# CERTAIN FACTORS AFFECTING THE GROWTH OF AZOTOBACTER IN THE SOIL

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During the many years which have elapsed since the discovery of the nitrogen-fixing organisms of the genus *Azotobacter*, much experimental work has been done dealing with their nutritional requirements and their probable economic importance in the maintenance of soil fertility. It is generally agreed that *Azotobacter* may, under favorable conditions, be an important factor in the maintenance of soil fertility. It is not definitely known how much nitrogen will be fixed by these organisms under field conditions, but the evidence available indicates that these bacteria may be important in adding to the nitrogen content of the soil and therefore in furnishing growing crops with the nitrogen they need.

The experiments reported here were undertaken to study the influence of various factors on nitrogen fixation by *Azotobacter*, and to determine if possible whether any of these factors can be controlled by artificial means.

In order that one might determine what influence soil treatment had on the occurrence and activity of *Azotobacter*, soil samples were collected from twenty-five experimental fields located in the five major soil divisions of Iowa and representing eleven soil types. These fields had been under observation for many years. Manure had been applied to these soils at the rate of eight tons an acre every four or five years. Lime had been added as needed according to the Truog lime requirement test, while twenty percent superphosphate had been applied at the rate of 160 pounds an acre a year. Composite samples were collected from three plots in each field. One sample was collected from the check plot, a second sample from the plot which had been treated with lime and manure, and another from the plot which had received manure, lime, and superphosphate. These soil samples were tested according to the following methods: by Winogradsky's spontaneous culture test, by nitrogen fixation in solution and on silica gel, by the soluble phosphorous test, by pH determination, and by the Truog lime requirement test.

The results secured indicate that soil reaction is probably the most important factor affecting the distribution of *Azotobacter* in these soils. Different results were secured, however, with the two methods employed for establishing the presence of these organisms. Many of

the soils which gave positive results with the silica gel method failed to show the presence of these bacteria when tested by the Winogradsky spontaneous culture method. The results obtained indicate that the silica gel method is more reliable for testing the presence of *Azotobacter* in soils than the spontaneous method, which is more widely employed. It is our belief that the silica gel exerts some beneficial effect on the growth of these bacteria. On the basis of the Winogradsky test many investigators have concluded that *Azotobacter* do not occur in soils more acid than pH 6.00. In the work reported here, however, these bacteria were detected in soil as acid as pH 5.6.

Many investigators have reported that *Azotobacter* can live in culture media far more acid than pH 6.00 if combined nitrogen is present. Since many of the soils used in this investigation had been manured, there may have been sufficient nitrogen present to support these organisms even though the soils were quite acid. Rather recently some research workers have been able to demonstrate the presence of *Azotobacter* in soils as acid as pH 4.00. From their results it appears that these organisms will live in extremely acid soils if other soil factors are favorable for their growth. In fact, it has been reported that the addition of calcium carbonate to soil may, under certain conditions, inhibit the growth of these organisms. It has been observed that the addition of hydrochloric acid, sulphuric acid, or phosphoric acid to soils which had been limed increased the activity of *Azotobacter* in these soils. Furthermore, some of these research workers have concluded that there is no close correlation between soil reaction and the occurrence of *Azotobacter*, but that the carbonate phosphate ratio is more important than the soil reaction. Other investigators, however, have concluded that the soil complex is the most important factor affecting the occurrence of the non-symbiotic nitrogen-fixing organisms in soils. It is interesting to note that many of the extremely acid soils which contained *Azotobacter* were soils with a relatively high organic matter content. One might assume from the results that soil organic matter contains some factor or factors which stimulate these organisms. In fact, it has been observed that humus and soil extracts from high humus soils will stimulate these bacteria.

It seems that there is no general agreement among research workers concerning the influence of soil reaction on the occurrence of *Azotobacter*. I believe, however, it is safe to say that the majority of evidence available supports the belief that *Azotobacter* are more likely to occur in soils more alkaline than pH 6.00, than in soils more acid than pH 6.00.

It has already been pointed out that in the work reported here many of the soils more acid than pH 6.00 contained *Azotobacter*. In the majority of cases, however, the organisms isolated from these acid soils grew rather feebly on culture media and had a low nitrogen-fixing power. These results seem to indicate that if these bacteria occur in soils more acid than pH 6.00, they are likely to be rather inactive and probably do not fix as much nitrogen as those organisms living in soils with a more favorable reaction.

There is much evidence, therefore, that if enough lime is added to an acid soil to bring the reaction to near the neutral point, and if other soil conditions are favorable, an *Azotobacter* flora can be established without artificial inoculation. These organisms appear to be rather widely distributed, and if soil conditions are made favorable they will probably find their way into the soil by natural means.

In addition to soil reaction there are apparently many other factors which influence the occurrence and activity of *Azotobacter* in soils. Many research workers have found that the soil minerals are necessary for nitrogen fixation by the aerobic non-symbiotic nitrogen-fixers. The bacteria of this group are said to be particularly sensitive to a deficiency of phosphates. There is, however, some difference of opinion relative to the type and quantity of phosphate needed. Many investigators have reported that the addition of phosphate fertilizers to soil had no influence on *Azotobacter*, while others have observed a marked stimulation following the application of such materials. In the work reported here the addition of superphosphate, in addition to lime and manure, did not apparently increase the nitrogen-fixing power of the soil. None of the soils were, however, very low in

available phosphorus. If smaller amounts of soluble phosphorus had been present in these soils, different results might have been secured. The soils which contained *Azotobacter* had a higher average quantity of soluble phosphorus than the soils in which the presence of these organisms was not established. From these data and from many other reports it appears that if farmers followed the soil management methods recommended by their experiment stations, they would, in the majority of cases, provide soil conditions favorable for the aerobic non-symbiotic nitrogen-fixing organisms. In the work reported here sixteen percent of the untreated soils contained *Azotobacter*, while sixty-four percent of the fertilized soils showed the presence of these bacteria. Furthermore, the untreated soils fixed much less nitrogen on silica gel and in solution than did the fertilized soils. It should be pointed out, however, that many of the soils which had a high crop-producing power and possessed conditions which are generally considered favorable for these bacteria, either failed to show the presence of these organisms, or had a low nitrogen-fixing power.

We must admit, therefore, that our knowledge of the conditions necessary for maximum growth of *Azotobacter* is far from complete. Quite recently, many substances have been found which stimulate these bacteria. In addition to ordinary soil minerals, many of the rare minerals, such as molybdenum and vanadium, have been found to stimulate this group of bacteria. In addition to the minerals many organic compounds seem to have a beneficial effect. There is also some evidence that some materials contain accessory growth factors which influence this group of organisms. It is not known just how these materials influence the growth of *Azotobacter* and the nitrogen fixation by this group of organisms, nor is it known what quantities are needed for best results. The answers to many of these questions will have to await further investigation.