THE OXYGEN REQUIREMENTS OF BIOLOGICAL SOIL PROCESSES[1]

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The aim of this work was to find out whether the fundamental processes carried on by soil bacteria proceed better under aerobic or anaerobic conditions.

The work was carried out with three soils, first a greenhouse loam soil, rich in organic matter, second a field soil from the experiment station plats,—a Hagerstown silt loam, and third, a clay soil—a Hagerstown clay taken from the side of a hill sloping down to a brook. These were selected to represent three different types of soil and to secure different flora and different conditions of microbic development.

The biological processes in soil are influenced according to Lipman (1911) by moisture, temperature, aeration, reaction, and food supply. It seems to the writer that the relation of oxygen to the fundamental soil processes has not been thoroughly investigated; and that it has not been fully established—taking soil or synthetic solutions as media—whether nitrogen fixation, nitrification, ammonification and denitrification will go on under aerobic or anaerobic conditions only, and whether these processes will take place better in the presence or in the absence of air.

Preliminary experiments showed that ammonification, denitrification, and nitrogen fixation took place readily with or without air. Nitrification on the other hand would not take place under anaerobic conditions, either in soils or in solution in the preliminary or subsequent experiments. The results of the work on nitrification are therefore not included in this paper.

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AMMONIFICATION

Kelley (1915) found that anaerobic conditions greatly retard the formation of ammonia from all materials except casein and under anaerobic conditions the formation of ammonia has usually been found to be considerably less than under aerobic conditions. Aeration (Kelley, McGeorge and Thompson, 1915) stimulates ammonification but is not essential to the process as it is to nitrification.

Lohnis and Green (1913) found that aeration is of great importance in the ammonification of organic matter. "The most significant cause of variation appears to be that of aeration. Ammonification as a whole proceeds much more rapidly under aerobic than under anaerobic conditions, but it is believed that aerobic conditions favor more specifically those latter stages in the breakdown, which result in the formation of ammonia itself." Later (Lohnis and Green, 1914) they report that while aeration is not of preponderating importance ammonification of such substances as flesh meal, bone meal and blood meal proceeds better under aerobic than anaerobic conditions.

In my experiments the process of ammonification was tested both in soils and in solution. One hundred grams of soil of the different varieties was inoculated with 1 gram of the ammonifiable substance. Blood meal sterilized with CS_2 and sterile casein (Brown, 1913) solution were used. For solution work, Dunham's solution (1 per cent peptone plus 0.5 per cent salt) and urea bouillon (nutrient bouillon and 1 per cent urea) were used. In each case an easily and a less readily ammonified substance were used, casein and urea being easily ammonified.

Anaerobic conditions were obtained with the soils by placing them under a bell jar in a somewhat larger dish with pyrogallic acid and caustic soda solution. The pyrogallic acid was placed in the bottom of the dish, the soil in tumblers, and the bell jar put on, the caustic soda solution added, and then some paraffin oil. The bell jar was lifted slightly to let the caustic soda come in contact with the pyrogallic acid. The oil prevented the mixture from absorbing oxygen from the atmosphere. In solution

anaerobic conditions were obtained by adding one inch of sterile paraffin oil. The materials were incubated a week at 30° C. and the ammonia determined by distillation with MgO.

Experiment I. Ammonification of blood meal and casein under aerobic and anaerobic conditions in soils. Ammonia in milligrams per 100 grams of soil.

Casein

SOIL	AEROBIC	ANAEROBIC
Greenhouse	90.10	97.26
Loam	78.05	77.46
Clay	71.25	43.18
Blood meal		
Greenhouse	21.42	20.70
Loam	21.08	11.90
Clay	10.88	7.60

Experiment II. Ammonification in sterile soil by mass cultures under aerobic and anaerobic conditions. Ammonia in milligrams per 100 grams of soil.

Casein

SOIL	AEROBIC	ANAEROBIC
Greenhouse	96.39	95.61
Loam	82.96	81.26
Clay	91.29	81.94
Blood meal		
Greenhouse	98.94	105.23
Loam	70.72	87.72
Clay	8.89	14.62

In experiment II the mass cultures were obtained by inoculating Lipman's synthetic media (Lipman and Brown 1911) with soils of the different types and growing one week at 30° C. The soil was sterilized in the autoclave. Twenty cubic centimeters of this mass culture were added to each 100 grams of soil.

It is at once apparent that ammonification in soil proceeds just as readily under anaerobic conditions as it does under

aerobic conditions. The process in general proceeds better in richer soils. It is best with the greenhouse type and poorest with the clay. Casein is more easily broken down than blood meal. Blood meal is ammonified to a greater extent under aerobic and anaerobic conditions in sterile inoculated soil than in fresh soil. The soils kept under anaerobic conditions gave a strong disagreeable odor, showing that other processes were going on. This was not noticeable with the soils kept under aerobic conditions.

Experiment III. Ammonification in solution. Ammonia in milligrams per 100 cc. of solution.

Urea bouillon

SOIL	AEROBIC	ANAEROBIC
Greenhouse	554.71	679.66
Loam	448.75	687.65
Clay	553.69	684.22
Dunham's solution		
Greenhouse	136.85	141.78
Loam	134.30	125.12
Clay	147.90	148.24

One hundred cubic centimeters of the solutions in 250 cc. Erlenmeyer flasks were inoculated, respectively with 2 grams of soil of each type. One inch of sterile paraffin oil was added to half of them, the other half being kept under aerobic conditions.

Ammonification in solution goes on under anaerobic conditions as well as under aerobic conditions. The urea is more easily ammonified than the peptone. The ammonification of urea proceeds better under anaerobic conditions than under aerobic conditions. The ammonification of peptone proceeds just as readily in the presence as in the absence of air. Larger amounts of ammonia were formed in solution than in soil.

It was thought that an excess of air might inhibit or increase the amount of ammonia formed. A preliminary experiment was carried out by bubbling washed air through 100 cc. of Dunham's solution inoculated with 2 grams of garden soil (greenhouse).

Air was bubbled through sterile water, then through the Dunham solution, and then through 50 cc. of $\frac{N}{10}$ H_2SO_4, colored with methyl red, by means of a water air pump. Any ammonia that was drawn across in the process was caught in the acid. As the acid lost color, more acid was added.

Milligrams of ammonia formed per 100 cc. Dunham's solution

No. I. Excess air	120.53
No. II. Air	155.72
No. III. Without air (oil)	163.20

From these data it would appear that ammonification proceeded best without air, next with air and least with an excess of air.

Experiments were carried out with urea bouillon (no peptone) and Dunham solution with the bacteria of the three types of soil, under anaerobic, aerobic and excess aerobic conditions.

Experiment IV. Ammonification in solution. Milligrams ammonia per 100 cc. of solution.

Urea bouillon

SOIL	EXCESS AIR	AIR	WITHOUT AIR
Greenhouse	357.85	313.48	570.80
Loam	302.94	306.51	563.04
Clay	291.29	271.53	550.80

Dunham solution

Greenhouse	130.17	149.36	161.23
Loam	145.71	148.89	154.17
Clay	55.11	93.31	137.17

It is again noticeable that urea is more readily ammonified than peptone. Ammonification proceeds best under anaerobic conditions. The process seems to proceed equally well with air or with an excess of air. With urea bouillon the production of ammonia is slightly higher with an excess of air than under ordinary air conditions. With Dunham's solution the production of ammonia is slightly higher without an excess of air.

Some further experiments with pure cultures were carried out. A pure culture was isolated from an ammonified urea solution and grown on urea agar under anaerobic conditions. A very simple method was devised for anaerobic plate work. It consisted in adding sterile paraffin oil to agar that had been cooled, inoculated and poured. Oil was added to the level of the rim of the plate. This avoided the use of the anaerobic jar and proved very effective. No spreading colonies were observed, and colonies were as well isolated as on an aerobic plate. The plates may be removed from the incubator and examined for growth at any time. This is a decided advantage over the anaerobic jar method. The oil may be poured off the plate and the colonies exposed for further study.

The organism isolated by this method was a diplo-bacillus. It would not grow on nutrient agar under aerobic or anaerobic conditions. It grew very well on urea agar under aerobic and anaerobic conditions, although it had been isolated purely by anaerobic technique.

Cultures of this organism were inoculated into 100 cc., respectively of urea bouillon (no peptone), urea solution (glucose, 10 per cent, K_2HPO_4 5 per cent, $MgSO_4$ 0.05 per cent, urea 1 per cent), and Dunham solution. These inoculated flasks were placed under aerobic and anaerobic conditions.

Experiment V. Ammonification in solution by pure culture. Ammonia in milligrams per 100 cc. of solution.

MEDIA	AEROBIC	ANAEROBIC
Urea bouillon	308.69	530.53
Urea solution	130.63	79.31
Dunham solution	—	—

In this experiment urea bouillon seemed to be the best medium. Ammonification with this material proceeded better under anaerobic conditions. The reaction did not take place with Dunham's solution. It did not proceed as well with the urea solution as with urea bouillon, although the process went on under aerobic and anaerobic conditions.

Some further experiments were carried out with pure cultures of *B. mycoides* and *B. subtilis* freshly isolated from the soil.

Experiment VI. Ammonification in solution by B. mycoides and B. subtilis. Ammonia in milligrams per 100 grams of solution.

Urea bouillon

ORGANISM	AEROBIC	ANAEROBIC
B. mycoides	42.46	62.19
B. subtilis	41.28	62.67
Dunham solution		
B. mycoides	35.71	14.37
B. subtilis	9.95	6.07

These two facultative anaerobes not only live under anaerobic conditions, but carry out their activities as well. Urea bouillon is more easily ammonified than the Dunham solution by these organisms. More ammonia is produced under anaerobic conditions with urea and less with peptone.

In general, from a perusal of the preceding experiments, it appears that ammonification of the substances tested under laboratory conditions, proceeds readily under aerobic or anaerobic conditions in mass cultures using soil as a medium or in media inoculated with soil or when pure cultures isolated from the soil are used.

The ammonification of blood meal and casein proceeds as well under anaerobic as under aerobic conditions in the soil. The same is true of ammonification in solutions of urea and peptone. More urea is, however, broken down under anaerobic conditions. Excess air bubbled through inoculated liquid media does not inhibit the production of ammonia, although less ammonia was produced with Dunham's solution under these conditions than under ordinary air conditions. Pure cultures of *B. mycoides* and *B. subtilis* readily form ammonia under anaerobic conditions. More ammonia is however produced from urea under anaerobic conditions by these organisms.

NITROGEN FIXATION

The ability of microörganisms to fix atmospheric nitrogen was first definitely demonstrated to be due to an anaerobic bacillus, *B. Pasteurianus*, in 1893 by Winogradski. It remained for Beyerinck in 1901 to demonstrate an aerobic organism that also assimilated free nitrogen. Non-symbiotic fixation of nitrogen in the soil is due to both types of organisms but at times may be due only to one type. Lipman and Burgess (1915) found two-thirds of the soils examined by them free from *Azotobacter*. Yet these soils were capable of fixing nitrogen when inoculated into solutions. They ascribed the nitrogen fixation to clostridium forms. Haselhoff and Bredemann (1906) investigated anaerobic nitrogen-collecting bacteria and found results approximating those of Winogradski. The amount of nitrogen fixed varies with the amount of carbonaceous matter present, the more carbon the higher the assimilation. Working with pure and mixed cultures, they found from 0.42 to 2.74 mgm. of nitrogen fixed per gram of mannite. Lipman (1908), working with pure cultures of *Azotobacter*, found from 0.39 to 10.45 mgm. of nitrogen per gram of mannite formed in four weeks in mannite solution. In this work mass cultures were used, either by inoculating solution with soil or by adding the source of carbon to the soil.

In my work nitrogen fixation was carried out in soils and in solution. For the solution work, 100 cc. of a nitrogen poor medium was inoculated with from 2 to 5 grams of soil. The following solution (N. J. 1908) was used:

H_2O	1000.0 grams
K_2HPO_4	0.2 gram
$MgSO_4$	0.2 gram
NaCl	0.5 gram
Mannite	20.0 grams
$FeCl_3$	1 drop of 10 per cent solution

The solution was neutralized with KOH using phenolphthalein as an indicator. Anaerobic conditions were obtained by adding about an inch of sterile paraffin oil or by placing the material in the anaerobic apparatus described under the ammonification experiments.

For nitrogen fixation in soils, the three types of soil used for ammonification were again studied. One gram of mannite was added to 100 grams of soil in a beaker. Anaerobic conditions were again obtained by the absorption of oxygen with pyrogallic acid and caustic soda solution.

The materials were incubated at about 30° C. for twenty-one days and then the total nitrogen was determined by the modified Gunning method, (Hibbard, 1910). Blanks were run at the beginning and the difference between these blanks and the total nitrogen at the end of twenty-one days gives the amount of nitrogen fixed.

Experiment VII. Nitrogen fixation in solution. Nitrogen in milligrams per 100 cc. of solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Aerobic			
Greenhouse	12.00	9.38	2.62
Loam	7.60	3.75	3.85
Clay	5.60	1.74	3.86
Anaerobic			
Greenhouse	12.60	9.38	3.22
Loam	6.30	3.75	2.55
Clay	5.10	1.74	3.36

In this experiment 100 cc. of the solution contained in 250 cc. Erlenmeyer flasks was inoculated with 5 grams of the soils of the different types. About one inch of sterile paraffin oil was added in order to insure anaerobic conditions.

All of the soils used are capable of fixing nitrogen under both aerobic and anaerobic conditions. The greenhouse type of soil is richer than the loam and the loam richer than the clay in total nitrogen both at the beginning and at the end of the experiment. The actual increase in nitrogen does not vary much with any of the three soils. Lipman and Burgess (1915) also noticed this and remarked in a conclusion that as a rule a high nitrogen content in the soil seems to mitigate against a vigorous nitrogen fixation. The nitrogen fixation seems to proceed as readily with or without the presence of air in this experiment.

In the following two experiments nitrogen fixation was carried out in solution under anaerobic conditions by placing narrow bottles containing the mannite solution, inoculated with 5 grams of soil, in the anaerobic apparatus and absorbing the oxygen with pyrogallic acid and caustic soda solution. In one case thin bottles containing a solution in which denitrification was going on, were added.

Experiment VIII. Anaerobic nitrogen fixation in solution. Nitrogen in milligrams per 100 cc. of solution.

A

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Greenhouse	11.34	9.38	1.96
Loam	7.00	3.75	3.25
Clay	8.68	1.74	6.94
B			
Greenhouse	9.80	4.18	5.62
Loam	12.88	1.50	11.38
Clay	5.18	0.70	4.58

In Series B denitrification was going on in the same apparatus in three other samples. The nitrogen fixing solution was inoculated with 2 grams of the soils. There was more nitrogen fixed in B, probably due to the fact that nitrogen was being continually given off in the denitrification experiments. This might indicate that the more nitrogen present the more nitrogen is fixed. It may be that as denitrification takes place in the soil some part of this nitrogen may be again fixed immediately. Except in the last experiment anaerobic conditions do not seem to favor the production of more nitrogen than is produced under aerobic conditions in solutions. Nitrogen fixation in solution proceeds as well with or without the presence of air.

Further experiments were carried out in soils by adding 1 gram of mannite per 100 grams of soil. Anaerobic conditions were obtained with pyrogallic acid and caustic soda solution. The total nitrogen was determined in 10 gram samples at the beginning and end of the experiment.

Experiment IX. Nitrogen fixation in soils. Nitrogen in milligrams per 10 grams of soil.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Greenhouse	27.16	26.32	0.84
Loam	12.46	9.38	3.08
Clay	5.18	4.34	0.84
Anaerobic			
Greenhouse	34.82	26.32	8.50
Loam	14.66	9.38	5.29
Clay	9.03	4.34	4.69

In these experiments greater amounts of nitrogen are fixed under anaerobic conditions than under aerobic conditions. There is a gradation shown under anaerobic conditions, most nitrogen being fixed by the greenhouse soil, less by the loam and least by the clay.

In the following experiment soil was sterilized in the autoclave and mass cultures grown under aerobic and anaerobic conditions in mannite solution were added.

Experiment X. Nitrogen fixation in sterile soil. Nitrogen in milligrams per 10 grams of soil.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	LOSS OR GAIN
Greenhouse	24.67	26.32	−1.65
Loam	12.50	9.32	3.10
Clay	5.60	4.34	1.26
Anaerobic			
Greenhouse	27.86	26.32	1.54
Loam	11.48	9.38	2.10
Clay	5.04	4.34	.70

The number of bacteria per gram of soil, capable of growing on nitrogen poor media, was estimated on three different media under aerobic and anaerobic conditions. The following media were used:

Mannite agar		*Ashby agar*		*Winogradski agar*	
K_2HPO_4	0.2	K_2HPO_4	0.2	K_2HPO_4	1.00 gram
$MgSO_4$	0.2	$MgSO_4$	0.2	$MgSO_4$	3.00 grams
NaCl	0.5	NaCl	0.2	NaCl	0.01 gram
Mannite	20.0	$CaSO_4$	0.2	$MnSO_4$	0.01 gram
H_2O	1000.0	$CaCO_3$	5.0	$CaCO_3$	10.00 grams
Agar	15.0	H_2O	1000.0	$FeCl_3$	.2 drops 10 per cent
$FeCl_3$, 1 drop 10 per cent		Agar	15.0	H_2O	1000 grams
Neutralized with KOH.				Agar	15 grams

The soil was plated and the plates were incubated at 30° C. under aerobic and anaerobic (pyrogallic acid and caustic soda) conditions for seven days and then counted.

Experiment XI. Number of bacteria per gram on nitrogen-poor media.

Aerobes

SOIL	MANNITE AGAR	WINOGRADSKI AGAR	ASHBY AGAR
Greenhouse	26,000,000	29,000,000	4,000,000
Loam	1,150,000	1,000,000	1,190,000
Clay	40,000	21,000	32,000
Anaerobes			
Greenhouse	1,050,000	1,020,000	310,000
Loam	240,000	240,000	190,000
Clay	11,800	9,600	3,500

Greater numbers of bacteria develop under aerobic than anaerobic conditions. There are more aerobic than anaerobic bacteria capable of growing on nitrogen-poor media. The number of bacteria varies with the type of soil, the greatest number being present in the greenhouse type and fewest in the clay.

Two cultures of *Azotobacter* were picked from the aerobic plates and two cultures were picked from the anaerobic plates. These cultures were inoculated into 100 cc. of nitrogen poor media,—Mannite solution, Winogradski solution and Ashby solution (same composition as the agars without the agar). The two cultures taken from the anaerobic plates were kept, with and without, oil. All inoculated material was incubated twenty-one days at 30°C.

Experiment XII. Nitrogen fixation in solution by pure cultures. Nitrogen in milligrams per 100 cc. of solution.

Aerobes

MEDIA	CULTURE NUMBER	NITROGEN FIXED
Mannite	1	2.02
	2	1.86
Ashby	1	3.23
	2	1.86
Winogradski	1	3.23
	2	3.39
Anaerobes (oil)		
Mannite	3	
	4	1.75
Ashby	3	1.90
	4	1.75
Winogradski	3	2.02
	4	2.21

Experiment XIII. Nitrogen fixation in solution by pure cultures. Nitrogen in milligrams per 100 cc. of solution.

Anaerobes

MEDIA	CULTURE NUMBER	NITROGEN FIXED UNDER OIL	NITROGEN FIXED WITHOUT OIL
Mannite	3	—	2.63
	4	1.75	2.63
Ashby	3	1.90	2.32
	4	1.75	2.39
Winogradski	3	2.02	7.21
	4	2.21	3.54

More nitrogen is fixed by the anaerobic organisms. More nitrogen is fixed by the anaerobic organisms when inoculated into media with no oil added to insure anaerobic conditions than when oil is added. In the last case more nitrogen is fixed by the anaerobes without oil than is fixed by the aerobes. The Winogradski medium seems to be the best for nitrogen fixation by pure cultures. More nitrogen is fixed in Winogradski media than in the other two materials used.

In general, fixation proceeds better in soils than in solution, more nitrogen being fixed in soils. The nitrogen fixed per gram of mannite is higher with the soils than in solution. The greater amount of nitrogen fixed in soils may be due of course to other forms of energy in the soil in the shape of decomposed plant tissue. Nitrogen is fixed readily under aerobic or anaerobic conditions in solutions. In soils, nitrogen fixation proceeds better under anaerobic conditions.

DENITRIFICATION

Broadly speaking, denitrification is the breaking down of nitrates to nitrites and ammonia and the liberation of free nitrogen. More narrowly it includes only the latter phase, the liberation of free nitrogen by microörganisms acting on nitrates or nitrites. It is with this latter phase that my experiments were concerned. The importance for agriculture of denitrification in the soil has been greatly exaggerated. It is important if large amounts of fresh manure are added to soil rich in nitrates, but not otherwise.

Denitrification is carried out by a variety of microörganisms, chief among which are *B. denitrificans, B. pyocyaneus, B. fluorescens-liquefaciens,* and *B. Hartlebii.* Lipman (1902) found from 1.3 per cent to 25.6 per cent loss in nitrogen with pure cultures and as high as 35 per cent loss with mixed cultures. He also states that denitrifying organisms are found in all soils. These denitrifying organisms live in the presence of air but may live anaerobically.

Koch and Pettit (1910) found that denitrification varies with the moisture present. With an increase in moisture there is an increase in denitrification, and as the moisture is increased under laboratory conditions there is an increase in the nitrogen lost.

Jensen (1909) has pointed out that denitrification is always accompanied by oxidative processes.

My study of denitrification was carried out both in soils and in solutions. For the solution work the following medium was used—Giltay and Aberson's solution:

H_2O	1000.00	K_2HPO_4	2.00
KNO_3	2.00	CaCl	0.20
$MgSO_4$	2.00	Na_2CO_3	4.25
Citric acid	5,00		

For the soil work, 100 grams of soil were inoculated with 0.2 gram of KNO_3. Anaerobic conditions were again obtained with sterile paraffin oil for the solution work, and by the absorption of oxygen with pyrogallic acid and caustic soda for the soil work. In the following experiment 200 cc. of Giltay and Aberson's solution were inoculated with 2 grams of the soils of the three types and then incubated for twenty-one days at 30° C. under aerobic and anaerobic conditions.

Experiment XIV. Denitrification in solution. Nitrogen in milligrams per 200 cc. of solution.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse	20.10	56.19	−36.09
Loam	22.47	52.19	−29.72
Clay	18.84	51.82	−32.98
Anaerobic			
Greenhouse	20.10	56.19	−36.09
Loam	16.82	52.19	−35.37
Clay	16.74	51.82	−35.08

Denitrification seems to proceed better under anaerobic conditions than under aerobic conditions. The difference is not very marked, though noticeable. Again the process seems to proceed best in the richest soil, probably due to the fact that there are more bacteria of the denitrifying type present.

In the following experiment washed air was bubbled through 200 cc. of the solution for twenty-one days at 30°C.

Experiment XV. Denitrification in solution. Nitrogen in milligrams per 200 cc. of solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse	20.34	56.19	−35.85
Loam	24.33	52.19	−27.86
Clay	20.48	51.82	−30.34

Bubbling air through the solution does not inhibit the liberation of free nitrogen into the air by bacteria to any marked extent. In every case there is less nitrogen lost when air is bubbled through than under ordinary aerobic and anaerobic conditions, but there is not enough difference to be of any marked importance.

Loss of nitrogen under different conditions. Nitrogen in milligrams per 200 cc. of solution.

SOIL	EXCESS AIR	AIR	WITHOUT AIR
Greenhouse	−35.85	−36.09	−36.09
Loam	−27.86	−29.72	−35.37
Clay	−30.34	−32.98	−35.08

From a study of these figures it is evident in each case, with each type of soil, that there is least nitrogen lost under excess air conditions and most lost under anaerobic conditions.

In the following experiment denitrification was carried on in solution (100 cc. of solution and 2 grams of soil) under anaerobic conditions, in the anaerobic apparatus (pyrogallic acid and caustic soda) along with a nitrogen fixation experiment.

Experiment XVI. Denitrification in solution. Nitrogen in milligrams per 100 cc. solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse	11.76	28.10	−16.34
Loam	12.88	26.09	−13.11
Clay	16.24	25.91	− 9.67

Most denitrification goes on in the greenhouse soil and least in the clay.

Some further experiments were carried out with soil as a medium. One hundred grams of soil were inoculated with 10 cc. of a 2 per cent solution of KNO_3, and incubated twenty-one days at 30°C. under aerobic and anaerobic conditions.

Experiment XVII. Denitrification in soils. Nitrogen in milligrams per 10 grams of soil.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Aerobic			
Greenhouse	25.58	29.09	−3.51
Loam	12.59	12.15	0.44
Clay	10.57	7.11	3.46
Anaerobic			
Greenhouse	18.05	29.09	−11.04
Loam	13.05	12.15	0.90
Clay	11.31	7.11	4.20

With the greenhouse type of soil, denitrification took place, to a greater extent under anaerobic than under aerobic conditions. With the other two types of soil there was no loss of nitrogen. It is evident that soil as a medium does not give as good results as the solutions in regard to denitrification.

In general, denitrification goes on under aerobic and anaerobic conditions. An excess of air does not seriously inhibit the production of nitrogen, although slightly less nitrogen is lost. The process proceeds slightly better under anaerobic conditions, although almost as much nitrogen is lost under aerobic conditions. Denitrification proceeds better in solution than in soils, nitrogen being lost only in the greenhouse type of soil and not in the other two types.

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