THE RELATION OF BACTERIA TO NITRIFICATION.

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The Origin of the Soil.

Very long ago--so far back that the time which has elapsed, even were it capable of ~~of~~ being expressed in a definite number of years, would be quite incomprehensible by us--the globe on which we live and which we call the earth, was in a highly heated condition, the intensity of the heat being so great that the materials composing the rocks which we now see around us were in molten condition. In the course of ages much of the earth's heat was radiated into space; and this went on till at length the earth became sufficiently cooled for some portion of it to assume a solid state. It was probably in this manner that the first hard rock masses made their appearance on the earth's surface. As the cooling continued, the water vapor, or steam, which must have been present in the hot atmosphere, became condensed into a liquid; the water itself was then subjected to the cooling influence of radiation, and in course of time the earth's surface became inhabited by low forms of life. The effect of the sun's heat in those far distant ages would be, as it is now, to cause the water on the earth's surface to rise in the form of vapor, and so to form clouds. These clouds floating about in the higher atmosphere, would become sufficiently cooled for their water-vapor to be condensed and fall in the form of rain-drops to the earth. And from the amount of water that surrounded the barren earth at that time the evaporation and the condensation must have been intense and thus we have first evidence of wearing away and disintegration of rock.

The direct mechanical action of the rains, the effect of freezing and thawing of water, the solvent action of various waters, especially if they contain carbonic acid in solution, the direct oxidation of the rocks by the oxygen of the atmosphere, are all important factors in the disintegration of rocks. We have, in past years, regarded these physical and chemical forces as the sole agencies in the breaking up of rocks to form soils.

Although the weathering of rocks, which results in their being broken up and somewhat changed in chemical nature, it to be attributed chiefly to chemical and physical agencies, we are fast learning that micro-organisms play an important part in these processes.

Even before this fact was known it had been suggested that micro-organisms aided in rock disintegration, since bacteria, which require no organic food for their sustenance, being able to live wholly upon mineral matter, by the aid of ammonia salts as a source of nitrogen, were found in abundance upon the surface of bare disintegrating rocks. These bacteria are active oxidizing agents and as a result of their life processes they produce a variety of by-products which are known to have an important chemical effect upon the rocks.

In addition to their aid in rock disintegration bacteria are known to play an important part in the subsequent changes which occur in the soil formed from the debris. Chemical changes are going on continually in the soil and many of them are produced by living organisms.

Sulphur and Iron Bacteria.

In regard to some of the mineral products of the soil, bacterial action can be even more closely associated than in their disintegration of rocks. Organisms have been isolated and examined that have contained within their bodies compounds of iron and sulphur and it is thought that others contain some of the other minerals. The bacteriological investigators have not gone very far into this as yet but special attention has been given to those organisms which aid in chemical transformation of sulphur and iron. The reason of this is obvious, as the compounds of these metals add directly or indirectly to the fertility of the soil.

Becuase bacteria produce decomposition of proteid material, and proteids contain among some other elements a certain amount of sulphur. As the proteids are decomposed the sulphur is loosened from it combinations with the other elements and set free in the form of a gas, hydrogen sulphide (H_2S). Proteids are not the only source of this gas. Some species of bacteria are able to decompose sulphates and sulphites and other low sulphur compounds and some can even produce hydrogen-sulphide (H_2S) from the pure element. Thus it is obvious that a great variety of sulphur compounds may under the influence of the several species of bacteria be a source of sulphuretted hydrogen.

After the gas has been extracted from its various sources it readily enters into new combinations, sulphates and sulphuric acid (H_2SO_4) for example. It may form sulphuriceacid by simple oxidation and additioncof water according to the following formula:

$$2H_2S + O = 2H_2O + S_2$$

$$S_2 + 2H_2O + 3O_2 = 2H_2SO_4$$

But this direct oxidation is not the chief method by which hydrogen sulphide (H_2S) is converted into sulphuric acid and sulphates. There are certain bacteria which have such close relation to this sulphur problem that they have been called sulphur bacteria. The two best known of these were found by Winegradsky and were called Beggiatoa and Ophidomonas and each show sulphur masses in their bodies. In addition to these are others which are frequently called "red bacteria" and belong to a more common type. Both of these classes, however, use hydrogen sulphide (H_2S) in much the same way that other bacteria and plants in general use carbohydrates as a source of energy liberated from the compound by oxidation, and sulphur, in a combined state carbon-bi-sulphide (CS_2), is deposited with the body of the organism. It does not remain long in the bodies of growing bacteria until it is oxidized and converted into sulphuric acid (H_2SO_4), the acid is then liberated from the bacteria and enters into combination with some soil base as ammonia to form ammonium sulphate or with calcium carbonate to form calcium sulphate--two salts which contribute largely to the fertility of the soil.

The iron bacteria, as one class is called, make use of iron compounds as sources of energy just as the last group make use of sulphur compounds. Their chief source of iron is from iron carbonate. The iron carbonates are oxidized by the bacteria as follows:-

$$2FeCO_3 + 3H_2O = Fe_2(OH)_6 + CO_2$$

The iron hydroxid, Fe (OH) is deposited in the cell of the bacterium and afterwards forms combinations outside of the cell. Iron hydroxid is an active chemical agent and readily unites with phosphorous or silicia of the soil to form phosphates or silicates of iron. These salts are essential for plant food.

It has been shown how some of the most important of the elements are changed in condition so that they may be used by the plants there-by adding to the fertility of the soil, and we acknowledge they are indispensable but at the same time we know they are inexhaustible in most soils and are a great deal more cheaply restored to soils that have lost some of their mineral constituents than one other very important factor to plant life; that is organic material which contains that all important element--nitrogen.

Transformation of Nitrogen.

The fertility of any soil depends almost entirely upon the organic material present. A soil may have all the mineral needed and in an available condition but would be barren without the presence of organic substances.

In nature's world after the plant and animal life become thoroughly established there was little or no demand upon the earth for any of these plant foods as all life flourished during its era and then decayed and returned from whence it came. Conditions have changed now and the elements of living things do not all return to the soil it was taken from and large crops of one kind are now grown on a field and when it is mature it is taken off, perhaps transported to some other country. The methods are numerous by which the farmer is getting rid of nitrogen. This produces an inevitable drain upon the soil and a tendency toward nitrogen starvation.

But we need not be alarmed about our soil becoming devoid of nitrogen. Although there may be a constant drain of nitrogen it is evident that it may be replaced in some way thus allowing plant life to continue.

The two most important ingredients of plant foods are carbon and nitrogen. The carbon is furnished from the atmospehere in the form of carbon dioxide and is used by the plants by the aid of sunlight. The nitrogen problem is a far more important one. Plants obtain their nitrogen from the soil but not all the nitrogen in the soil is in an available condition for plant use. Of the various nitrogen compounds in the soil there are but two of any consequence that are available to plants. They are the nitrates and ammonium salts. The more highly complex compounds such as proteids and other organic substances and the simple compounds of ammonia and compounds of nitrous acid and the free nitrogen of the air, of which it is composed of about 77% can not be used by the plants until it has been converted into soluble compounds.

Several different processes are known which can fix free nitrogen in soil and which may have contributed to the formation of the first nitrates in the soil. It is known that certain alkaline bodies in porous soil can be held and fix a small quantity of nitrogen. Electric discharges are known to induce atmospheric nitrogen into the soil and form compounds of the element. But owing to the inadequacy of those two and to the fact that positive experiments have shown that bacteria do fix considerable amounts of nitrogen in the soil and their action is regarded as the most important of the three.

Restoration of Nitrogen to the Soil.

As we have seen, the problem of the transformation of nitrogen is of far more significance to the fertility of the soil than that of carbon. Plants assimilate nitrates from the soil and build them into proteids. Some of these proteids are stored in various plant products and after the death of the plant undergo decomposition through the agency of the bacteria. As animals are dependent upon plants for their food they build up the material in much the same compounds as they are in plants. They do not make any higher compounds than plants do but their bodies contain considerably more nitrogen and considerable less carbonaceous material. The nitrogen is much more concentrated in the animal body and is in a condition wholly removed from the possibility of serving plants as food. The question of restoration of nitrogen to soil then is, How is it that these highly complex bodies, built by plants, utilized and stored by animals, are reduced to simple compounds in which they can once more serve as plant food.

The ways in which these compounds of plants and animals get back to the soil are many and varied but sooner or later all the elements that have been taken from the soil and atmosphere must return and before they return to their former states they must be decomposed.

Decomposition of Nitrogenous Compounds.

We now come to one of the most important functions of the soil bacteria. The decomposition of nitrogenous compounds, (urea, proteids, gelatins and other bodies built up by plants and animals) takes place principally as a result of bacterial action. The species of bacteria that decompose these bodies are many and varied and consequently decompose the bodies in various ways. Some are said to perform their work only in an abundance of oxygen and the result is decay--a complete chemical disintegration of the organic substances, the carbon oxidizing into carbon dioxide, the hydrogen becoming mostly water. Another mode of decomposition occurs only in the absence of an abundance of oxygen and is called putrifaction. This is caused by bacteria that work without free access of oxygen and their action does not result in complete decomposition.

The chemical nature of these changes is very complicated and highly varied. We know that as a result of decomposition many new products are formed and these products must belong to at least two types as far as bacteria are concerned. Some of these are products excreted from the bacteria and are the result of metabolism--tearing down process--of the micro-organisms. The others are what are called by-products of decomposition. By-products are parts of molecules that remain or combine with other substances to form new compounds after certain atoms have been taken from the complex molecules of some previous composition or compound. These by-products have never been in or a part of the bacteria and are not the direct result of metabolism.

The Ammonical Fermentation.

As the nitrogen products are most intimately related to our subject we shall confine our attention to a few of the chief products of nitrogen decomposition. One of the most common is that which produces ammonia, and the first nitrogenous body to undergo ammonical fermentation is urea. Urea is a white solid excreted from the kidneys of animals and is contaminated in the ducts from the bladder with certain species of bacteria. The result of the fermentation that follows is that urea ($CO\ H_4N_2$) is split up into first ammonia, carbon dioxide and water and that forms Ammonium Carbonate ($(NH_4)_2CO_3$)

$$\underset{\text{urea.}}{\underline{COH_4N_2}} + 2H_2O = CO_2 + \underset{\text{ammonia}}{\underline{2NH_3}} + H_2O = \underset{\text{ammonium carbonate.}}{\underline{(NH_4)_2CO_3}}$$

and ammonium carbonate is a soluble salt, therefore an available plant food.

Building up of Nitrogen Compounds, Nitrification.

The process of nitrification is in general an oxidation of nitrogen compounds of the soil, the result of which is nitric acid. The nitric acid unites with the bases present in the soil to form nitrates which are the most useful nitrogenous foods. We have noticed that ammonia salts are available for plants, though less so than nitrates. We have seen that ammonical fermentation is the result of the action of putrefactive bacteria upon proteids and furnishes some food for plants. Where the ammonical fermentation occurs in the soil in connection with the formation of acids which can hold the ammonia in the form of salts it will render available some of the nitrogen which was previously out of reach of the plants. The ammonical fermentation takes place where ever complex nitrogenous compounds in the soil are acted upon by common putrefactive bacteria and this is one of the means if rendering nitrogen directly available. Ammonia products are not directly used by plants. It has been proven that in sterilized soils plants make less use of these salts than they do in unsterilized soils, a fact that indicates that possibly all of the ammonia compounds must be first converted into nitrates before they are directly available.

Ammonia salts are not the only source of nitrogen. In the humus there is a variety of compounds which have to be changed in their chemical nature before they can be utilized by plants. It has been found that to render these compounds available for plant food it is only necessary to oxidize the lower compounds of nitrogen to convert them into nitrates. The oxidation of ammonia or its salts takes place in the following manner:-

$$2NH_3 + 2O = 2HNO_2 + 3H_2O$$

By replacing the hydrogen of the nitrous acid (HNO_2) by potassium and oxidizing it further we get potassium nitrate (KNO_3), the best fertilizer known .

The Nitrification Bacteria.

This process is called nitrification. The next question that presents itself is, how does the oxidation take place? It was first thought to be a simple chemical process as such oxidation can take place when the material is brought into very intimate contact with oxygen but it was found by Schlossing and Maintz in 1877 that this nitrification is intimately associated with living matter in the soil. Nothing very definite, however, could be proved in regard to it as the germ was not isolated or a pure culture made from it until a Russian Naturalist Winogradsky (1890) discovered that the germ would not grow in the usual organic media. He then made a series of experiments and was finally able to isolate from the soil an organism, which proved to be a definite species of bacteria, and which was able under proper conditions to carry on this oxidation of nitrites and ammonia in such a way as to result in the production of nitrates. He proved further that the oxidation is a double process, that there are two independent actions of two distinct species of bacteria to convert ammonia products into nitrates. The first step as we saw in the formula was to convert ammonia into nitrous acid and the next step was to oxidize the nitrites into nitric acid. Then, of course, the remainder of reaction is purely chemical. These bacteria are called Nitrobacteria.

Nitrous Bacteria;-These bacteria perform the first step in the nitrification, oxidizing the ammonia compounds into the form of nitrous acid which forms nitrous salts by combining with the alkalies of the soil. At least two species are known, one being a bacillus (nitrobacteria) and the other a coccus (nitrococcus).

Nitric bacteria, called Nitro bacteria is the second species which completes the nitrification. Both these types are commonly found in soils, so that the nitrification if it occurs will be complete. Their importance in the soil can hardly be overestimated, since they complete the final transformation of nitrogen compounds into the form most available for plants, and thus unlock much of the soil nitrogen from its useless combinations.

Denitrification.

This general decomposition of compounds under the influence of bacteria produces evil as well as good results. Decomposition bacteria are not only able to destroy the urea and proteids but they also have the power of setting nitrogen free and of extracting the oxygen from any nitrates reducing them to a lower form and rendering them useless for plant food.

The Relation of Nitrification and Denitrification.

Denitrification bacteria are very thrifty while the nitrifying bacteria are comparatively weak, but the conditions favoring the two are so different they are not active to any great extent in the same material at the same time. The building of nitrates will not take place in soil where there is a considerable amount of organic matter or ammonia while that is the only media in which the denitrifyers will work. The denitrifyers cease to grow after they have used up all the organic material that nourishes them, and even if the nitrates are subsequently produced they will not be reduced by the nitrifying organisms. On the other hand when this condition is reached, when the high organic compounds have all disappeared and in their place has formed a quantity of ammonis salts and other simple nitrogen compounds we have just the proper conditions for action of nitrification.

Distribution of Nitrifying Bacteria.

Although only a few of these bacteria are yet known they are widely distributed. They are found in water in sewage and in ordinary soil. They do not occur when the soil has a strong acid reaction and are frequently few or wanting in meadows. They are abundant in well fermented manure and one of the advantages of adding manure to certain soils seems to be rather from the bacteria thus inocluated in the soil than the actual plant food in the manure.

Can Nitrification be Increased.

To the agriculturist it has become a practical question to learn how these bacteria, if not already present, may be added to the soil, and how they may be stimulated into activity if they are present but inactive.

It would be impractical to try to cultivate bacteria by laboratory methods to use in inoculating the soil because the organisms are too difficult to grow and moreover it is not necessary. Most soils have the nitrification bacteria so that a stimulation of their action is needed rather than the addition of more bacteria.

It is therefore more important to have the conditions for their best development than to try to increase their number by inoculation. This can be brought about, to a certain extent, by knowing conditions most favorable for them and correcting present conditions as much as possible.

The soil should of course be moist but not saturated with water so oxygen will not be excluded. The temperature must be above 5°C. and nitrification increases with temperature up to a certain degree. The activity of the nitrobacteria demands an alkaline medium, hence, lime and similar fertilizers aid in fertility by correcting the acidity of the soil. The same may be said of manure in a great many cases, as manure is always alkaline and this neutralizes the acid in the soil. And as nitrification demands a good supply of oxygen it is stimulated by keeping soil well stirred allowing air to circulate through it. This may be brought about by better and more frequent cultivation. These facts have entirely done away with the old theory of fallowing. In case of fallowed land the process of nitrification is very slight and what nitrates are formed are drained out of the land because there are no plants to take them up.

Reclaiming Lost Nitrogen.

Bacterial action on Leguminous plants.

The greatest means at the disposal of the farmer for increasing his store of nitrogen is to grow leguminous crops. This is also a bacterial action and it seems for every family of the legumes there is a peculiar species of bacteria that effects its roots. It has been demonstrated very conclusively that peas and beans growing in a soil practically free from nitrogen and fed upon feed containing no nitrogen, increased the amount of nitrogenous material in the plant and the only source of nitrogen at its command was the atmosphere.

Root Tubercles.

On close investigation it was found that the fixation of the nitrogen was closely associated with the growth upon the roots of the plant of little nodules known as tubercles. These tubercles are little abnormal growths on the roots, sometimes numerous and varying from the size of a pin head and smaller to the size of a pea. The nature and cause of these tubercles were for a long time unknown but they are now known to be caused by bacteria attacking the roots and making a growth there much the same as some of the fungi attacks leaves of trees although these growths on the root do not in any way injure the plant. The bacteria are thought to enter into the root tissue and these develop and stimulate the tissue and cause an abnormal growth. They are the result of the action of of bacteria and do not normally belong to the leguminous plant itself.

Assimilation of Nitrogen.

It is still an open question whether it is the legume or the bacterium that fixes the nitrogen or whether it is the result of the two organisms combined. It is certain that the plant alone without the bacterium to cause the nodules cannot fix the atmospheric nitrogen neither can the bacterium do the work without the aid of the plant but the two working together are able to fix atmospheric nitrogen in considerable amounts.

Importance of Leguminous Crops to Agriculture.

When we realize that in the older communities many farmers are paying from $6 to $10 an acre for fertilizers, which in the aggregate amounts to a tax of millions of dollars, and as we in the West are fast tending in the same direction it would be well for us to awaken to the fact that we can maintain and even increase the fertility of our soil if we but aid nature in her great work. We can do this by raising leguminous crops and if the bacteria that invade the roots of the plant we wish to produce are absent from the soil they can be brought from soils in which they do now exist and be introduced into our soil. Thus we see that the whole problem of the soil fertility is inseparable from bacterial fermentation. From the origin of the soil, through its use by plants and the subsequent destruction to their original condition of the products formed, we find nearly every step accompanied by bacterial action. The continued fertility of the soil is thus associated with bacterial life. In the future the problem of the proper treatment of the soil for the use of agriculture will be, in a very large degree, a problem of the proper control of bacteria.

Agriculturists must learn to stimulate the bacterial actions which are advantageous and check those which are disadvantageous, if they would insure the continuance of soil fertility.