UNIVERSITY OF GLASGOW.

THESIS FOR THE DEGREE

----- of -----

DOCTOR OF MEDICINE.

NITRIFICATION in SOIL.

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Various opinions have been expressed in explanation of the changes which take place in dead organic matter, commonly called putrefaction. It was the belief of the ancient agricultural chemists that putrefaction was the origin of vegetation, but it is now clear that low forms of life, bacteria - unicellular living things, are the principal factors in putrefaction. In the field of bacteriology, it has been established by many experiments that these organisms account for the splitting up of all organic compounds in soil, sewage, etc; and that the end of this metamorphosis results in mineralisation.

Liebig enunciated about the year 1840 in a paper to the British Association on "Chemistry in its application to Agriculture and Physiology", that nitrogenous organic matter

decayed in the soil by a chemical process, with the formation of ammonia, a small part of which was converted into nitric acid, which apparently served as plant food. During the period between the years 1860 and 1880 it was definitely established that decomposition is brought about by bacteria, and that the process is not purely chemical in the sense in which Liebig believed it to be.

To Pasteur must be ceded the honour of first suggesting that the changes in soil might be due to micro organisms; and later in showing experimentally that they were the results of an entirely bacterial process. This suggestion was practically applied to sewage by Schloesing and Müntz in 1877 in an experiment which has been so far reaching in its effects that it may be described in detail.

A continuous stream of sewage was

allowed to trickle down a column of sand and limestone in a tube so slowly that it took 8 days to pass. For the first 20 days the ammonia in the sewage was not affected, thereafter it began to be converted into nitrate, and finally all the ammonia was converted during the passage through the column, and nitrates alone were found in the issuing liquid. This filtered sewage contained no ammonia. After this process had continued for some weeks chloroform vapour was passed through the column, with the result that in 10 days after the introduction of the chloroform all nitrates had disappeared from the effluent. Subsequently the passage of chloroform vapour was stopped. Nitrification did not recur until the washings from 10 grms garden soil were added, and 8 days after this addition had been made nitrates again appeared.

in the effluent. Nitrification was thus shown to be due to micro-organisms, organised ferments as they were then called. The chloroform vapour, it would appear, acted as an antiseptic and killed the nitrifying organisms, while the addition of the soil washings reinoculated the material in the column.

The results of this experiment were confirmed by Warington in his investigations on soil at the Rothamstead station, Harpenden, (Journ. Chem. Society 1891. p 484.). He showed that nitrification in the soil could be stopped by chloroform and Carbondisulphide, and that solutions of ammonia salts could be nitrified by adding a trace of soil.

This was an advance of very great importance and formed the basis of further work,

namely the task of isolating the organisms which were active in the digestion and mineralisation of the substances dealt with.

After many trials using ordinary agargelatine media, Winogradsky successfully isolated the nitrifying bacteria and published his discovery in the Annals of the Pasteur Institut,

1890. IV. 1e. Memoire 213. — 31.
2e. Memoire 257. — 75.
3e. Memoire. 760. — 71.

The discovery by Munro that organisms will grow in purely inorganic solutions has been used for the isolation of the different species. Frankland succeded in isolating nitrous organisms by the dilution method. Omeliansky confirmed the fact that two types of organisms are necessary to convert ammonia to

Nitrate, - Nitrite being an intermediate stage. Thus it was finally established on a scientific basis that the splitting up of all organic compounds is the work of micro-organisms.

In connection with the application of these principles to Public Health M. Louis Mouras was one of the pioneers. He invented a self emptying cesspool about the year 1860, and in 1883 the results of his observations were published by a Priest, The Abbé Moigno in Paris.

Briefly stated the gist of Mouras' work was in noting that the contents of old closed cesspools were clear water, a little sediment, and no bad odour. He thought that the agents which changed the original contents might be Vibrios or the Anaerobes of Pasteur. Cameron of Exeter in 1895 formed a cesspool after the manner of Mouras and called it "The Septic Tank".

During the transformation of nitrogenous organic matter into nitrates whether in soil or artificial sewage beds, it is found that there is a continual diminution in the total number of bacteria and that there is a constant change of type. As the environment changes from phase to phase, groups of organisms disappear and new types take their place. Each type of organism has an optimum set of conditions such as temperature, moisture, quality and quantity of food, amount of oxygen, etc.

It is known that when sewage reaches the purification ground much of the proteins has been converted into albumoses and peptones and that these quickly pass through the stage of amino-acids into ammonia, nitrogen, and other gases. There is now quite a mass of experimental evidence to show that most of the bacteria found in sewage and soil are capable

of forming ammonia from nitrogenous organic matter. The work of Warington, Chester, Leube, Miguel, and others has demonstrated this point. There is evidence to show further that nitrogen is liberated from nitrated solutions, and that this gas is produced by the interaction of the oxides of nitrogen and amino-acids. The conversion of ammonia into nitrites and nitrites into nitrates was first studied in England by Warington and later on the Continent by Winogradsky. Two groups of microbes were carefully studied and named after their functions nitrous and nitric bacteria. It appears from more recent work by Massol that the nitrite-producers take on symbiotic properties with ordinary soil bacteria whereby organic matter fails to retard the growth of the bacteria; and that in the case of the nitrate-formers ammonia has little power of interference once the

organisms have had a vigorous start in growth.

Inasmuch as it requires weeks or months to transform dead proteins in the soil into nitric acid, soils or nitrifying beds doing poor work may be improved by seeding with soil from older and ripe beds. Such a bed is the scene of many physical and chemical changes occurring side by side with various biological operations. Microscopic particles are caught in the meshes of the filtering material, whilst ultra-microscopic particles fall out on the surfaces of the same. But it must not be concluded that the transparent and odourless effluent produced contains the previous nitrogenous matters in the form of nitrates. Sommerville has shown that it is possible in 5 minutes to transform the foulest sewage into a passable effluent by two or three filtrations through animal charcoal; but the charcoal at the

end of a month will still contain undissociated proteins which at the end of a second month have not all reached the stage of nitric acid. If the total nitrogen be estimated in the sewage and effluent a large deficit will always be found in the effluent. Further the nitrates found in such an effluent may have been formed in the filtering material a month ago and are now merely washed out. Alongside this process of nitrification the opposite process of de-nitrification is in operation. Maasen has studied more than a hundred species of bacteria capable of reducing nitrates. Stephen Gage states that of 5,000 cultures isolated at the Massachusets State Board of Health experimental station, 85 per cent were capable of reducing nitrates more or less completely. Some of these reduce nitrates to nitrites and ammonia; others reduce nitrates to NO and N_2O; and others evolve free nitrogen gas from ammonia. It is interesting in this

connection to note that Stutzer has separated a species which he has named B. denitrificans which decomposes nitrites but not nitrates. B. coli reduces nitrates to nitrites. Hence a mixture of these organisms will reduce nitrates to nitrogen gas. Denitrifying bacteria carry on their functions aerobically and anaerobically.

In soil and in sewage beds the formation of nitrates takes place in two stages,

(1) Transformation of ammonia to nitrites - by the action of the group of bacteria named after their function Nitrous bacteria.

(2) The oxidation of nitrites to nitrates through the action of nitric bacteria. To enable this oxidation to take place there must be present :-

Pabulum for the bacteria, an alkaline media, Oxygen, a base (calcium) with which the

nitric acid can combine, Water, and a temperature between 5° C. and 50° C.

In organic solutions, nitrous organisms grow well, while Nitric organisms do not, and in inorganic solutions containing nitrites the reverse is true because in these solutions nitrous organisms are incapable of carrying out the oxidation process. The presence of humus or peat aids nitrification by the evolution of carbon-dioxide, which reduces the number of bacteria belonging to other groups, and establishes conditions under which it is possible for the nitric and nitrous groups to grow together, and at the same time it allows the stronger organisms to survive, develop, and become more active in their work.

The large protein molecule containing nitrogen, is abundant in soil and in sewage.

In the presence of water and putrefactive bacteria cleavage takes place. The hydroxyl group (OH) controlled by the enzyme acts as a wedge in splitting the protein molecule, with formation of Proteoses; further digestion takes place into Peptones, Amino-acids, Ammonia, free Nitrogen, and organic acids. Oxidation is now carried a step further by the action of the nitrifying bacteria, Ammonia is oxidised to nitrous acid, and later Nitrates are formed. The change from Ammonia to nitrous acid is comparatively slow, no intermediate product has been discovered, the higher oxidation to nitric acid is rapid. In over two hundred determinations made by the writer only a trace of nitrite was discovered by the methods employed.

Hydrolysis of Protein Molecule results in

↓

Proteoses

↓

Peptones

↓

Polypeptides

↓

Amino-acids

↓

Ammonia.

Ammonia is later oxidised by the nitrifying bacteria to

Nitrites

↓

Nitrates.

It is believed that these changes are effected through the enzymes of the bacteria engaged acting as catalysts.

A catalyst is an agent which influences a chemical reaction, and either accelerates, directs, or retards it, while the catalyst is not itself changed. An example of a catalysed reaction is found in the solubility of Iodine in a solution of potassium Iodide at ordinary temperature. In this change K.I. is the catalyst.

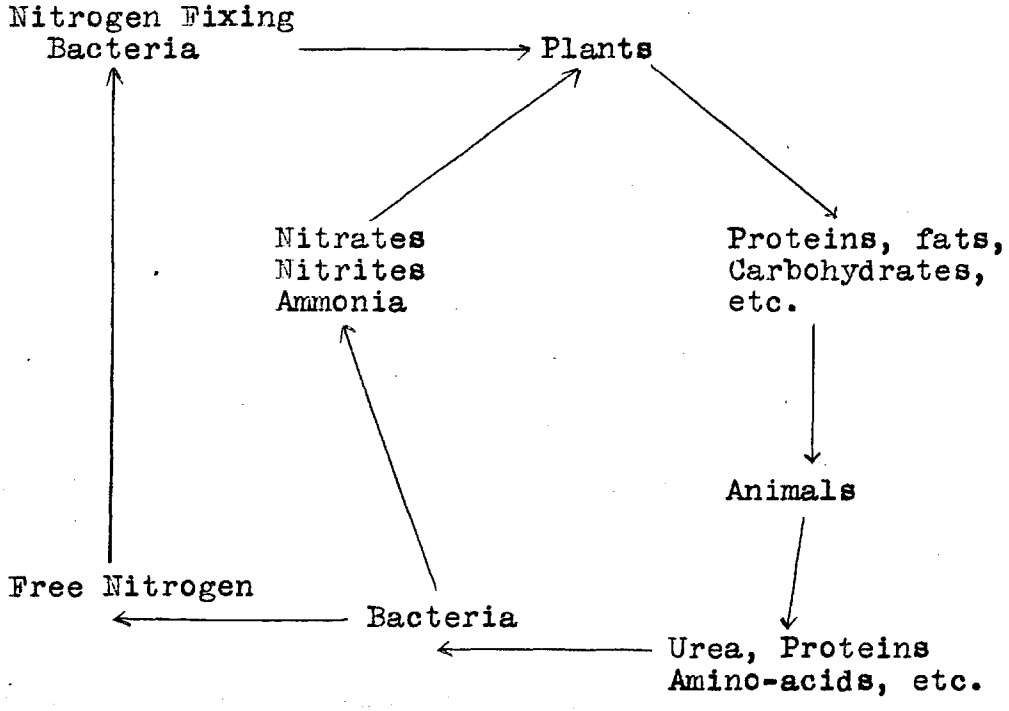
Again there are numerous combinations effected by the catalytic agency of finely divided platinum such as the combination of Oxygen and Hydrogen to form Water, and the hydrolysis of cane sugar by Ionic Hydrogen.

Furthermore, oxidations effected by Hydrogen peroxide are accelerated by the presenee of certain salts of iron and manganese. The action of the catalyst is compared to that of blacklead on a summer ice table, — A wooden table coated with blacklead, om which curling stones of iron are used. The Plumbous oxide reduces the co-efficient of friction, and allows free movement of the iron on the wooden surface, and thus accelerates the motion. Or again the action of the catalyst is like that of chalk used on the leather tip of a billiard cue to prevent slipping, but whether the real effect of the catalyst is to accelerate, retard,

or to prevent slipping, it seems to direct the action and retire, as it is not found in the end product. As regards the part which the catalyst plays in a reversible reaction, it is possible that all reactions are reversible if we knew how to establish suitable conditions, and that the catalysing agent takes the same part in denitrification as it does in nitrification.

Incubation at certain temperatures in presence of moisture stimulates the bacterial process; above 50° C. however the formation of nitric acid by bacteria ceases. Antiseptics of definite strengths also stop the action. Nitric acid may be formed but the formation is slow and must be purely chemical.

The Nitrogen Cycle.



The following experiments show the effects of

(1) Temperature.

(2) Water.

(3) Addition of humus.

(4) Addition of sulphur.

(5) Addition of CS_2.

(6) Addition of fat.

(7) Addition of basic slag.

upon the activities of the nitrifying organisms in certain soils.

The method of estimating nitrates which I used was that of Sprengel.

The basis of this method consists in the nitration of phenol-sulphonic acid to form picric acid and the conversion of the picric acid into an alkaline salt (I used the ammonium salt) which is highly coloured. The sample under examination was treated in parallel with a

standard solution of KNO_3 (1 cc. -= 0.1 mgm nitric N).

Phenol sulphonic acid was prepared by heating pure phenol and pure sulphuric acid in the necessary quantities for several hours on a water bath at 100° C.

Ten cc. of the sample nitrate and 10 cc. of the standard nitrate solution were evaporated to dryness in porcelain basins on a water bath at 100° C.. To each of the dry residues were added 1.5 cc. phenol sulphonic acid and the nitration completed on the water bath. When cool the contents of each dish were carefully washed into a marked nessler glass with distilled water, saturated with NH_4OH, and the volumes made up to 100 cc. The colour of the sample was matched by the requisite quantity taken from the more deeply coloured standard and diluted up to the same volume, viz 100 cc.

Where slight differences occurred in the shade of the tint, as did occasionally, the match was made by viewing the tints in the clear glass of the bottoms of the nessler glasses as they stood on a white tile side by side in a north light.

Taking of Soil.

Samples were taken at a uniform depth with an iron tool making a clean vertical cut, no sample was taken from freshly-ploughed or recently-manured land. In the laboratory the soil was spread out to dry, and the gross matters and stones removed, after which it was passed through a 3 MM sieve, slightly heated and ground in a mortar. Samples of 5 grammes were carefully weighed out and dissolved in 25 cc. distilled water over a water bath, stirred, and washed several times until all nitrates were extracted. The solution was then filtered. Ten cc. of the filtrate were evaporated in a porcelain dish.

Nitrates in soils at 16° C and at 37° C.

Table 1.

Nov: 1913.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
1. Surface soil, arable land in Ayrshire. 2 samples.	3.5 3.8	6.2 6.52
2. Surface soil, arable land, in Middlesex.	3.3	7.6
3. Surface soil, arable land, manured with sea weed for 25 yrs. Ayrshire.	5.6	9.8

Table 1. (continued).

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
4. Arable land, red sandy loam. Surrey.	3.5	4.7
5. Surface Arable Land. Hertford.	2.3	3.38
6. Made soil. Middlesex.	3.3	4.52
7. Loam from arable land. Middlesex. 2 samples.	0.82 1.4	1.85 2.64
8. Surface, arable land. Middlesex.	1.54	3.43

Table 2.

December 1913.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
9. Garden Soil, surface uncultivated. Middlesex. Three samples.	9.0 7.8 8.5	11.5 10.6 11.7
10. Garden Soil, 18" below surface uncultivated. Middlesex. Three samples.	3.3 3.8 3.67	4.82 5.91 4.25
11. Garden Soil, surface, uncultivated. Middlesex. Two samples.	3.5 3.8	5.5 6.4
12. Garden soil with Humus. Two samples.	6.3 6.75	9.5 9.85

Table 3.

January 1914.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
13. Limestone surface soil, grey colour. Surrey. Two samples.	6.2 4.1	1.7 0.75
14. Surface sandy soil from the Downs Surrey. White chalk with flints. Two samples.	2.0 1.3	0.38 0.4
15. Surface sandy soil from the Downs Surrey, red sand without flints. Two samples.	2.8 1.7	0.85 0.8

Table 3. (continued).

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
16. Upper green sands deposits known as Black Down. Surrey. Two samples.	8.7 7.5	0.28 1.6
17. Poor surface sandy soil. Surrey.	1.5	0.7
18. Sandy substance with lime, from a pit 10 feet deep. Middlesex.	4.3	0.3

Table 4.

January 1914.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
19. Surface soil from Woodlands at Crystal Palace. Kent. Two samples.	5.2 5.8	9.5 8.75
20. From a wood uncultivated for ages. Kent. Two samples.	2.5 3.8	5.4 5.85
21. Surface from woodland Epping Forest. Essex. Two samples.	1.8 1.83	2.6 2.35

Table 4. (continued).

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
22. From Woodland, Norwood, S.E., with much Humus. (surface) Two samples.	4.48 4.61	7.6 8.9
23. Sub-surface 12", same place. (dry). Two samples.	0.85 1.23	2.7 2.15

Table 5.

December 1913.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
24. River bank bathed with sewage. Ayrshire. Five samples.	1.5	5.6
	2.8	7.5
	3.2	5.58
	5.5	7.51
	2.85	5.67

Table 6.

February 1914.

Nitrates parts per million.

Soil, sample 5 grams.	At 16° C.	After incubating in moist condition at 37° C for 3 days.
25. Peat.	8.6	11.3
Renfrewshire.	8.74	10.9
Five samples.	7.98	10.4
	8.54	11.8
	7.68	11.35

Table 7.

Nitrification in water-logged soils.

April and May 1914.

20 samples of 50 grms, saturated with distilled water, put in glass cylinders, air excluded by stopper. Kept 10 days at 18° C.

Nitrates parts per million.

Before Saturation.	After 10 days. water was carefully filtered off, and N determined in residue.
3.5	0.6
1.5	0.5
8.6	0.8
5.6	0.3
1.5	0.2
9.0	0.81
3.3	0.3
3.5	0.25
3.3	0.31
1.8	0.7
3.8	0.13
5.2	0.53
3.5	0.16

Table 9.

Nitrification in a mixture of soil and Humus.

(Humus from an old sewage bed.)

March 1914.

Soil 75 grams.

Humus 25 grams.

Water 25 grammes.

Kept 21 days at 16° C.

Nitrates parts per million.

Humus.	Mixture after 21 days.
0.38	0.7
0.46	1.5
0.35	1.2
0.14	1.3
0.27	1.3
0.32	1.5
0.15	1.8
0.28	2.4
0.14	2.3
0.13	0.36

Table 9. (continued).

Humus.	Mixture after 21 days.
0.24	0.42
0.13	0.35
0.14	0.43
0.3	1.7
0.12	2.5
0.14	0.7
0.2	0.9
0.3	1.3
0.32	1.1
0.4	0.75

Table 10.

April 1914.

The influence of sulphur on nitrification in soil from arable land.

Soil 100 grms. Sulphur (sublimed) 1 grm.

Mixed and moistened with 25 cc. distilled water.

Kept 14 days at 16°C.

Nitrates parts per million.

Before S. added.	After 14 days.
2.5	0.2
1.85	0.3
2.0	0.3
1.76	0.05
1.8	0.035
2.1	0.12
1.75	0.07
1.76	0.4
2.5	0.8
1.6	0.84
1.2	0.7
1.5	0.74
1.6	0.55

Table 10. (continued).

Before S. added.	After 14 days.
1.8	0.48
1.7	0.34
2.4	0.33
1.8	0.33
1.54	0.32
1.6	0.25
1.54	0.15
1.7	0.18
2.4	0.16
2.7	0.2
1.8	0.12
1.7	0.42

Table 11.

April 1914.

The influence of sulphur on nitrification in a mixture of soil and Humus from sewage bed.

Soil 75 grams.

Humus 25 grams.

Sulphur (sublimed) 1 gram.

Well mixed; moistened with 25 cc. distilled water.

Kept 14 days at 18° C.

Nitrates parts per million.

Before S. added.	After 14 days.
1.5	0.8
1.7	0.5
1.2	0.35
1.3	0.53
1.3	0.72
1.5	0.33
1.8	0.63
2.4	0.54
2.3	0.44
1.36	0.34
1.42	0.48

Table 11. (continued).

Before S. added.	After 14 days.
1.35	0.47
1.43	0.38
1.7	0.37
2.5	0.35
0.7	0.37
0.9	0.56
1.3	0.37
1.1	0.35
0.75	0.36

Table 12.

February, March 1914.

The influence of CS_2 on nitrification in a mixture of soil from arable land and Humus from sewage bed.

Soil 75 grms. Humus 25 grms. Water 25 cc.

CS_2 10 grams.

Well mixed, kept 10 days at 18° C.

Nitrates parts per million.

Untreated soil.	Mixture soil, Humus and CS_2.
3.5	0.15
3.8	0.06
4.1	0.2
3.6	0.05
2.9	0.01
4.2	0.13
3.5	0.07
2.6	0.14
3.1	0.3
1.9	0.07

Table 13.

April, May 1914.

The effect of fat on nitrification in soil.

Soil 100 grms.

Liquid fat composed of equal parts mutton fat olive oil and castor oil, 10 grams. gently heated and thoroughly mixed, moistened with 25 cc sterile water, - kept in a dark place for fourteen days at 18° C.

Nitrates parts per million.

Soil.	Soil 100 grms, fat 10cc after 14 days.	Soil 100 grms. Fat 5 cc. after 21 days.
1.8	0.22	0.12
2.5	0.13	0.1
1.85	0.14	0.12
2.0	0.14	0.14
1.76	0.12	0.22
1.8	0.17	0.21
2.1	0.13	0.08
1.75	0.17	0.05
1.76	0.18	0.17

Table 14.

May 1914.

The influence of Basic Slag on nitrification in Humus.

(from old sewage bed.)

Humus 75 grams.

Basic Slag 25 grams.

Water 25 cc.

Kept at 18° C. 3 days.

Nitrates parts per million.

Humus.	Humus and Slag.
0.38	5.21
0.46	4.5
0.35	4.2
0.14	4.2
0.27	4.3
0.32	3.1
0.15	3.03
0.28	3.15
0.14	5.63
0.13	4.52

Table 14. (continued.)

Humus.	Humus and Slag.
0.24	3.03
0.13	3.02
0.14	3.14
0.3	3.06
0.12	3.2
0.14	3.26
0.2	3.02
0.3	3.03
0.32	2.86
0.4	3.04

Conclusions.

These tables show :-

(1) That the nitrifying bacteria do much more work at 37° C. than at 16° C.

(Tables 1, 2, 4, 5).

(2) That in sandy soils nitrification is lessened at 37° C. as compared with that at 16° C.

(Table 3.)

(3) That in peat the nitrifying bacteria are more active at 37° C. than at 16° C.

(Table 6).

(4) That in water-logged soils nitrification falls off very much.

(Table 7.)

(5) That heating soil to 100° C. for half an hour largely increases nitrification; and this increase at the end of 3 months is nearly double that found at the end of 1 month.

(Table 8.)

P.T.O.

(6) That the addition of old humus (one third) to soil largely increases nitrification.

(Table 9.)

(7) That 1% Sulphur and 10% Carbon disulphide greatly diminish nitrification.

(Tables 10, 11, 12.)

(8) That the addition of 10% fat largely inhibits nitrification.

(Table 13.)

(9) That addition of one third by weight of basic slag to humus greatly increases nitrification.

(Table 14.)

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