microorganisms, as well as the higher plants, compete for the fixed nitrogen that is present. As a result, the Azotobacter, if they cannot compete for this material, must get their nitrogen from some other source. This source is the nitrogen of the air. If the Azotobacter lose this ability, therefore, at a pH of 6.0 or less, they would not generally occur in normal field soils below this pH.

In addition to the acidity, the available phosphate content of the samples was found to influence the presence of Azotobacter in Iowa soils. The influence, however, was not so great as that of pH. The limiting content of available phosphate for Azotobacter in Iowa soils was shown to approach 35 pounds per acre; few of the soils with lower amounts than this, regardless of the pH, contained the organisms. That the Azotobacter should be sensitive to an available supply of phosphate has been indicated by the work of many investigators. The phosphorus content of Azotobacter cells has been recorded as varying from 2.51 percent to 4.93 percent of the dry material (17, 27) which indicates a rather pressing need for phosphorus during the growth of the cells. In the mineral field soils, therefore,
an available supply of phosphorus ought to be maintained if the organisms are to survive.

In addition to the determination of factors which limited the occurrence of the Azotobacter in Iowa soils, a study was made of the factors which influenced the amount of growth which the Azotobacter would make in the soil. For this study only those samples were considered which contained the bacteria. In these samples, therefore, phosphate was not a limiting factor for the bacteria. The results showed that a further supply of phosphate would not necessarily bring about an increased growth. The results for pH showed that an increase in the pH of the sample improved conditions so that the Azotobacter could make a better growth. This is logical and in keeping with the ideas developed concerning the so-called optimum pH. The most interesting result obtained, however, was the close relationship that existed between the quantity of Azotobacter growth and the total nitrogen content of the samples. Nitrogen exerted a greater influence upon the growth of the bacteria than any of the other variables considered.

In the determinations which were made for total nitrogen, only that nitrogen was determined which was in the organic form. Reed and Williams (22) found that the nitrogen of complex organic compounds was not available for use by the Azotobacter and that, as a result, it did not appreciably influence their growth. Fuller and Rettger (13) and Bonazzi (4) arrived at essentially the same conclusion. It does not appear probable, therefore, that the total nitrogen content of the sample influenced the growth of the Azotobacter directly.

In the presence of a high total nitrogen content, particularly in soils above a pH of 6.0, ammonification and nitrification are likely to be increased so that the amount of available nitrogen would be expected to be higher. That such is the case has recently been emphasized by Remezov (23) who found that the water soluble fraction of the organic nitrogen of the soil depended upon the total nitrogen content and the preponderance of anions over cations.

Burk and Lineweaver (7) using the Warburg technique found that the influence of available nitrogen upon the Azotobacter was to prohibit nitrogen fixation, but at the same time it increased the number of viable Azotobacter cells. From this finding it was concluded that: "From the agronomic point of view, it is reasonably certain that applications of nitrogenous fertilizers to soils under field conditions will often increase the absolute numbers of Azotobacter therein, as in the case of other organisms." This finding is supported by the work of Hills (16) who studied the effect of nitrates on soil cultures of Azotobacter. He observed that a marked stimulation in numbers of Azotobacter occurred from the addition of nitrates to the soil but that the efficiency of the organisms as far as the nitrogen fixation was concerned was actually decreased to a marked degree.
In Iowa soils, therefore, it might conceivably be that the association of the large amounts of Azotobacter growth with the high total nitrogen contents represents a dependence of the Azotobacter on the high available nitrogen contents measured indirectly by the total nitrogen determinations. But if such were the case, little fixation of nitrogen by the organisms might be expected.

Winogradsky (36), however, has shown that the influence of available nitrogen upon the Azotobacter when growing in their natural habitat, the soil, was to depress and often entirely eliminate them. The smallest dose of nitrogen added, 5 parts per 100,000 parts of soil, was sufficient to retard the growth of the Azotobacter and reduce the numbers to one-twenty-fifth of what they normally would have been without the addition of nitrogen. Ten parts per one hundred thousand parts of soil and all larger doses completely suppressed the growth of the bacteria. This conclusion was supported by the work of Ziemiecka (38).

The results of Winogradsky are contrary to those of Hill who observed a stimulative effect of available nitrogen upon the Azotobacter. This discrepancy may perhaps be explained by the work of Winters (37) who found that small applications of available nitrogen to the soil, 80 to 100 pounds per acre of NaNO₃ or an equivalent amount of nitrogen in (NH₄)₂SO₄ or Ca(NO₃)₂, stimulated nitrogen fixation whereas larger applications depressed it.

To test out this idea, the following simple experiment was run: To the regular nitrogen-free agar plates used throughout this investigation different amounts of available nitrogen were added as potassium ammonium phosphate as follows: (a) No addition, (b) 0.09 grams per liter, (c) 0.18 gm. per liter, (d) 0.36 gm. per liter and (e) 1.36 gm. per liter. Two different Clinton silt loam soils which contained the Azotobacter were sprinkled upon the surface of the agar plates in duplicate and incubated for 7 days at 28°C. At the end of this time, the amount of nitrogen fixed was determined in the usual manner by the Kjeldahl method. The results were calculated as nitrogen-fixed per gram of soil and are shown in table 14.

These results show that the smallest amount of available nitrogen added to the agar plates materially stimulated nitrogen fixation by the Azotobacter. The second smallest amount of nitrogen added also stimulated nitrogen fixation but not to the extent of the first addition. The two final additions of available nitrogen, how-

<table>
<thead>
<tr>
<th>KNH₄HPO₄·4H₂O added to the regular medium per liter</th>
<th>Milligrams of nitrogen fixed per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No. 1</td>
<td>Sample No. 2</td>
</tr>
<tr>
<td>0.0 grams</td>
<td>9.32</td>
</tr>
<tr>
<td>0.09 grams</td>
<td>13.12</td>
</tr>
<tr>
<td>0.18 grams</td>
<td>9.65</td>
</tr>
<tr>
<td>0.36 grams</td>
<td>1.74</td>
</tr>
<tr>
<td>1.36 grams</td>
<td>None</td>
</tr>
</tbody>
</table>
ever, first greatly depressed the fixation and finally eliminated it altogether. This verifies the results which Winters (37) obtained.

The increase in quantity of Azotobacter growth with total nitrogen content of the samples of Iowa soils, therefore, may possibly be explained on the basis of a stimulation of growth with an increase in the available nitrogen supply.

Another, and perhaps a more logical explanation for the close correlation that existed between the total nitrogen content of Iowa soils and the amount of Azotobacter growth therein, is that the total nitrogen content of Iowa soils gives an indirect measure of the organic matter content, the latter having been shown to increase the growth of the Azotobacter materially. Walker and Brown (31) calculated the correlation coefficient for total nitrogen and organic carbon in the soils of Iowa and found that it was 0.95 for the drift soils (384 samples) and 0.93 for the loess soils (229 samples). The coefficient of variability in each case was very small. Under these conditions, the organic carbon content of Iowa soils may be calculated from the figure for total nitrogen, the total nitrogen content figure, in this case, acting as a coded value for the organic carbon.

The favorable influence of organic matter upon the Azotobacter has been generally recognized. Some explanations for this beneficial effect which have been given are that some of the constituents of the soil organic matter act as sources of energy for the Azotobacter (11, 24), that growth-promoting substances for Azotobacter are found in the organic matter (2, 18, 19, 21), that the inorganic constituents of organic matter such as manganese, iron, silicic acid and molybdenum stimulate Azotobacter growth (5, 20) and that the general improvement of the soil structure, buffer capacity, protective action against poisons which results from an increase in the soil organic matter also influences the growth of the organisms. They all probably aid in the resultant beneficial effect upon the Azotobacter which comes with an increase in the soil content of organic matter.

On the basis of the previous results, a group of experimental plots was sampled to determine whether or not soils which did not originally contain the organisms could be treated with lime and rock phosphate to correct the acidity and deficiency in available phosphate and thereby support a flora of Azotobacter. Also, a group of experimental plots on high-lime soils which contained large amounts of organic matter as indicated by total nitrogen determinations was sampled to determine whether or not the expected vigorous growth of the bacteria would be found in such soils.

While the results were far from conclusive, they indicated that a treatment of lime to some Iowa soils which did not contain the bacteria had induced the Azotobacter to develop and that a treatment of rock phosphate in addition to the lime treatment had not induced a better growth of the Azotobacter than on the soils treated with lime alone. For the high-lime plot soils, the Azotobacter were found growing vigorously regardless of fertilizer treatment as was expected.
It may be concluded, therefore, that Azotobacter do not occur to any great extent in Iowa soils, that the high acidity of the majority of these soils is the most important factor limiting their occurrence although the available phosphate content may also be of importance in this respect, and that, other factors being favorable, the amount of growth which the Azotobacter will make depends largely upon the organic matter content of the soil and upon the pH.

LITERATURE CITED


