Preliminary Investigation of the Occurrence and Distribution of Azotobacter on the Soils in Iowa

William P. Martin
Iowa State College

R. H. Walker
Iowa State College

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PRELIMINARY INVESTIGATION OF THE OCCURRENCE AND DISTRIBUTION OF AZOTOBACTER IN THE SOILS OF IOWA

WILLIAM P. MARTIN AND R. H. WALKER

Berthelot in 1888 was the first to suggest that nitrogen may be added to field soils through the activity of microorganisms. In 1901 Beijerinck isolated a non-symbiotic, aerobic, nitrogen-fixing organism which he called *Azotobacter chroococcum*. Since this time five other species belonging to this genus have been isolated and likewise have been found capable of fixing atmospheric nitrogen.

The discovery of these nitrogen-fixing bacteria has since 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 azotobacter may fix from 15 to 40 pounds of nitrogen per acre per year in the soil. While this is not a large amount, nevertheless, it is an economically important aid in keeping up the nitrogen supply of the soil and in supplying the growing crops with the nitrogen they need. The occurrence of azotobacter in soils may thus be of considerable economic importance.

Azotobacter are widely distributed over the earth's surface, having been found in the soils examined from practically every nation in the world. Nevertheless, approximately half of all soils examined have failed to show the presence of the azotobacter. There must, therefore, be a factor or factors limiting the growth and thus the distribution of these organisms in soils.

A preliminary investigation of the occurrence and distribution of azotobacter in the soils of Iowa has been conducted. Forty-two soils of twenty-two different soil types were collected from Osceola, Ida, Marion, Franklin, Davis and Boone counties in Iowa and examined for the presence of azotobacter. The spontaneous-culture-method for the determination of the occurrence of azotobacter in soil, introduced by Winogradsky in 1928, was employed in this investigation.

The essential procedure with this method consists in the intimate mixing of certain materials with the soil, and the addition of enough water so that when the soil is worked thoroughly a moist...
pasty combination is 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 the azotobacter were present, macroscopic colonies were readily observable on the surface of the soil. The presence of such colonies served as a criterion of the presence of the azotobacter in the soil. The materials added are given in connection with the results of the individual sample analysis.

The results of this investigation are given in Table I and summarized in Table II. Of the forty-two soils examined, only those having a pH of 6.0 and above contained azotobacter. The macroscopic colonies appeared in all but one case only upon the addition of a soluble phosphate.

**Factors Affecting the Occurrence and Distribution of Azotobacter in Soils**

The work of many investigators has indicated that the azotobacter are sensitive to acidity. Gainey (4, 5, 6 & 7), Niklas, Poschenrieder and Hock (12), and others have shown that if the reaction of the soil is more acid than pH 6.0, the azotobacter are seldom present. Using Yule's association formula, Gainey, after an investigation of over 400 normal field soils, reached the very high coefficient of 0.96 which indicates an unmistakable correlation between hydrogen-ion concentration and the growth of the azotobacter.

On the other hand, Burk and Lineweaver (2), using the manometric technique developed by Warburg, have shown 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. If fixed nitrogen is not present in the medium, the azotobacter must fix nitrogen from the atmosphere in order to live. Burk, Lineweaver and Horner (1) found that the ability to fix nitrogen from the atmosphere stopped at a pH of 6.0 and that fixed nitrogen in the medium also inhibited nitrogen fixation.

The fact that the azotobacter do not generally occur in mineral field soils below a pH of 6.0 may thus be explained as follows: The natural organic materials added to soils in plant residues and other organic materials are largely made up of cellulose, lignin, proteins, hemicelluloses, and a limited amount of carbohydrates.
Table I — Macroscopic Azotobacter Colony Growth on Soil Plaques for 42 Iowa Soils of Differing Soil Types and Having Various Reactions.

<table>
<thead>
<tr>
<th>Location</th>
<th>Series Name</th>
<th>pH</th>
<th>Soil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis County</td>
<td>Lindley</td>
<td>5.05</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shelby</td>
<td>5.14</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Marion</td>
<td>5.17</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Calhoun</td>
<td>5.24</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Grundy</td>
<td>5.24</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Putman</td>
<td>5.40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Edina</td>
<td>5.45</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Clinton</td>
<td>6.08</td>
<td>+</td>
</tr>
<tr>
<td>Marion County</td>
<td>Muscatine</td>
<td>5.05</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shelby</td>
<td>5.29</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Putman</td>
<td>5.33</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Grundy</td>
<td>5.34</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Calhoun</td>
<td>5.48</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tama</td>
<td>5.82</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Waukesha</td>
<td>5.85</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Clinton</td>
<td>5.95</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lindley</td>
<td>6.09</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>O’Neill</td>
<td>6.50</td>
<td>+</td>
</tr>
<tr>
<td>Ida County</td>
<td>Marshall</td>
<td>5.82</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Waukesha</td>
<td>5.82</td>
<td>+</td>
</tr>
<tr>
<td>Osceola County</td>
<td>Webster</td>
<td>5.55</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Waukesha</td>
<td>5.75</td>
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<td></td>
<td>Marshall</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Clarion</td>
<td>7.75</td>
<td>+</td>
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<tr>
<td></td>
<td>Webster</td>
<td>7.77</td>
<td>+</td>
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<td>Franklin County</td>
<td>Carrington</td>
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<td></td>
<td>Dickinson</td>
<td>5.51</td>
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<tr>
<td></td>
<td>Clyde</td>
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<td></td>
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<td>Dodgeville</td>
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<tr>
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<td>Clarion</td>
<td>5.62</td>
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</tr>
<tr>
<td></td>
<td>Clarion</td>
<td>5.82</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Clarion</td>
<td>5.99</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Clarion</td>
<td>6.17</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Clarion</td>
<td>6.32</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Webster</td>
<td>6.93</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Webster</td>
<td>7.96</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Webster</td>
<td>7.96</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Webster</td>
<td>8.01</td>
<td>+</td>
</tr>
</tbody>
</table>

These substances act as the carbon or energy source for a great many microorganisms such as bacteria, fungi, and actinomycetes. The addition of these materials should, therefore, stimulate the growth of these organisms. That such stimulation does occur, has
Table II — Macroscopic Azotobacter Colony Growth on Soil Plaques of Iowa Soils of Varying pH Values.

<table>
<thead>
<tr>
<th>pH Range</th>
<th>Number of Samples</th>
<th>Number of Soils Containing Azotobacter Upon Addition of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mannite + CaCO₃</td>
</tr>
<tr>
<td>5.05 - 5.49</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>5.50 - 5.99</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>6.00 - 6.49</td>
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<td>0</td>
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<tr>
<td>6.50 - 6.99</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>7.00 - 7.49</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>7.49 - 7.99</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

been demonstrated in a number of experiments conducted by different investigators. To break down these compounds, however, 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 the soil, 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 and they obtain it by fixing it in an available form in their cells. Because the azotobacter lose this ability, therefore, at a pH of 6.0 or less, they generally do not occur in the normal field soil below this point.

In addition to soil acidity, the growth and nitrogen-fixing ability of the azotobacter are affected by other soil deficiencies, and particularly by a lack of available phosphorus. Waksman and Starkey (14) in summing up the work of many investigators state that the amount of phosphorus required by the bacteria is in direct proportion to the amount of nitrogen fixed, and that phosphorus is by far the most important of the mineral substances absorbed during the growth of the cell.

If, then, available phosphate becomes rather limited in a soil, the growth of the azotobacter may be rather limited. Heck (9) and Teakle (13) have shown that soluble phosphate is fixed in an unavailable form in soils as insoluble iron, aluminum, and manganese phosphates under slightly acid to acid conditions. In the normal mineral soils, then, which contain iron, aluminum, and manganese and which have an acid reaction, soluble phosphate may become limited with a subsequent limiting effect upon the growth of the azotobacter.

Greene's experiments (8) seem to show that, as in the case of
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the higher plants, the phosphate is absorbed by the azotobacter in the form of the \( \text{H}_2\text{PO}_4^- \) ion. This ion is most abundant in the soil at a pH of about 6.8. Greene also points out the possibility that the azotobacter cells may be brought to their isoelectric point at a pH of 6.0. Greene's preliminary experiments indicate a rather wide isoelectric range from pH 4.0 to 6.0, thereby preventing the intake of ions at this point. This may also offer an explanation as to why a pH of 6.0 limits the growth of the azotobacter.

The work of Wilson and Wilson (15) offers a rather contradictory result in the light of the preceding discussion. Working with the peat soils of New York, they found that azotobacter were present throughout a range of pH from 3.6 to 7.6. The evidence presented indicated that a pH of 6.0 was not a limiting factor in the growth of the azotobacter but that the soil complex was the more important. These investigators hint at a carbonate-phosphate ratio as the controlling factor in the growth of the azotobacter.

There are a number of explanations that might be given to explain this apparent discrepancy. In the first place, and in contrast to the situation in normal mineral soils, available phosphates would most probably be present throughout the pH range. An analysis of these peat soils by Wilson and Staker (16) showed that iron, aluminum, and manganese were present only in very small amounts. Hence, fixation of available phosphate under these acid soil conditions would not be very great. Also, in these peat soils, a large amount of organic matter or humus would necessarily be present. Nemec (10 & 11) has shown that there is a general parallelism between the citric acid solubility of soil phosphate and the humus content of the soil. In these soils, therefore, available phosphate may not be limited.

Also, because these peat soils contain a large amount of organic matter, fixed nitrogen would necessarily be high. Wilson and Staker (16) pointed out this fact. In the presence of fixed nitrogen, as was previously pointed out, the azotobacter may exist at pH values well below pH 6.0. In most of these peat soils, then, the environment should be such that the azotobacter may live. Nitrogen fixation, however, may not take place in this case. The presence of azotobacter in this case may thus not be of economic importance.

In the third place, as Wilson and Wilson pointed out, it may be possible that these soils, being peat soils and thus having a greater heterogeneity than normal mineral soils, might contain small nuclei in which an active azotobacter flora would exist and fix nitrogen. In this case, the pH and environment of the whole soil mass would
not represent the environment of the small nuclei in which the azotobacter were growing and in which the environment would be favorable for growth.

In addition to available phosphate, the growth and nitrogen-fixing ability of the azotobacter are affected by other mineral substances. Burk and Lineweaver (3) have shown that calcium or strontium in relatively large concentrations, 25 to 50 p.p.m., is necessary to give normal rates of nitrogen fixation. In the presence of fixed nitrogen, calcium or strontium are not necessary for normal growth providing certain other elements, such as magnesium, barium, or beryllium are present in sufficient concentration. As these elements occur in the soil in sufficient quantities, it is reasonable to assume that they would in all probability not become limited to such an extent that they would hinder the growth and nitrogen-fixing ability of the azotobacter.

CONCLUSIONS

The results of this preliminary investigation indicate that acidity is an important factor in limiting the growth of azotobacter but that other factors more or less controlled by the pH or associated with it may also be of importance. Especially may this be true in the case of phosphate.

It is also important that emphasis be placed upon the fact that even though the azotobacter may be present in a soil, they may not fix any nitrogen in which case sufficient fixed nitrogen is present to supply the needs of the organisms.

Nevertheless, the desirable azotobacter do not seem to occur extensively in the soils of Iowa. Perhaps soil inoculation with the azotobacter after the addition of sufficient lime to raise the pH of the soil above a pH of 6.0 together with a soluble phosphate fertilizer may be practiced in Iowa. This is a problem that could profitably be investigated.

LITERATURE CITED

3. BURK, DEAN; AND LINWEAVER, HANS. The Influence of Calcium and Strontium upon the Catalysis of Nitrogen Fixation by Azotobacter. Archiv fur Mikrob., 2:155-186, 1931.

DEPARTMENT OF AGRONOMY,
IOWA STATE COLLEGE,
AMES, IOWA.