Some Effects of Organic Growth-Promoting Substances (Auximones) on the Soil Organisms concerned in the Nitrogen Cycle.

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(Communicated by F. W. Oliver, F.R.S. Received January 2, 1917.)

It is a well-established fact that the presence of a certain amount of humus in soil is essential to complete fertility, and it is equally well known that this organic matter, whether supplied in the form of stable or green manure, is far more effective when decomposed, or "rotted," than when fresh. This has been attributed to the soluble humus formed during the rotting, but wherein lies the peculiar merit of this soluble humus has long been a debatable point, some considering that it serves primarily as a plant nutrient, while others claim that its most important effect is upon the bacterial flora of the soil.

It is well known that raw peat, although rich in humus, is practically useless as a manure, on account of its acid and insoluble nature. This insoluble humus can be neutralised and rendered largely soluble by extraction with alkalies, and almost all experiments on the effect of soluble humus on bacteria have hitherto been carried out with such extracts. Too much reliance must not be placed on the results obtained with these chemically prepared substances, for they are not strictly comparable with the soluble humus produced in the soil by natural processes. However, Bottomley* has shown that it is possible, by inoculating peat with certain aërobic soil bacteria, and keeping it under suitable conditions, to convert it into a partially soluble humus in a comparatively short time. A similar bacterial action is taking place more slowly in every rotting manure heap and in all soils, for the longer stable and farm manures are kept, the more water-soluble brown humus can be extracted from them. Bottomley's bacterial treatment of the peat simply reproduces and hastens these natural processes, and the product of the treatment, which is known as "bacterised peat," is practically the counterpart, in a more concentrated form, of rotting stable manure or of green manures which are undergoing decomposition in the soil. The soluble humus which can be extracted from it may be justly considered to approximate more closely to the natural product than any extracts obtained by chemical processes, and the

* Bottomley, W. B., 'Journ. Roy. Soc. Arts,' vol. 62, No. 3199 (1914).

effects of this soluble humus on soil bacteria accordingly reproduce more exactly the influence of the organic matter of soils upon the bacterial flora.

Recently Bottomley* has shown that a water extract of bacterised peat, and certain fractions obtained therefrom, produce an increase in the growth of plants which cannot be attributed to any purely nutritive effect caused by the recognised manurial constituents present, and he suggested that during the decomposition of the peat, certain accessory food substances are formed, which fulfil a similar function to that of the vitamines known to be so important in animal nutrition. His recent work on the influence of these growth-promoting substances obtained from bacterised peat, which he has called "auximones," has lent confirmation to this suggestion, and it was with the object of investigating the effect of these auximones upon the four chief groups of soil organisms concerned in the nitrogen cycle, that is, upon the nitrogen-fixing, the nitrifying, the ammonifying, and the denitrifying bacteria, that the present research was undertaken.

Nitrogen Fixation.

It was first pointed out three years ago by Bottomley† that the addition of bacterised peat to soil results in a marked increase in the rate of nitrogen fixation, and this result was attributed purely to the activity of the nitrogen-fixing organisms introduced into the soil with the material. He‡ has since found that, apart from the organisms which the material contains, the bacterised peat itself has certain inherent properties which have the effect of increasing the rate of nitrogen fixation by soil organisms to a marked degree, and that these properties are not possessed by either raw or chemically treated peat. The experiments hitherto recorded, however, have been more or less isolated, and it appeared advisable to undertake a more extensive investigation of the effect of bacterised peat, and the various fractions obtained from it, upon nitrogen fixation in soil and in both crude and pure liquid culture.

A preliminary experiment was carried out to determine whether bacterised peat which had been sterilised at 135° C., to kill off the nitrogen-fixing organisms which it contained, would stimulate nitrogen fixation in soils. Six portions, each consisting of 24 oz. of a uniform sample of a loamy soil, were weighed out. Two were mixed with one part in ten by volume of sterilised bacterised peat, and two with a similar

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

† Bottomley, W. B., 'Report Brit. Assoc.,' 1913.

‡ Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914), and vol. 89, pp. 102–108 (1915).

quantity of the normal material containing living organisms, the remaining two portions serving as controls. A similar experiment was also arranged with a clay soil. When three samples had been taken from each for analysis of their nitrogen content, all the soils were placed in large glass bottles loosely corked, so that they could be well shaken daily to ensure aëration, and were placed on top of an incubator kept at 26° C., the average temperature of the soils being about 20–22° C. The moisture content was kept as uniform as possible, and after two weeks' incubation the soils were again sampled, the total nitrogen being determined by the Kjeldahl process. The results obtained were :—

Table I.

	Original nitrogen content (mgrm. N per 100 grm. soil).		Nitrogen content after two weeks (mgrm. N per 100 grm. soil).		Gain in two weeks (mgrm. N per 100 grm. soil).	
	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.
1. Soil alone	220, 224, 227 } 224	295, 306, 299 } 300	236, 230, 238 } 235	313, 320, 322 } 318	11	18
2. „ „	229, 224, 225 } 226	299, 302, 296 } 299	237, 241, 240 } 239	316, 323, 319 } 319	13	20
3. Soil + sterilised bacterised peat	362, 372, 371 } 368	465, 462, 454 } 460	410, 403, 400 } 404	513, 509, 502 } 508	36	48
4. „ „	375, 368, 368 } 370	464, 468, 462 } 465	398, 405, 400 } 401	504, 514, 510 } 509	31	44
5. Soil + normal bacterised peat	368, 360, 365 } 364	458, 464, 465 } 462	424, 430, 428 } 427	527, 522, 524 } 524	63	62
6. „ „	364, 371, 372 } 369	466, 458, 457 } 460	432, 428, 434 } 431	526, 518, 517 } 520	62	60

The three analyses of each soil gave results which approximated very closely to the mean, and therefore, in the following Tables of soil analyses, only the mean of the three determinations will be given in each case, in order to avoid unnecessary figures. The maximum deviation from the mean for nitrogen fixation was ± 6.

It is thus apparent that the addition of bacterised peat has increased the activities of the nitrogen-fixing organisms already in the soil, quite apart from any bacteria introduced. This result may have been due to (1) the physical effect of the organic matter in improving the aëration of the soil, and thus facilitating the activities of the aërobic bacteria; (2) the effect of

the soluble humate alone; (3) the effect of the organic products, other than soluble humate, formed during the bacterisation of the peat. If the first be the case, then a similar result might be expected upon the addition of raw peat to the soil, if the soil be limed to correct the acidity of the peat; and, if the second be true, then chemically treated peat should produce a like result. Accordingly, another six portions of soil from each of the same localities were weighed out, two of each being mixed with one part in ten by volume of raw peat and 1 per cent. of their weight of powdered calcium carbonate. Two other portions of each were mixed with one part in ten by volume of peat which had been treated with 2 per cent. of its weight of sodium carbonate, and which was absolutely neutral, while the remaining two portions served as controls. Since the soils already contained sufficient lime, the chalk was added only to those containing raw peat. The soils were sampled, as before, for their nitrogen content, and then placed on top of the incubator at about 20° C. for a fortnight, at the end of which period they were again analysed, with the following results :—

Table II.

	Original nitrogen content (mgrm. N per 100 grm. soil—mean of three determinations).		Nitrogen content after two weeks (mgrm. per 100 grm.—mean of three determinations).		Gain in two weeks (mgrm. N per 100 grm. soil).	
	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.
1. Soil alone	220	300	230	318	10	18
2. „ „	221	299	232	319	11	20
3. Soil + raw peat ...	285	369	273	381	−12	12
4. „ „ ...	290	371	270	387	−20	16
5. Soil + carbonated peat	292	365	304	382	12	17
6. „ „	286	368	300	388	14	20

In these soils it is evident that neither the aëration nor the chemically formed soluble humus are capable of producing an effect comparable with that of the bacterised peat. The addition of raw peat appears to result in one case in a loss of nitrogen, while the chemically treated peat does not appreciably affect the nitrogen-fixing organisms.

These and other results obtained from time to time all tend to show that bacterised peat has the property of increasing nitrogen fixation in soils, independently of the organisms which it contains, and quite apart from any physical effect or any purely stimulating property of the soluble humates

as such. In order to investigate further this property a fractionation of the bacterised peat was made according to the methods already described by Bottomley,* and the decomposed phosphotungstic-acid fraction obtained. Four portions, each consisting of 40 oz. of soil from Chelsea Physic Garden, were weighed out, and to each of two of them was added, in solution in distilled water, the phosphotungstic fraction of that weight of bacterised peat which, if mixed with the soil, would give a proportion of one part of peat in ten of soil by bulk; the other two served as controls. The four soils were sampled at once for their nitrogen content, and were then placed in loosely corked glass bottles on top of the incubator as before, for about seven weeks, moisture being added when necessary, and the bottles being shaken daily. Samples analysed twice during that period gave the following results:—

Table III.

	Original nitrogen content (mgrm. per 100 grm.—mean of three determinations).	Nitrogen content after 28 days (mean of three determinations).	Nitrogen content after 52 days (mean of three determinations).	Gain in 28 days.	Gain in 52 days.
1. Soil alone.....	312	321	333	9	21
2. „ „	316	322	335	6	19
3. Soil + phosphotungstic fraction	314	333	373	19	59
4. „ „	317	337	380	20	53

The figures here given show that the addition of even the phosphotungstic fraction to the soil results in an increase in nitrogen fixation, although it is not to be expected that this fraction would be as effective as the bacterised peat itself. This substance certainly contains neither organisms nor soluble humus as such, nor can it have any effect on the physical condition of the soil. As a result of these investigations, a comparison was made between the effect of humus from raw peat and that from bacterised peat and its various fractions, upon the nitrogen-fixing organisms in liquid culture.

It has already been shown by Krzemieniewski† that the addition of natural humus to the culture medium in which *Azotobacter chroococcum* is growing results in a greatly increased nitrogen fixation, so a solution of this natural humus was prepared by treating some of the raw peat used in the preparation of the bacterised peat with just sufficient sodium carbonate solution to

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

† Krzemieniewski, 'Bull. Acad. Sci. Cracovie,' No. 9, pp. 929–1050 (1908).

extract all the brown soluble matter. Part of this crude sodium humate was set aside for use in the culture media. The remainder was acidified with dilute hydrochloric acid, the precipitate filtered off, washed thoroughly, and redissolved in just sufficient sodium carbonate solution. Part of this purer sodium humate was preserved for experiment, and part was treated with a little calcium chloride solution, the precipitate of calcium humate formed being thoroughly washed. A crude ammonium humate was also prepared by extracting raw peat with a slight excess of ammonium hydrate solution, filtering the extract, and removing the excess of ammonia by evaporation on the water-bath. A solution of artificial sodium humate was obtained by dissolving artificial humic acid, prepared by boiling sucrose with dilute hydrochloric acid in the usual way, in the requisite amount of sodium carbonate solution. All of these preparations were neutral when added to the culture media.

A pure culture of *Azotobacter chroococcum* was isolated from soil and cultivated in a solution consisting of 100 c.c. distilled water, 1 grm. mannite, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$. A number of flasks each containing 100 c.c. of this medium were divided into series of six each. To the various series the additions shown in the Table below were made, and all were inoculated with 1 c.c. of a uniform suspension of *Azotobacter*. Two flasks of each series were then sterilised in an autoclave at 135° C. to serve as controls, and the whole set was incubated for ten days at 26° C. Analysis by the Kjeldahl method for the nitrogen content then gave the following results:—

Table IV.

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.	Gain in nitrogen.
		mgrm.	mgrm.
1	Control	0·4	
2	,,	0·4	0·4
3	100 c.c. mannite solution	5·6	5·2
4	,, ,, ,,	5·4	5·0
5	,, ,, ,,	5·3	4·9
6	,, ,, ,,	5·4	5·0
			5·0
7	Control	2·4	
8	,,	2·6	2·5
9	100 c.c. mannite solution + crude sodium humate from 0·25 grm. peat	11·1	8·6
10	,, ,, ,, ,,	11·5	9·0
11	,, ,, ,, ,,	11·4	8·9
12	,, ,, ,, ,,	11·7	9·2
			8·8

Table IV—*continued.*

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.	Gain in nitrogen.
		mgrm.	mgrm.
13	Control	1·8	
14	,,	2·0 } 1·9	
15	100 c.c. mannite solution + purer sodium humate from 0·25 grm. peat	7·3	5·4
16	,, ,, ,, ,,	6·9	5·0 } 5·2
17	,, ,, ,, ,,	7·4	5·5
18	,, ,, ,, ,,	7·0	5·1
19	Control	6·4	
20	,,	6·4 } 6·4	
21	100 c.c. mannite solution + crude ammonium humate from 0·25 grm. peat	15·0	8·6
22	,, ,, ,, ,,	14·8	8·4 } 8·3
23	,, ,, ,, ,,	14·4	8·0
24	,, ,, ,, ,,	14·5	8·1
25	Control	1·6	
26	,,	1·6 } 1·6	
27	100 c.c. mannite solution + purer calcium humate from 0·25 grm. peat	6·5	4·9
28	,, ,, ,, ,,	6·2	4·6 } 5·0
29	,, ,, ,, ,,	6·6	5·0
30	,, ,, ,, ,,	7·0	5·4
31	Control	0·7	
32	,,	0·5 } 0·6	
33	100 c.c. mannite solution + artificial sodium humate from 0·1 grm. artificial humic acid	5·9	5·3
34	,, ,, ,, ,,	6·2	5·6 } 5·1
35	,, ,, ,, ,,	5·6	5·0
36	,, ,, ,, ,,	5·3	4·7
37	Control	5·1	
38	,,	5·1 } 5·1	
39	100 c.c. mannite solution + water extract of 0·25 grm. bacterised peat (sterilised)	18·9	13·8
40	,, ,, ,, ,,	19·7	14·6 } 14·1
41	,, ,, ,, ,,	19·2	14·1
42	,, ,, ,, ,,	19·0	13·9
43	Control	2·0	
44	,,	2·2 } 2·1	
45	100 c.c. mannite solution + water extract of 0·25 grm. raw peat	5·5	3·4
46	,, ,, ,, ,,	6·1	4·0 } 3·6
47	,, ,, ,, ,,	5·6	3·5
48	,, ,, ,, ,,	5·8	3·7

It is apparent from these figures that the crude humus extracted from the raw peat by alkalies has the effect of increasing to some extent the nitrogen fixation of *Azotobacter* in culture solution, and that this property is not retained when the humus is further purified. Remy and Rösing* obtained

* Remy and Rösing, 'Centr. Bakt. Par.,' Abt. II, vol. 30, pp. 349–384 (1911).

comparable results, but they attributed the beneficial effect of the humus solely to the iron which it contains. In view of this statement, it must be pointed out that both the purified sodium humate and the calcium humate used in the above experiments contained appreciable quantities of iron, which was presumably adsorbed by the colloidal humus, and was by no means entirely removed by the subsequent solution and re-precipitation. Further, while the sodium humate must contain quite as much iron as does the water extract of bacterised peat, since both have been prepared from the same kind of raw peat, yet the effect of the bacterised peat is markedly greater than that of the chemical preparations from raw peat. The inference seems to be that during the bacterisation some soluble organic substances are produced, besides the soluble humates, which have the effect of greatly increasing the nitrogen fixation by *Azotobacter*. These substances appear to be formed in comparatively small quantities during treatment of the peat with weak alkalies, since such a preparation increases fixation to some extent, but they also appear to be lost during further purification of the crude humate. Such substances are not present in raw peat, at least in a water-soluble condition, since a water extract of such peat depresses the rate of nitrogen fixation. That the beneficial effect of the crude humus is not due to any physical action of the colloidal extract is shown by the results with artificial humus, which appears to have practically no effect. This is in accordance with the results of Krzemieniewski.*

The addition of natural humus to the culture medium of *Azotobacter*, in the hands of practically all investigators, has proved to be beneficial to nitrogen fixation, but the degree of benefit obtained differs very widely, rising in Krzemieniewski's* researches from 2·32 mgrm. nitrogen fixed without humus to 21·52 mgrm. with sodium humate from soil. This divergence is very probably due to the varying degree of bacterial decomposition which has taken place in the humus before extraction; the greater the decomposition, the better being the result; and it is most probable that in bacterised peat the bacterial action has taken place under the circumstances most favourable for the production of the essential organic substances. This conclusion receives support from the work of Löhnis and Green†, who found that the humus from fresh stable manure increased fixation by 9·8 mgrm., while that from similar manure which had been "humified" resulted in an increase of 14·4 mgrm. A similar experiment, which they carried out with peat, showed no difference between the effect of fresh and

* Krzemieniewski, 'Bull. Acad. Sci. Cracovie,' No. 9, pp. 929–1050 (1908).

† Löhnis and Green, 'Centr. Bakt. Par.,' Abt. II, vol. 40, pp. 52–60 (1914).

humified peat, evidently owing to the fact that they simply mixed the materials with sand, and allowed them to remain thus for 4½ months, depending solely upon the bacteria which they contained for the "humifying" process. Stable manure is teeming with such organisms, and conditions were ideal for their further action, but peat is practically devoid of them, and they must be added under suitable conditions before decomposition can take place. Hence the difference in the results obtained.

In order to investigate further the effect of these organic substances in bacterised peat, an alcoholic extract, and also the phosphotungstic and silver fractions, were employed. In all cases where an alcoholic extract was used, the alcohol was driven off at a low temperature by means of a fan before use, and the residue taken up in distilled water. Where a water extract was employed, this was sterilised in an autoclave at 135° C., in order to kill off any bacteria already present.

As already shown by Bottomley, the addition of the alcoholic extract of 1 grm. of bacterised peat to every 100 c.c. of culture solution resulted in a marked increase in the rate of nitrogen fixation, so a comparison was made of the action of the different fractions of the peat. A set of 30 flasks was prepared, all containing 100 c.c. of the mannite solution, and divided into five series of six flasks each. To the various series were made the additions described below, and all the flasks were inoculated with 1 c.c. of a suspension of a crude culture of *Azotobacter* from soil. A similar set of 30 flasks was also prepared and inoculated with a pure culture. Two flasks of each series were sterilised for controls, and all were incubated for 10 days, when upon analysis the results set out in Table V were obtained.

Throughout the experiments, the beneficial effect of the fractions from bacterised peat was manifested by the fact that, after about three days, while the cultures in mannite alone showed still only a faint cloudiness, a definite scum was already produced on the surface of those containing these extracts. The figures obtained show a progressive superiority in the effect of the phosphotungstic, silver, alcoholic and water extracts, and, while they point to the conclusion that the active substances are not separated quantitatively by the methods so far adopted, yet it is obvious that all the auximone fractions have the power of increasing the rate of nitrogen fixation of *Azotobacter*. It should be noted that none of the alcoholic, phosphotungstic, or silver fractions contained any trace of iron.

The effect of the auximone fractions upon *Bacillus radicicola*, the nitrogen-fixing organism of the leguminous nodules, was then investigated. Pure cultures were obtained from the nodules of various leguminous plants, notably, broad bean, sweet pea, lucerne, clover and hop trefoil, and the

Table V.

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.		Gain in nitrogen in 10 days.	
		Crude.	Pure.	Crude.	Pure.
		mgrm.	mgrm.	mgrm.	mgrm.
1	Control	0·2 } 0·2	0·1 } 0·1		
2	,, ,, ...	0·2 }	0·1 }		
3	100 c.c. mannite solution	6·5	4·1	6·3 ⎫	4·0 ⎫
4	,, ,, ,,	6·1	4·3	5·9 } 6·0	4·2 } 4·3
5	,, ,, ,,	6·2	4·4	6·0	4·3
6	,, ,, ,,	5·9	4·7	5·7 ⎭	4·6 ⎭
7	Control	10·4 } 10·5	10·3 } 10·3		
8	,, ...	10·6 }	10·3 }		
9	100 c.c. mannite solution + water extract of 0·5 grm. bacterised peat	24·8	26·3	14·3 ⎫	16·0 ⎫
10	,, ,, ,,	25·5	25·6	15·0 } 14·5	15·3 } 15·8
11	,, ,, ,,	25·1	26·2	14·6	15·9
12	,, ,, ,,	24·7	26·3	14·2 ⎭	16·0 ⎭
13	Control	2·1 } 2·2	2·2 } 2·1		
14	,, ...	2·3 }	2·0 }		
15	100 c.c. mannite solution + alcoholic extract of 1 grm. bacterised peat	15·1	17·9	13·9 ⎫	15·8 ⎫
16	,, ,, ,,	16·4	17·7	14·2 } 14·1	15·6 } 15·6
17	,, ,, ,,	16·6	18·1	14·4	16·0
18	,, ,, ,,	16·0	17·3	13·8 ⎭	15·2 ⎭
19	Control	0·4 } 0·4	0·3 } 0·3		
20	,, ...	0·4 }	0·3 }		
21	100 c.c. mannite solution + phosphotungstic fraction of 1 grm. bacterised peat	10·6	10·1	10·2 ⎫	9·8 ⎫
22	,, ,, ,,	10·3	9·9	9·9 } 10·2	9·6 } 9·6
23	,, ,, ,,	10·7	9·5	10·3	9·2
24	,, ,, ,,	10·8	10·2	10·4 ⎭	9·9 ⎭
25	Control	0·2 } 0·2	0·2 } 0·2		
26	,, ...	0·2 }	0·2 }		
27	100 c.c. mannite solution + silver fraction of 1 grm. bacterised peat	10·8	10·0	10·6 ⎫	9·8 ⎫
28	,, ,, ,,	11·0	10·2	10·8 } 10·4	10·0 } 10·0
29	,, ,, ,,	10·1	10·3	9·9	10·1
30	,, ,, ,,	10·5	10·4	10·3 ⎭	10·2 ⎭

effect of the auximones on all these varieties was tested by cultivating them in a solution consisting of 100 c.c. distilled water, 1 grm. sucrose, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$. Similar results to those shown with *Azotobacter* were obtained with every variety of the organism tested. The following mean fixations obtained with broad bean

organisms, from six series each consisting of six flasks, are typical of the results with *Bacillus radicicola* :—

	Mgrm.
Sucrose solution	2·6
„ „ + water extract bacterised peat	7·7
„ „ + alcoholic extract bacterised peat	6·5
„ „ + phosphotungstic fraction bacterised peat ...	5·6
„ „ + silver fraction bacterised peat	6·0
„ „ + water extract raw peat	1·9

The difference in the density of the cultures containing the fractions from bacterised peat, and those containing only sucrose, or sucrose with raw peat, was very marked throughout the experiments, and afforded a very sure indication of the greater activity of the organisms in those cultures containing the auximone fractions.

Both from these experiments and from those with *Azotobacter*, it is evident that certain organic substances, the nature of which has not yet been investigated, can be separated from the prepared peat, and that these substances have an appreciable effect upon the rate of nitrogen fixation by these organisms.

Nitrification.

Experiments on the second group of bacteria concerned in the nitrogen cycle, that is, the nitrifying organisms, were also carried out in soil and in liquid culture. Ever since the isolation, in purely inorganic media, by Winogradsky,* of the particular organisms concerned, the effect of organic matter upon the rate of nitrification has provoked a considerable amount of discussion. Winogradsky* himself stated that the presence of nitrogenous organic matter is inhibitory to the organisms, but further investigation by Müntz and Lainé† revealed the fact that humus in soil has no deleterious effect upon the process. Very soon Coleman‡ showed that organic matter is injurious only in culture solutions, and subsequent research by Stevens and Withers§ fully confirmed this statement, and proved that in soils the presence of organic matter may even help the process. Karpinski and Niklewski‖ have further stated that the presence of small amounts of some

* Winogradsky, 'Ann. de l'Inst. Pasteur,' vol. 4, 1. Mem., pp. 213–231 ; 2. Mem., pp. 257–275 ; 3. Mem., pp. 760–771 (1890).

† Müntz and Lainé, 'Compt. Rend.,' vol. 142, pp. 430–435 (1906).

‡ Coleman, 'Centr. Bakt. Par.,' Abt. II, vol. 20, pp. 401–420 (1908).

§ Stevens and Withers, 'Centr. Bakt. Par.,' Abt. II, vol. 27, pp. 169–186 (1910).

‖ Karpinski and Niklewski, 'Bull. Acad. Sci. Cracovie,' 1907, pp. 596–615.

organic substances is favourable to nitrification in impure culture, and that especially good effects are produced by humates in very small amounts.

In order to examine the effect of bacterised peat upon nitrification in soil a preliminary experiment was carried out upon a rich garden soil from Kew. Four portions of soil, each weighing 24 oz., were taken, and each of two of them was mixed with one part in ten by volume of bacterised peat. Two portions of another soil were also taken, one being treated and the other serving as control. Three samples of each were weighed out for nitrate determination, and the six soils were kept in wide-mouthed glass bottles at about 20° C. as before. They were aërated daily, and the moisture content kept as uniform as possible. At intervals they were all examined for nitrate, the determinations being made by the phenol-sulphonic acid method. The figures obtained by this method were very concordant, and at the outset a comparison was made between the results thus obtained and those given by the method of reduction by sodium amalgam, the ammonia being separately determined when the latter process was adopted. A close agreement was shown between the figures, and the more cumbersome sodium amalgam method was therefore discarded.

When dealing with solutions containing the water extract of the peat, the deep colour could readily be discharged by precipitating the humic acid with a drop of dilute hydrochloric acid and shaking the filtrate with carbon black (D. Elf brand). The colourless liquid thus obtained was then analysed, and concordant results obtained by this method of procedure.

The figures obtained at intervals from the above soils were:—

Table VI.

	Nitrate content (parts per million of nitric nitrogen—mean of three determinations).				
	Originally.	After 7 days.	14 days.	21 days.	28 days.
1. Soil A alone	30	43	57	107	208
2. „ „	31	45	56	112	200
3. Soil B alone	35	45	81	95	99
4. Soil A + bacterised peat	27	87	450	540	539
5. „ „ „	29	91	439	528	541
6. Soil B + bacterised peat ...	30	156	248	319	372

Each of the figures in the above and following Tables of soil analyses represents the mean of three determinations, the maximum deviation from the mean being ± 5.

The fact that the increase in nitrate content in the soils A containing

bacterised peat in the above Table had practically ceased during the last week, suggested that either the nitrate had accumulated to such an extent that it had become inhibitory to the organisms concerned, or that the whole of the available nitrogen had been nitrified.

In order to test this, each of the two soils containing bacterised peat was placed on a filter paper in a large Buchner funnel fitted into a vacuum flask. The soils were covered with distilled water, and the flasks exhausted as rapidly as possible by means of a pump. They were then dried down to a suitable moisture content as rapidly as possible at a low temperature by means of a fan, and were re-incubated after samples had been taken for analysis. By this time two days had elapsed since the sampling on the 28th day, and during this time the control soils had been incubated as usual. These soils were not re-examined on the 30th day, but in the Table below the figures are given as for the 28th day. The subsequent results obtained were:—

Table VII.

	Nitrate content (parts per million—mean of three determinations).			
	After 30 days.	35 days.	42 days.	49 days.
1. Soil alone	208	222	234	177
2. „ „	200	230	179	175
3. Soil + bacterised peat ...	204	230	298	337
4. „ „ „ ...	189	199	310	350

These results indicated that the addition of bacterised peat to a fertile soil results in a rapid increase in nitrate content up to a maximum, when the concentration of nitrate becomes inhibitory to the growth of the organisms, and nitrification ceases. If this accumulation be partially removed by rapid washing, nitrification again proceeds.

As in the case of nitrogen fixation, nitrification is essentially an aërobic process, and may have been facilitated by the better aëration of the soil, so a comparison was made of the effect of introducing similar bulky matter in the form of raw peat and stable manure. An examination was also made of the effect of chemically produced soluble humate in the form of peat which had been treated with 2 per cent. of its weight of sodium carbonate. The soil chosen for this experiment was a fertile soil from Chelsea Physic Garden, and the organic manures were added in the proportion of one part in ten by bulk. The soils containing raw peat also received a dressing of 1 per cent. of powdered chalk, in order to counteract as far as possible any inhibitory

effect due to the natural acidity of the peat. The various soils were incubated precisely as before, and at the end of 28 days it was found that the nitrate-content of the soil containing bacterised peat had approached very near to the maximum quantity shown in the previous experiment. These two samples of soil were accordingly leached with distilled water, as described above, to remove the accumulations of nitrate. The nitric nitrogen which they contained was then estimated before they were re-incubated, and the figures obtained are shown in the following Table :—

Table VIII.

	Nitrate content (parts per million—mean of three determinations).						
	Originally.	After 7 days.	21 days.	28 days.	35 days.	47 days.	54 days.
1. Soil alone	70	72	86	92	105	136	156
2. „ „	67	68	86	95	112	140	163
3. Soil + raw peat	66	73	77	84	79	81	59
4. „ „	64	76	80	89	80	80	66
5. Soil + carbonated peat	61	81	98	108	136	143	173
6. „ „ „	67	84	92	99	142	167	186
7. Soil + bacterised peat	59	90	356	460 leached = 37	94	158	195
8. „ „ „	60	110	380	450 leached = 62	101	174	213
9. Soil + stable manure ...	65	65	71	78	89	101	128
10. „ „ „	60	64	80	85	89	109	116

It is evident from the results so far given that the addition of bacterised peat to the soil results in an enormous accumulation of nitrate, which would naturally be removed fairly rapidly by any growing crops. The figures in the above Table also indicate that the introduction of the chemically produced humate results in a slight increase in nitrate production, while the addition of opening material in the form of raw peat and stable manure tends rather to depreciate than increase the rate of nitrification in the soil. The causes of this depreciation were not further investigated, since it had no particular bearing upon the work.

There is one other important factor which must be taken into consideration in connection with the rapid nitrification in soils containing bacterised peat. It is well known that this material contains a certain amount of soluble nitrogen in the form of ammonium humate, and the question arises as to whether this ammonia is merely being nitrified at a normal rate, or whether any stimulation of the soil organisms is taking place in addition.

In order to put this to the test, an estimation was made of the soluble nitrogen in bacterised peat, and a comparison was then made between the rate of nitrification in soil containing one-tenth of its volume of this material and in that containing an amount of ammonia equivalent to the soluble nitrogen thus introduced, in the form of ammonium sulphate. Portions consisting of 800 grm. of a fresh sample of Chelsea soil were used, and one-tenth of the volume of this of bacterised peat weighed 32 grm., containing 40 per cent. of moisture, the actual dry weight of the material introduced into the soil being thus equal to 19·2 grm. This contained 1·8 per cent. of soluble nitrogen as ammonia, so that the soluble ammonia introduced was equivalent to 1·63 grm. ammonium sulphate, and this addition was made to each of two portions of soil. All the soils were incubated just as before, and the results obtained were:—

Table IX.

	Nitrate content (parts per million—mean of three determinations).				
	Originally.	After 7 days.	14 days.	35 days.	49 days.
1. Soil alone	25	28	42	78	104
2. „ „	27	29	40	82	110
3. Soil + $(NH_4)_2SO_4$	26	45	67	195	276
4. „ „	25	49	75	190	259
5. Soil + bacterised peat	20	54	210	480	524
6. „ „ „	22	61	201	468	541

It is evident that the rate of nitrification in soil containing bacterised peat is greater than that in soil containing an equivalent quantity of soluble ammonia in the form considered most suitable for nitrification; so that it appears probable that, apart from supplying nitrifiable nitrogen, the addition of bacterised peat increases the activities of the soil organisms. That there is some factor in this material which has a beneficial effect upon nitrification in soils, apart from any nitrogen it contains, is shown by the effect of the phosphotungstic fraction upon nitrate formation. Attention has already been drawn to this effect by Bottomley,* but fuller experiments have since been made. To various uniform samples of the same soil were added the phosphotungstic fraction obtained from amounts of bacterised peat equivalent to (*a*) one-tenth the volume of soil, (*b*) one-fifth the volume of soil, (*c*) one-sixteenth the volume of soil, and (*d*) one-eighth the volume of soil

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 102–108 (1915).

respectively. The proportion of one part of bacterised peat in ten of soil is that used practically throughout the experiment, but it was anticipated that, since in all probability by no means the whole of the active substance is separated by the phosphotungstic acid method, an addition of this fraction equivalent to one part of peat in ten might possibly be too small to produce the maximum effect. For this reason, experiments were made with the phosphotungstic fraction from double the amount of peat normally used, and also from proportions of one-eighth and one-sixteenth the volume of soil. The soils, incubated and sampled from time to time, gave the following results :—

Table X.

	Nitrate content (parts per million—mean of three determinations).			
	Originally.	After 15 days.	29 days.	40 days.
1. Soil alone	31	50	88	112
2. „ „	35	48	96	120
3. Soil + phosphotungstic fraction from 1 in 10	36	84	220	320
4. „ „ „ „ 1 in 10	33	90	211	309
5. „ „ „ „ 1 in 5 ...	34	112	304	462
6. „ „ „ „ 1 in 5 ...	36	105	302	448
7. „ „ „ „ 1 in 8 ...	35	83	232	346
8. „ „ „ „ 1 in 8 ...	31	85	241	335
9. „ „ „ „ 1 in 16	32	49	130	198
10. „ „ „ „ 1 in 16	31	53	141	205

The addition, in sufficient quantity, of the phosphotungstic fraction thus appears to increase the rate of nitrification in soils. It has, however, been shown above that such addition also increases the nitrogen fixation, and the possibility arises that the increase in nitrate content may be due to the nitrification of the nitrogen fixed rather than to direct action upon the nitrifying organisms themselves.

This point could only be elucidated by examining the effect of the auximone fractions upon nitrification in liquid culture. Accordingly, a crude nitrifying culture was obtained by inoculating some good garden soil into Erlenmeyer flasks, each containing 100 c.c. of the following medium :— Tap water, 100 c.c.; ammonium sulphate, 0·1 grm.; dipotassium phosphate, 0·1 grm.; basic magnesium carbonate, 0·2 grm.; and incubating at 26° C. for seven days. This was sub-cultured three times before any attempt was made to experiment with the auximone fractions, but then it was found that the addition of the phosphotungstic or silver fraction to the culture resulted in the formation of the thick scum of some foreign organisms already described

by Bottomley,* and no nitrate was formed in the medium. It was only after long-continued sub-culturing that a nitrifying culture was obtained free from these organisms, and the experiments recorded were carried out with this comparatively pure culture.

A preliminary experiment showed that the water extract of bacterised peat is directly nitrifiable, hence the effect of this extract upon the rate of nitrification in normal nitrifying solution cannot be tested. An experiment was therefore made to determine whether the auximone fractions had any effect upon the rate of nitrification in ammonium sulphate solution. Portions of 100 c.c. of Winogradsky's medium, described above, were put into each of 24 flasks, divided into four series of six each, with the additions shown in the Table below. When all had been inoculated, and two of each series sterilised for controls, the whole set was incubated at 26° C. for seven days. The nitrate content of 25 c.c. of each was then estimated as before, with the following results :—

Table XI.

Flask.	Contents.	Nitric nitrogen in 25 c.c.	Nitric nitrogen in whole.	
		mgrm.	mgrm.	
1	Control	0·01	0·04	0·038
2	,,	0·009	0·036	
3	100 c.c. Winogradsky's medium	0·18	0·72	0·77
4	,, ,, ,,	0·21	0·84	
5	,, ,, ,,	0·20	0·80	
6	,, ,, ,,	0·18	0·72	
7	Control	0·01	0·04	0·04
8	,,	0·01	0·04	
9	100 c.c. Winogradsky's medium + alcoholic extract of 1 grm. bacterised peat	0·31	1·24	1·16
10	,, ,, ,, ,,	0·30	1·20	
11	,, ,, ,, ,,	0·27	1·08	
12	,, ,, ,, ,,	0·28	1·12	
13	Control	0·009	0·036	0·036
14	,,	0·009	0·036	
15	100 c.c. Winogradsky's medium + phosphotungstic fraction of 1 grm. bacterised peat	0·27	1·08	1·14
16	,, ,, ,, ,,	0·31	1·24	
17	,, ,, ,, ,,	0·27	1·08	
18	,, ,, ,, ,,	0·29	1·16	
19	Control	0·01	0·04	0·04
20	,,	0·01	0·04	
21	100 c.c. Winogradsky's medium + silver fraction of 1 grm. bacterised peat	0·34	1·36	1·44
22	,, ,, ,, ,,	0·36	1·44	
23	,, ,, ,, ,,	0·39	1·56	
24	,, ,, ,, ,,	0·35	1·40	

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 102–108 (1915).

It is apparent from these figures that the auximone fractions have the power of increasing the rate of nitrification in culture solution. If organic matter in general has the effect of depressing nitrification, the additions made in these fractions are evidently too slight to produce any such result. However, since the water extract of bacterised peat, a highly organic solution, nitrifies so readily, an experiment was made to compare the rate of nitrification in Winogradsky's solution with that in bacterised peat extract containing an equivalent quantity of nitrogen, and also with nitrification in a solution of ammonium humate containing an equal quantity of nitrogen. The humic acid for the ammonium humate was extracted from raw peat in the usual manner, purified by re-dissolving in ammonium hydrate, and re-precipitating with hydrochloric acid twice over, finally dissolving up in excess of ammonia, and then expelling the excess by evaporation on the water-bath. The ammonium humate thus obtained was dissolved in distilled water and the ammonia in an aliquot part determined. The concentrated solution was then diluted until it contained the same proportion of ammonia as Winogradsky's medium. The requisite amounts of potassium phosphate and magnesium carbonate were added to the flasks containing this solution. The bacterised peat extract was prepared by making a concentrated extract, determining the nitrogen it contained, and diluting until it contained the same proportion as Winogradsky's medium and the ammonium humate, the phosphate and base being added as before. A series of 30 flasks was prepared, as shown in the Table below, and after inoculation and incubation for seven days 25 c.c. from each flask was analysed for nitric nitrogen. The whole set was then re-incubated for a further period of seven days, when 10 c.c. of each was again examined. The figures obtained are given in Table XII (p. 526).

Although the greater part of the nitrogen in the water extract of bacterised peat occurs in the form of ammonium humate, it appears from the above figures that this substance, in the pure condition, is comparatively slowly nitrifiable, and even the addition of auximones, which increases the rate of nitrification of the ammonium sulphate to an appreciable extent, does not render that of ammonium humate equal to that of ammonium sulphate alone. This is probably due to the depressing effect of the organic matter, although the readily nitrifiable bacterised peat extract contains a similar quantity. It follows that, in this extract, there must be some other factor to be taken into consideration besides the ammonia content and the auximone fraction thus far isolated.

It appears most probable, from all the results obtained, that the methods

employed fail to extract the total amount of the activating substances present in the bacterised peat, although they show that the material separated in the various fractions has the effect of appreciably increasing the rate of nitrification as well as nitrogen fixation.

Table XII.

Flask.	Contents.	7 days.		14 days.	
		Nitric nitrogen in 25 c.c.	Equivalent to nitric nitrogen in whole.	Nitric nitrogen in 10 c.c.	Equivalent to nitric nitrogen in whole.
		mgrm.	mgrm.	mgrm.	mgrm.
1	Control	0·01	0·04 } 0·04	0·005	0·05 } 0·05
2	,,	0·01	0·04 }	0·005	0·05 }
3	100 c.c. Winogradsky's medium	0·18	0·72 }	0·51	5·1 }
4	,, ,, ,,	0·19	0·76 } 0·72	0·55	5·5 } 5·3
5	,, ,, ,,	0·18	0·72 }	0·54	5·4 }
6	,, ,, ,,	0·17	0·68 }	0·52	5·2 }
7	Control	0·01	0·04 } 0·04	0·004	0·04 } 0·045
8	,,	0·01	0·04 }	0·005	0·05 }
9	100 c.c. Winogradsky's medium + silver fraction from 1 grm. bacterised peat	0·31	1·24 }	0·75	7·5 }
10	,, ,, ,,	0·30	1·20 } 1·17	0·77	7·7 } 7·6
11	,, ,, ,,	0·28	1·12 }	0·79	7·9 }
12	,, ,, ,,	0·28	1·12 }	0·73	7·3 }
13	Control	0·006	0·024 } 0·028	0·003	0·03 } 0·03
14	,,	0·008	0·032 }	0·003	0·03 }
15	100 c.c. ammonium humate solution	0·11	0·44 }	0·25	2·5 }
16	,, ,, ,,	0·12	0·48 } 0·45	0·22	2·2 } 2·4
17	,, ,, ,,	0·12	0·48 }	0·22	2·2 }
18	,, ,, ,,	0·10	0·40 }	0·26	2·6 }
19	Control	0·006	0·024 } 0·024	0·003	0·03 } 0·025
20	,,	0·006	0·024 }	0·002	0·02 }
21	100 c.c. ammonium humate solution + silver fraction from 1 grm. bacterised peat	0·16	0·64 }	0·37	3·7 }
22	,, ,, ,,	0·15	0·60 } 0·65	0·35	3·5 } 3·4
23	,, ,, ,,	0·18	0·72 }	0·36	3·6 }
24	,, ,, ,,	0·16	0·64 }	0·30	3·0 }
25	Control	0·016	0·064 } 0·056	0·007	0·07 } 0·06
26	,,	0·012	0·048 }	0·005	0·05 }
27	100 c.c. bacterised peat extract	0·35	1·40 }	0·79	7·9 }
28	,, ,, ,,	0·35	1·40 } 1·42	0·81	8·1 } 7·8
29	,, ,, ,,	0·38	1·52 }	0·78	7·8 }
30	,, ,, ,,	0·34	1·36 }	0·76	7·6 }

Ammonification.

The cycle of changes which nitrogenous substances undergo in the soil is a complex one, and in marked contrast to the two processes investigated above

are the two chief decomposition processes, ammonification and denitrification. These result in the breaking down of the soil organic matter and nitrates, with the liberation in the form of ammonia and free gaseous nitrogen of the element which has been "fixed" and oxidised in the two processes of nitrogen fixation and nitrification. As these two decomposition processes involve reactions which are directly opposed to those concerned in the two already considered, it was thought possible that an investigation of the effect of auximones upon the bacteria concerned in them would give some indication as to whether the auximones merely stimulate all classes of bacteria equally, or whether they play some definite part in the building up of the nitrogenous molecule.

It was practically impossible to carry out experiments on ammonification in a soil which had been mixed with bacterised peat, in the same way as had been done for nitrogen fixation and nitrification, on account of the ammonia content of the bacterised peat and the rapid nitrate formation and other changes in the nitrogen compounds in such a mixture. The only alternative was to depend upon the results obtained in liquid culture, and here again, on account of the ammonia contained in the water extract of bacterised peat, it became difficult to test the effect of this extract upon the process of ammonification. It appears, however, from the results obtained in the experiments recorded above, that the auximone fractions are largely responsible for the increased activity of the bacteria hitherto investigated; hence tests were made of the effect upon the ammonifying organisms of the alcoholic, phosphotungstic and silver fractions alone.

An investigation was first made of the influence of the addition of these fractions upon the "ammonifying" or "putrefactive" power of the soils used in the previous experiments, this putrefactive power being determined by the method in general use described by Remy.*

Sixteen flasks were prepared, each containing 100 c.c. of 1 per cent. peptone solution and 10 grm. of fine air-dried Chelsea soil. To four of these was added the alcoholic extract, to another four the phosphotungstic fraction, and to a third four the silver fraction, of 1 grm. of bacterised peat. The whole set was incubated at 22° C. for five days, then 2 grm. of calcined magnesia and a few drops of paraffin (to prevent frothing) were added to each. The contents of each flask were distilled, and the distillate received in decinormal sulphuric acid, which was then titrated with decinormal sodium hydrate solution. The figures obtained were:—

* Remy, 'Centr. Bakt. Par.,' Abt. II, vol. 8, pp. 657–662 (1902).

Table XIII.

Flask.	Contents.	Acid neutralised.	NH_3 present.	
		c.c.	mgrm.	
1	Soil + 1 per cent. peptone	35·0	59·50	60·60
2	,, ,, ,, ...	36·1	61·37	
3	,, ,, ,, ...	35·5	60·35	
4	,, ,, ,, ...	36·0	61·20	
5	Soil + 1 per cent. peptone + alcoholic extract of 1 grm. bacterised peat	35·9	61·03	61·11
6	,, ,, ,, ,,	35·7	60·69	
7	,, ,, ,, ,,	36·0	61·20	
8	,, ,, ,, ,,	36·2	61·54	
9	Soil + 1 per cent. peptone + phosphotungstic fraction from 1 grm. bacterised peat	36·2	61·54	61·16
10	,, ,, ,, ,,	36·3	61·71	
11	,, ,, ,, ,,	35·8	60·86	
12	,, ,, ,, ,,	35·6	60·52	
13	Soil + 1 per cent. peptone + silver fraction from 1 grm. bacterised peat	36·1	61·37	60·94
14	,, ,, ,, ,,	35·8	60·86	
15	,, ,, ,, ,,	35·6	60·52	
16	,, ,, ,, ,,	35·9	61·03	

An identical series of experiments was carried out with each of the soils obtained from Kew, and these yielded similar results, all failing to show any effect of the auximones, stimulating or otherwise, upon the ammonifying power of the soils. In view of these results, an examination was made of the influence of these substances upon the ammoniacal fermentation of urea.

For this purpose, a mixed culture of ammonifying organisms was obtained from rotting manure by inoculating the latter into a culture medium consisting of Witte's peptone 1 grm., urea 10 grm., Lemco 5 grm., distilled water 100 c.c., the whole being neutralised with ammonium carbonate solution. A drop of this mixed culture was then inoculated into a solution of urea 50 grm., mono-potassium phosphate 25 grm., sodium acetate 10 grm., distilled water 1000 c.c., this solution also being just neutralised with ammonium carbonate solution. The culture thus obtained was sub-cultured three successive times into flasks of the same medium, three days' incubation elapsing between each sub-culture. Sixteen flasks, each containing 100 c.c. of the same medium, with the additions shown in the Table below, were then inoculated each with 1 c.c. of the culture of urea-splitting organisms thus obtained. The whole set was incubated at 22° C., 10 c.c. of each being withdrawn after periods of 24 and 48 hours respectively, and titrated with decinormal sulphuric acid, methyl orange being used as indicator. The figures obtained were as follows :—

Table XIV.

Flask.	Contents.	In 24 hours, 10 c.c. required		In 48 hours, 10 c.c. required		
		c.c. acid =	mgrm. NH_3.	c.c. acid =	mgrm. NH_3.	
1	100 c.c. ammonifying culture	22·5	38·25	22·2	37·74	38·33
2	,, ,, ,, ...	22·0	37·40	23·0	39·10	
3	,, ,, ,, ...	22·4	38·18	22·5	38·25	
4	,, ,, ,, ...	22·5	38·25	22·5	38·25	
5	100 c.c. ammonifying culture + alcoholic extract of 1 grm. bacterised peat	21·8	37·06	22·3	37·91	38·13
6	,, ,, ,,	22·4	38·18	22·4	38·18	
7	,, ,, ,,	22·5	38·25	22·5	38·25	
8	,, ,, ,,	22·0	37·40	22·4	38·18	
9	100 c.c. ammonifying culture + phosphotungstic fraction of 1 grm. bacterised peat	21·8	37·06	22·5	38·25	38·33
10	,, ,, ,,	22·0	37·40	22·8	38·76	
11	,, ,, ,,	22·0	37·40	22·3	37·91	
12	,, ,, ,,	22·3	37·91	22·6	38·42	
13	100 c.c. ammonifying culture + silver fraction of 1 grm. bacterised peat	21·8	37·06	22·4	38·18	37·83
14	,, ,, ,,	22·2	37·74	22·4	38·18	
15	,, ,, ,,	21·9	37·23	21·6	36·72	
16	,, ,, ,,	22·0	37·40	22·5	38·25	

These experiments were repeated over and over again, examinations being made of the ammonia produced at the end of periods varying from 6 to 96 hours, but always with similar results. No effect whatever was observed upon the rate of ammonia production, and, in view of the increased activities of the nitrogen-fixing and nitrifying organisms following upon the addition of the auximones to their culture solutions, these results were at first surprising. However, it should be pointed out that these substances are produced in the peat during its "bacterisation," which results in the formation of a certain quantity of ammonium humate, and that they are therefore, at least partially, the products of a bacterial action somewhat similar in nature to the ammonifying process itself. It is scarcely to be expected therefore that the activities of the organisms would be affected by substances bearing a close relation to their own products, unless, as in the case of the nitrifying bacteria, these products had accumulated in such amounts as to bring about an inhibitory effect.

The auximone fractions, however, as has been shown above, have the effect of increasing the rate of nitrification in soil and in culture solution, yet they have no effect upon the rate of ammonification. These results appear to be

directly opposed to the statement by Russell* that "a measure of the speed at which nitrates are formed does not measure the rate of nitrification, but the rate of ammonia production." If this statement that the oxidation of ammonia to nitrates is normally proceeding as fast as ammonia is being formed be true, then the possible sources of nitrifiable nitrogen must be considered, for the auximones themselves introduce only a negligible quantity. There is the possibility that the addition of the auximones may have an effect upon ammonification in soil widely different from that in liquid culture, owing to divergence of conditions; but, apart from this consideration, the fixation of nitrogen introduces an appreciable quantity of this element into the soil, probably in a nitrifiable form. Thus the increase in nitrate content following upon the addition of the auximone fractions to the soil is probably partly accounted for by the nitrification of the element introduced by the nitrogen-fixing organisms, whose activities are also shown to be increased by this addition. Experiments in pure culture, however, show that in the presence of sufficient quantities of nitrifiable nitrogen, the activities of the nitrifying organisms are increased beyond their normal rate by the addition of auximones.

Denitrification.

For the purpose of investigating the effect of the auximone fractions of bacterised peat upon denitrification, methods of liquid culture were again employed. The bacteria which are concerned in this process are responsible for the loss of nitrogen which often follows upon the addition of decomposing organic manures to soil containing nitrates. Since the nitrogen is liberated in the free gaseous form, an estimation of the activity of the organisms can be made approximately by measuring the volume of gas to which they give rise.

For the isolation of these organisms in impure culture, Giltay's solution is most generally employed, but equally good and very uniform results have been obtained during the present work with a medium consisting of calcium tartrate 10 grm., potassium nitrate 10 grm., di-potassium phosphate 0·25 grm., and tap-water 500 c.c. This has the advantage of being simple and very readily made up, so it was used throughout the following experiments.

In order to obtain a culture of the organisms, small portions of decomposing stable manure were introduced into Erlenmeyer flasks of 150 c.c. capacity. The flasks were then filled to the brim with the above medium,

* Russell, E. J., 'Soil Conditions and Plant Growth,' 1915, p. 88.

and each was closed by a well-fitting cork, through which passed a glass tube, reaching almost to the bottom of the flask, and bent at an angle of about 60° just above the cork. The corks were all coated with melted paraffin to render them air-tight. The process of denitrification is an anaërobic one, and the inert gases collected at the top of the flasks, forcing the liquid out through the bent tube and maintaining the anaërobic conditions.

When a crude culture had been obtained in this way, it was sub-cultured four successive times into fresh media before being used for purposes of experiment, and a comparatively pure culture of mixed denitrifying organisms was thus obtained; 1 c.c. of this culture was then transferred to each of the 20 flasks shown in the Table below, and after filling with their respective solutions these flasks were all corked, great care being taken that none of the medium should be spilt, and at the same time that all air bubbles should be excluded.

After 36 hours' incubation at 22° C., the corks were carefully removed, and the volume of gas which had collected in each was measured approximately by filling each flask again with water from a burette.

The results obtained were :—

Table XV.

Flask.	Contents.	Gas formed in 36 hours.	
		c.c.	
1	Culture solution	49	
2	,, ,, ...	58	54
3	,, ,, ...	54	
4	,, ,, ...	55	
5	Culture solution + water extract of 0·5 grm. bacterised peat	54	
6	,, ,, ,, ,, ,, ...	56	54
7	,, ,, ,, ,, ,, ...	50	
8	,, ,, ,, ,, ,, ...	57	
9	Culture solution + alcoholic extract of 1 grm. bacterised peat.....	4·0	
10	,, ,, ,, ,, ,, ...	6·0	5·2
11	,, ,, ,, ,, ,, ...	6·5	
12	,, ,, ,, ,, ,, ...	4·5	
13	Culture solution + phosphotungstic fraction of 1 grm. bacterised peat	3·0	
14	,, ,, ,, ,, ,, ,, ...	2·5	3·0
15	,, ,, ,, ,, ,, ,, ...	4·0	
16	,, ,, ,, ,, ,, ,, ...	2·5	
17	Culture solution + silver fraction of 1 grm. bacterised peat.....	29	
18	,, ,, ,, ,, ,, ...	27	30
19	,, ,, ,, ,, ,, ...	33	
20	,, ,, ,, ,, ,, ...	30	

This experiment was repeated several times, always with similar results. At the conclusion of one of these repetitions 1 c.c. of the culture was extracted from each of the flasks and diluted to 100 c.c., and 1 c.c. of each of these was again taken and diluted to 100 c.c. A further similar dilution was made, and then 1 c.c. of each of the 20 equally diluted cultures was inoculated into a sterilised tube of the denitrifying medium, containing 1 per cent. of agar-agar. The inoculation was performed just before the media solidified, the tubes being well shaken to ensure distribution of the organisms. When this was accomplished, they were plugged with cotton wool and incubated at 22° C. The number of bubbles of gas formed in the solid media after a given time indicated the number of colonies in the tubes, and these numbers were practically equal in the tubes inoculated from the pure culture medium and in those from the water extract, very much fewer in number in those from the alcoholic and phosphotungstic fractions, these being again practically equal between themselves, while those from the silver fraction were intermediate in number between these two sets. These numbers bear out the figures obtained above, for it is to be expected that the number of bacteria should be proportional to the activity of the culture.

Similar results having been uniformly obtained in all experiments with a comparatively pure culture, the effect of the addition of the auximones upon the denitrifying power of the soils themselves was examined. The soils from Kew and Chelsea used in the above experiments were investigated, and the culture flasks were prepared precisely as before. Instead of being inoculated with a definite quantity of a pure culture, however, each was inoculated with 5 grm. of a finely sifted uniform sample of the soil to be tested, and was then incubated in the usual manner. The results obtained were concordant throughout with the figures given for the pure culture, a typical set of mean results, obtained with a Chelsea soil, being as follows:—

	Gas formed in 48 hours. c.c.
Culture solution—	
+ soil	63·0
+ soil + water extract 0·5 grm. bacterised peat	64·0
+ soil + alcoholic extract 1 grm. bacterised peat	9·5
+ soil + phosphotungstic fraction 1 grm. bacterised peat	8·1
+ soil + silver fraction 1 grm. bacterised peat	31·0

From the results obtained it appears evident that while the water extract of bacterised peat is practically without effect upon denitrification, the

auximone fractions definitely inhibit the process to a marked degree. Since the auximones are water-soluble, it might be expected that the water extract would also depress the rate of denitrification. However, the presence of some readily oxidisable organic matter is necessary in order that denitrification may proceed, and the medium used in these experiments contains such in the form of tartrate. In addition to this, the water extract supplies extra organic matter, and although the humus of the soil had been found by Stoklasa and Ernest* to be not very serviceable for denitrification, it is quite probable that such an addition might increase the rate of denitrification to a degree sufficient to counterbalance the depressing effect of the auximones contained.

From all the evidence collected in the present work it is apparent that soluble humus, and especially that produced by bacterial decomposition, is a very important factor from the point of view of the activities of soil bacteria. Its effect upon the organisms appears to be largely independent of any inorganic matter which it may contain, or any physical action brought about by its colloidal nature, and is shown to be due to the presence in the humus of growth-promoting substances or auximones. The influence of these auximones upon the organisms concerned in the nitrogen cycle may be briefly summed up in the general statement that they increase the rate of nitrogen fixation and nitrification, depress the rate of denitrification, and do not appreciably affect the rate of ammonification. These results are interesting from the indication they give of the specific rôle of the auximones. If these substances merely act as stimulants to the bacterial protoplasm, it is to be expected that similar effects would be produced by them upon all classes of bacteria. If, on the other hand, they play some definite part in the building up of the complex nitrogenous molecule, it follows that a directly opposite effect might be anticipated from the addition of these substances to two classes of bacteria whose activities are directed upon such widely divergent lines as those concerned in the constructive processes resulting in the oxidation and the fixation of nitrogen in an organic form, on the one hand, and those destructive organisms which bring about its decomposition and liberation in the form of the free element on the other.

In conclusion, I wish to express my sincere thanks to Prof. W. B. Bottomley for the valuable advice and help which he has so kindly given me during the progress of this work.

* Stoklasa and Ernest, 'Centr. Bakt. Par.,' Abt. II, vol. 17, pp. 27–33 (1907).