CXCII. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

VI. THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE RATE OF IMMOBILISATION OF NITROGEN BY STRAW.

By ARTHUR GEOFFREY NORMAN.

*From the Fermentation Department, Rothamsted Experimental Station,
Harpenden, Herts.*

(Received September 9th, 1931.)

THE total nitrogen requirements of any plant material in decomposition may be determined quite simply, and can conveniently be expressed by some such term as the "nitrogen factor." This term was first employed by Hutchinson and Richards [1921], and may be defined as the inorganic nitrogen temporarily immobilised by 100 g. of material in decomposition. Certain considerations which affect the nitrogen factor have been discussed in the preceding paper of this series [Richards and Norman, 1931]. The rate of immobilisation has, however, not been determined, nor has the influence of hydrogen ion concentration upon the rate been investigated. Any differences either in rate or extent of decomposition which may be observed will, of course, be due to a modification of the active flora. It might be expected, therefore, that both the rate of fermentation and the amounts of the various constituents removed might vary considerably with changes in the p_H of the medium.

It was necessary in the first place to devise some means by which the hydrogen ion concentration of the decomposing material could be kept approximately constant, and by which a sufficiency of available nitrogen would be present at all stages of the decomposition, without having a large excess in the initial stage since this might interfere with the normal course of decomposition. Both these requirements pointed to some arrangement for the continuous supply of nitrogen.

Richards showed that a solution of ammonium carbonate (10 parts of nitrogen per 100,000), could be passed through a percolating filter of wheat straw until, on the twentieth day, only 1 % of the ammonia nitrogen would be found in the effluent and all but a small percentage (3.4) of the balance retained in the filter as microbial protein. From the twentieth day the mature filter would continue to remove practically all the nitrogen fed to it until saturation point was reached, *i.e.* until the "nitrogen factor," 0.7 per 100 parts of dry straw, had been attained. This laboratory experiment and the subse-

quent successful trials on the large scale made by Richards and Weekes [1921] with sewage were carried out at a p_H of 7.0–8.0.

Accordingly straw was arranged in the form of a filter and supplied with a very dilute solution of an ammonium salt adjusted to the required p_H . This appeared to give very satisfactory results, and is a method suitable for other studies of a similar nature.

EXPERIMENTAL.

Known amounts of dry chaffed straw were packed into a series of lamp glasses as supplied for paraffin lamps, the wider end being covered by muslin held in place by rubber bands. The most convenient size of lamp glass held about 50 g. straw, but others rather larger holding 70 g. were also employed and were quite satisfactory.

A dilute solution of an ammonium salt together with certain mineral salts, adjusted to the required hydrogen ion concentration, was allowed to drop through at the rate of 1 litre per day. At the concentration of nitrogen employed (7 parts per 100,000) this was sufficiently in excess for all stages of the decomposition. Even at the most active point less than 50 % of the nitrogen was retained while in general 10 to 15 % only was immobilised. The mineral salts were added in case the leaching effect of a continuous flow removed any essential ions. The potash, for example, is easily washed out of straw¹. The rate of flow maintained was sufficient to keep the p_H close to the desired point, and quite insufficient to cause the development of anaerobic conditions. The distribution was found to be very satisfactory after the first two or three days. By means of the Nessler reaction the amount of ammonia in the effluent was checked daily. This was merely a precautionary measure to ensure the presence of sufficient nitrogen. The results obtained were not sufficiently accurate to be regarded as a measure of immobilisation, since they do not show the amount biologically immobilised by conversion to microbial protein, but merely the amount retained or lost by the filter.

At appropriate intervals, as indicated roughly by the Nessler figures, a whole filter was taken down and the following determinations made:

1. Loss of dry matter.
2. Total nitrogen content—Kjeldahl.
3. Ammoniacal nitrogen content—distillation with MgO.

After drying and grinding, the following determinations were made:

4. Total furfuraldehyde yield.
5. Cellulose—Cross and Bevan.
6. Furfuraldehyde from Cross and Bevan cellulose.
7. Carbon dioxide yield, uronic acids.

¹ Over 90 % of the total K_2O in wheat straw is soluble in water, and in barley straw 97 %. (Richards and Rege, unpublished.)

From the first three the nitrogen factor and nitrogen equivalent of the straw at any point could be calculated and compared with the losses sustained by the various constituents.

Four series of experiments were carried out, the variations of ingoing nitrogenous solution being indicated below. It was not found possible to hold the p_H of the solutions more closely to the desired figure than the limits given below, since it was undesirable to introduce buffering salts.

- Series I. $p_H = 8.5-8.8$, N as ammonium carbonate.
 „ II. $p_H = 7.4-7.8$, N as ammonium chloride + NaOH.
 „ III. $p_H = 5.0-5.5$, N as ammonium chloride + 2 cc. acetic acid per litre.
 „ IV. $p_H = 7.4-7.8$, N as ammonium chloride + sucrose to give 26 parts carbon per 100,000 + NaOH.

In each case the necessary amount of ammonium salt to give 7 parts per 100,000 on dilution was dissolved in 1 litre of distilled water together with the following mineral salts:

K_2HPO_4	...	...	2.0 g.
$MgSO_4$	...	...	1.0 g.
$NaCl$	...	...	0.2 g.
$FeCl_2$	...	...	0.05 g.

The p_H was adjusted so that when 10 cc. of the above solution were diluted to 1 litre the required p_H was given. This was a matter of some difficulty, since the distilled water was usually slightly acid and showed some variation, thereby accounting for the variations recorded above. The p_H of the effluent was determined from time to time. These figures will not be given in detail for their absolute value is doubtful. The following are the extreme limits of variation recorded. Usually the figure was nearer that of the ingoing solution. The alkaline filters tended to become more nearly neutral, the neutral filters slightly acid, and the acid filters less acid, though never reaching neutrality.

- Series I. Effluent $p_H = 7.8$.
 „ II. „ $p_H = 6.8$.
 „ III. „ $p_H = 6.6$.
 „ IV. „ $p_H = 6.4$.

When thoroughly wet each filter was inoculated with 100 cc. inoculum from soil containing decomposing organic matter.

The results of analyses are given in the following Tables. Table I shows the nitrogenous changes at various periods for the four series; Table II contains the analytical figures calculated on the basis of 100 g. of original straw.

Table I. *Nitrogen content of straw filters.*

Day	Total N	Am- moniacal N	Organic N	Loss of dry matter	Organic N on 100 g. original straw	"Nitrogen factor"	Nitrogen equivalent
SERIES I.							
—	0.302	—	0.30	—	—	—	—
6	0.47	0.03	0.44	3.19	0.43	0.13	4.07
12	0.70	0.07	0.63	3.88	0.60	0.30	7.73
16	0.80	0.10	0.70	5.10	0.66	0.36	7.06
19	0.85	0.09	0.76	11.00	0.68	0.38	3.45
23	0.87	0.12	0.75	19.77	0.60	0.30	1.52
28	0.75	0.12	0.63	21.04	0.50	0.20	0.95
34	0.82	0.13	0.69	22.69	0.53	0.23	1.01
38	0.83	0.11	0.72	24.56	0.54	0.24	0.98
42	0.98	0.11	0.87	27.91	0.63	0.30	1.07
72	1.90	0.16	1.74	48.08	0.90	0.60	1.25
SERIES II.							
—	0.302	—	0.30	—	—	—	—
10	0.59	0.04	0.55	10.38	0.49	0.19	1.82
17	0.77	0.03	0.74	16.57	0.62	0.32	1.93
23	0.90	0.03	0.87	19.15	0.70	0.40	2.06
29	1.07	0.02	1.05	27.37	0.76	0.46	1.68
35	1.15	0.10	1.05	28.10	0.76	0.46	1.64
51	1.28	0.11	1.17	31.81	0.81	0.51	1.60
72	1.38	0.08	1.30	40.67	0.77	0.47	1.16
SERIES III.							
—	0.302	—	0.30	—	—	—	—
10	0.57	0.03	0.54	9.74	0.49	0.19	1.99
19	0.69	0.05	0.64	15.22	0.54	0.24	1.57
23	0.74	0.04	0.70	16.98	0.58	0.28	1.65
29	0.80	0.05	0.75	20.91	0.59	0.29	1.38
37	0.90	0.04	0.86	23.48	0.66	0.36	1.53
51	0.85	0.08	0.77	30.84	0.53	0.23	0.74
72	1.15	0.07	1.08	37.48	0.68	0.38	1.01
SERIES IV.							
—	0.302	—	0.30	—	—	—	—
10	0.540	0.04	0.50	4.37	0.48	0.18	4.12
17	0.80	0.05	0.75	7.15	0.70	0.40	5.60
23	0.96	0.07	0.89	14.69	0.76	0.46	3.17

DISCUSSION OF RESULTS.

(a) *Nitrogen immobilisation.* Differences in the rate and extent of immobilisation of nitrogen in each series may most readily be seen by comparing the "nitrogen factor" at each stage, since by this is expressed the inorganic nitrogen transformed to organic on the basis of 100 g. of original straw. Comparing first Series II and III, the neutral and acid filters respectively, it is seen that over the first 10 or 12 days very similar amounts are retained, after which period rather more is immobilised under neutral conditions. The neutral filter attains a nitrogen factor of 0.51 after 51 days falling off slightly to 0.47 at the end of 72 days. The acid filter shows a peak of 0.36 in 35 days and after a slight loss finally reaches 0.38 at the end of 72 days. Neutral conditions, therefore, result in the retention of rather more nitrogen than acid. Under slightly alkaline conditions immobilisation is more rapid during the first fortnight than in either acid or neutral conditions. A peak of 0.38

Table II. *Analyses of straw filters.*

(Expressed on 100 g. original dry straw.)

Day	Loss of dry matter	Total furfurald. yield	C. and B. cellulose	Furfurald. yield from C. and B. cellulose	CO ₂ yield	Total uronic acid anhydride	Furfurald. from uronic acid anhydride	Furfurald. from pentose in hemi-cellulose	Xylan associated with C. and B. cellulose	"Pure" cellulose by difference
SERIES I.										
—	—	14.95	48.92	6.61	1.36	5.44	0.91	7.43	10.25	38.67
16	5.10	14.15	48.23	6.45	1.21	4.84	0.80	6.90	10.0	38.2
19	11.00	12.69	43.61	6.01	0.86	3.44	0.58	6.10	9.3	34.3
23	19.77	11.90	40.01	4.89	0.84	3.36	0.56	6.45	7.6	32.4
34	22.69	11.15	37.62	4.54	0.85	3.40	0.57	6.04	7.0	30.6
42	27.91	10.19	36.61	4.49	0.85	3.40	0.57	5.13	6.9	29.7
72	48.08	6.04	23.15	2.72	0.60	2.40	0.40	2.92	4.2	18.9
SERIES II.										
—	—	14.95	48.92	6.61	1.36	5.44	0.91	7.43	10.25	38.67
10	10.38	13.91	47.87	6.84	0.95	3.80	0.63	6.44	10.6	37.3
17	16.57	11.91	43.88	6.77	0.98	3.92	0.65	4.49	10.5	33.4
23	19.45	11.08	40.20	5.87	0.99	3.96	0.66	4.55	9.1	31.1
29	27.37	10.10	36.70	5.43	0.93	3.72	0.62	4.05	8.4	28.3
35	28.10	9.99	36.15	4.85	0.86	3.44	0.58	4.56	7.5	28.6
51	31.81	9.09	33.83	4.54	0.77	3.08	0.51	4.04	7.0	26.8
72	40.67	7.18	24.84	3.34	0.76	3.04	0.50	3.34	5.2	19.6
SERIES III.										
—	—	14.95	48.92	6.61	1.36	5.44	0.91	7.43	10.25	38.67
10	9.74	14.13	48.91	6.80	0.99	3.96	0.66	6.67	10.5	38.4
19	15.22	13.14	43.45	6.55	0.96	3.84	0.64	5.95	10.2	33.3
23	16.98	12.62	42.40	6.33	0.93	3.72	0.62	5.67	9.8	32.6
29	20.91	11.45	38.81	5.37	0.89	3.56	0.59	5.49	8.3	30.5
37	23.48	11.27	37.61	5.02	0.96	3.84	0.64	5.61	7.8	29.8
51	30.84	10.07	33.74	4.54	0.87	3.48	0.58	4.95	7.0	26.7
72	37.48	7.86	27.54	3.79	0.78	3.12	0.52	3.55	5.9	21.6
SERIES IV.										
—	—	14.95	48.92	6.61	1.36	5.44	0.91	7.43	10.25	38.67
10	4.37	14.05	49.64	7.09	1.17	4.68	0.78	6.08	10.99	38.7
17	7.15	13.49	47.77	6.32	1.08	4.32	0.72	6.45	9.80	38.0
23	14.69	12.26	44.26	5.72	0.95	3.80	0.63	5.91	8.87	35.4

is reached after 19 days and is followed by small losses. Finally over 72 days as much as 0.6 g. nitrogen is immobilised per 100 g. of straw. Alkaline conditions, therefore, seem ultimately even more favourable than neutral or acid, though in the intermediate period, from 4 to 5 weeks, less may be held than in the latter.

(b) *Loss of dry matter.* Under the conditions of the experiments the total loss of dry matter observed is not solely due to biological assimilation. In part it is due to the leaching out of soluble materials of the straw, and in part due to the removal of soluble intermediate products of microbial activity. A filter run solely on water for 51 days showed a total loss of dry matter of 21.26 %. This included biological action for no attempt was made to keep the filter sterile. There was in fact, a small "nitrogen factor" of 0.09 due to the picking up of ammonia from the air.

In the acid and neutral series losses of dry matter are very similar over the first fortnight, after which the neutral filter goes slightly ahead, and during the whole period loses rather over 40 % as opposed to 37 % in the case of the acid filter. Particularly noticeable is the regularity in the rate of decomposition.

In the alkaline filter in the early stages the rate of decomposition is slow, even though considerable immobilisation of nitrogen is taking place, and after 16 days only 5 % of dry matter has been lost. During the following weeks decomposition is very rapid, after which the rate becomes normal again. Over 72 days, 48 % is lost. The most notable feature is the well-established initial lag in decomposition, in spite of which the total final loss is greater than in either the neutral or acid series. Series IV was a short period run with a neutral sugar-nitrogen solution, in order to see how the addition of readily available carbohydrate affects the rate of decomposition of straw and the amount of nitrogen immobilised in the early stages. Even under these conditions with an excess of very readily available carbohydrate material, the loss of dry matter from the straw is as great as in the alkaline run over the same period, though the "nitrogen factor" is a little higher. Acid-production from the sugar is evident from the p_H figures. The addition of the sugar did not result in the immobilisation of as much nitrogen as might have been expected, and the experiment was discontinued.

(c) *Nitrogen equivalent.* The differences between the several series are well brought out by the nitrogen equivalent figures. The lower this figure, the less nitrogen has been immobilised in removing 100 g. of carbohydrate material from the straw. Both the neutral and the acid filters have similar nitrogen equivalents, that of the acid being just a little lower. The initial lag in decomposition in the alkaline filter is due to the unsuitability for cellulose decomposition of the flora developing in the opening stages, shown by the fact that the nitrogen equivalent is much higher then, being well over 7 as opposed to a maximum figure of 2 in either of the other series.

(d) *Loss of various constituents.* A comparison of the rate of loss of certain structural constituents shows no unusual features. The final loss of cellulose is rather greater in the alkaline filter than in either of the others as would be expected from the losses of organic matter observed. There is, however, an initial lag in the alkaline filter and even at the end of 6 weeks less has been removed than in the others. The loss of hemicellulose as measured by the disappearance of pentose presents precisely similar features, but is very different from that found to occur in straw compost heaps. Under such circumstances a very rapid fermentation of the hemicelluloses occurs in the early stages, before cellulose decomposition is appreciable. Later while the cellulose suffers a heavy loss, the hemicelluloses appear to remain at much the same level. In the filter it is true that decomposition of pentose precedes that of cellulose, but in no case does it exhibit an early peak, and losses continue at a fairly steady rate side by side with cellulose degradation

throughout the period of the experiments. The changes in the neutral filter approach most closely to those observed in compost heaps, in that the early loss of hemicelluloses is considerable but this loss is neither so large nor so rapid as would be found in the latter. The loss of xylan associated with the cellulose seems to follow closely the loss of the cellulose itself, as would be expected from its distribution.

CONCLUSIONS.

From the results presented it seems that changes in hydrogen ion concentration exercise a considerable effect on the rate and amount of nitrogen immobilised by straw undergoing decomposition. It is especially to be noted, however, that the total amount of organic matter lost may not be very different, nor the relative losses of individual constituents much affected by such changes. The wide range of organisms present in the soil and the co-operative nature of their activities make possible a large number of balanced reactions, each capable of bringing about the same ultimate result, though the route and rate of achieving it may differ. The effects and differences observed are undoubtedly due to a modification in nature of the active microflora. In the case of the acid filter, the decomposition of the straw was mainly the work of fungi, the growth of which would be favoured by such conditions; indeed a heavy growth of fungal tissue, mainly *Trichoderma*, was clearly visible in the earlier stages. There is probably a sequence of active forms as the straw undergoes modification in decomposition. The nitrogen factor in this series showed a peak of 0.36 after 5 weeks, followed by a slight fall and rise again to 0.38. The fall observed is direct evidence for the ammonification that proceeds side by side with the immobilisation of nitrogen. Ammonification may be due either to bacteria or fungi, but probably the latter. In the neutral filter the conditions are more suitable for bacterial development and not unfavourable to fungi. It is therefore not surprising that the nitrogen factor is consistently higher than under acid conditions since, in general, bacteria have a higher nitrogen content than the fungi. It is not unlikely that the fungi carry out the major part of the cellulose decomposition and that the bacteria are active in removing degradation products and synthesised fungal tissue.

Under slightly alkaline conditions it appears that the development of the fungi is hindered, and that, since the fungi are the more active in cellulose degradation, there is, in consequence, an initial lag in decomposition. It is possible that it is necessary for the conditions in the filter to become more nearly neutral before the fungi develop and that this is effected by the action of bacteria producing acid from the soluble constituents. The sudden rapid loss of organic matter which occurs at the end of a fortnight is accompanied by a considerable fall in nitrogen factor and this can be explained by fungal ammonification of the bacterial film built up in the early stages. The nitrogen

equivalent figures at this period are very significant, since they reveal the fact that the flora present suddenly becomes much more active in decomposition, implying a change of type of organism. Manure and compost heaps undergoing natural aerobic decompositions generally remain fairly alkaline or neutral, so that the maximum immobilisation of nitrogen probably occurs. The practice of adding calcium carbonate to ensure this has clearly much to recommend it, particularly in the case of composts to which ammonium sulphate has been added. However, it sometimes happens that through water-logging or too close packing, anaerobic or partially anaerobic conditions occur, in which circumstances organic acids are produced from cellulose and the heap becomes definitely acid. Nitrogen may then be lost by ammonification and washing out of ammonium salts or by evaporation in the air. When a source of nitrogen is added in composting, as in the Adco process for the production of artificial farmyard manure, the aim is to immobilise as much nitrogen as possible in a biologically available organic condition. To achieve this it is desirable, therefore, to keep the reaction of the heap on the alkaline side. This was mentioned by Richards and Hutchinson [1924] in their patent, and the biological reasons for it are now made clear in this paper.

It is proposed to extend this work to conditions which occur in abnormal soils of various types, such as alkaline soils, very acid soils, and soils containing considerable quantities of salts.

SUMMARY.

1. By the use of percolating straw filters supplied with available nitrogen at high dilutions, the rate of immobilisation of nitrogen during decomposition at various hydrogen ion concentrations has been determined.

2. Under slightly alkaline conditions immobilisation is more rapid in the early stages than in either slightly acid or neutral conditions, and ultimately more nitrogen is retained. Neutral conditions seem rather more favourable than acid for immobilisation.

3. In 10 weeks more organic matter is fermented away under alkaline than neutral conditions, and more under neutral than acid conditions. The alkaline filter shows an initial lag in decomposition not given by either of the others. Nitrogen equivalent figures show this to be due to the development of a primary flora relatively inactive as far as decomposition of cellulose is concerned.

4. In each case the loss of hemicelluloses is rather different from that found to occur in compost heaps, it being considerably more gradual in the filters.

5. The effects observed are due to differences in character of the active flora.

The author is indebted to Sir John Russell, Director of the Rothamsted Experimental Station for placing at his disposal the facilities of that Station,

and to the Department of Scientific and Industrial Research for a Senior Research Award, during the tenure of which this work was carried out.

Especially are his thanks due to Mr E. H. Richards, Head of the Fermentation Department, for his ready assistance and advice.

REFERENCES.

- Hutchinson and Richards (1921). *J. Min. Agric.* **28**, 398.
Richards and Hutchinson (1924). *Eng. Pat.* 219, 384.
—— and Norman (1931). *Biochem. J.* **25**, 1769.
—— and Weekes (1921). *Proceedings of Engineering Conference. Institute of Civil Engineers. Section VI.*