CXCI. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

V. SOME FACTORS DETERMINING THE QUANTITY OF NITROGEN IMMOBILISED DURING DECOMPOSITION.

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The decomposition of plant materials in the soil or in compost heaps involves the assimilation of the carbohydrate constituents of the tissues and the conversion of available nitrogen to microbial protein by the organisms concerned. In nature the supply of nitrogen is often a limiting factor in determining the rate and extent of decomposition. Mature plant tissues frequently contain rather a low percentage of nitrogen, and to effect a complete and speedy rot, an additional quantity of available nitrogen has to be supplied. If too much is provided, losses occur, or if too little, the decomposition is incomplete unless an unusually long period of time is allowed. Hutchinson and Richards [1921] were the first to recognise the quantitative importance of nitrogen in decomposition, and they determined the amount necessary for the decomposition of a number of materials. For common straws, for example, they found it to be between 0·7 and 0·8 g. nitrogen per 100 g. original material. This figure they termed the "nitrogen factor." It may be defined as the additional inorganic nitrogen immobilised as organic nitrogen by 100 g. of any material in the process of decomposition. This quantitative relationship forms the basis of Richards and Hutchinson's patents [1924] for the production of "artificial farmyard manure" (Adco), from waste plant materials. The "nitrogen factor" gives the maximum amount of nitrogen which when added to decomposing material will be retained without loss.

Table I gives the "nitrogen factors" for a number of common materials, and indicates the range of variation found.

No basis exists for any prediction of the "nitrogen factor" and it has to be determined by trial in each case. The variations which are observed clearly depend on the differences in composition of the various materials and their relative availabilities to the micro-flora present. Accordingly investigations were undertaken to ascertain the nature of the relationship, if any, between

Table I. *"Nitrogen factors" of some waste plant materials.*

Plant material	"Nitrogen factor"	Loss of dry matter %
Willow peelings	1·33	35·2
Sugar cane trash	1·06	27·9
Oat straw	0·85	53·1
Rye straw	0·71	45·0
Rice straw	0·64	35·2
Esparto grass waste	0·52	25·7
Rushes (Scilly Isles)	0·50	21·9
Almond husks	0·44	55·0
Banana leaf	0·42	45·7
Almond skins	0·30	52·2
Flax shives	0·12	19·6

composition and "nitrogen factor." A number of widely different materials, some extracted suitably to remove known constituents, were employed, and rotted under optimum and parallel conditions.

Experimental.

The materials were in each case cut to a suitable size for rotting, about that of chaffed straw. Equal amounts of each, of known moisture content, were taken and moistened thoroughly by spraying. Available nitrogen in the form of ammonium carbonate was added to the material when bottled, together with an inoculum from soil in which organic matter was rotting. The moisture content was kept equal amongst the members of each series. In the case of certain extracted materials moistening was carried out with a mineral salt solution, since the process of extraction was likely to remove necessary ions. Washing in many cases had to be very prolonged to remove all traces of the extracting agents.

For the determination of the "nitrogen factor" the following figures were necessary.

1. Original nitrogen content—Kjeldahl.
2. Final nitrogen content—Kjeldahl.
3. Final ammonia content—distillation with MgO.
4. Loss of organic matter in decomposition.

To determine the composition of the materials employed, the following estimations were carried out as described by Norman [1929].

5. Total furfuraldehyde yield.
6. Cellulose (Cross and Bevan).
7. Furfuraldehyde from Cross and Bevan cellulose.
8. Carbon dioxide yield (uronic acid).

The composition and the "nitrogen factor" at various stages were determined for the materials enumerated in Table II.

Willow peelings were also rotted without the addition of nitrogen and this series will be designated D 2.

Table II. *Percentage composition of plant materials employed.*

Series No.	Plant material	C. and B. cellulose	Furfurald. from C. and B. cellulose	CO_2 yield	Total uronic anhydride	Furfurald. from uronic acids	Total furfurald. yield	Furfurald. from pentoses in hemicelluloses	Pentoses in hemicelluloses as anhydroxylose	Xylan associated with C. and B. cellulose	"Pure" cellulose	Lignin	Total N
A	Flax straw	53·40	6·49	1·69	6·76	1·13	12·04	4·42	6·9	10·1	43·3	30·5	0·51
B	Flax shives (retted)	53·57	6·88	1·29	5·16	0·86	12·56	4·82	7·5	10·7	42·9	30·9	0·21
C	Flax fibre	87·50	1·19	0·22	0·88	0·15	1·85	0·51	0·8	1·8	85·7	Nil	0·55
D	Willow peelings	31·16	1·80	2·69	10·76	1·79	7·37	3·78	5·9	2·9	28·3	36·5	1·58
E	Oat straw	48·92	6·61	1·36	5·44	0·91	15·95	7·43	11·5	10·25	38·7	18·5	0·30
F	Oat straw extract with hot water	50·99	6·97	1·56	6·24	1·04	16·59	8·58	13·3	10·8	40·2	—	0·27
G	Oat straw extract with 4 % NaOH	84·07	7·86	0·26	1·04	0·17	9·01	0·98	1·5	12·8	71·3	11·1	0·12
H	Oat straw extract with 2 % HCl	61·98	1·53	0·61	2·44	0·40	2·57	0·64	1·05	2·4	59·6	25·6	0·34
I	Oat straw extract with 4 % alc. NaOH	65·60	8·70	0·80	3·20	0·53	14·08	4·85	7·5	13·5	52·1	8·2	0·11
J	Young barley straw extract with 4 % NaOH	89·10	2·66	0·31	1·24	0·21	3·55	0·68	1·1	4·1	85·0	—	Nil
K	Esparto grass waste	48·07	5·91	—	—	—	12·66	6·8 (approx.)	10·6	9·2	38·9	26·7	0·95
L	Esparto grass waste extract with 4 % alc. NaOH	55·37	9·45	—	—	—	14·65	5·2 (approx.)	8·1	14·6	40·7	12·3	0·23

The composition of these materials is given in Table II and the "nitrogen factor" determinations in Table III. In addition to the "nitrogen factor," the "nitrogen equivalent" has been calculated. This represents the amount of additional nitrogen immobilised per 100 g. of organic matter lost. The use of this figure as an aggregate measure of efficiency of the organisms has been described in Part IV of this series [Norman, 1931].

In Table II for the sake of comparison the furfuraldehyde from the Cross and Bevan cellulose fraction has been calculated in all cases as xylan. That it is xylan in every case is an unjustifiable assumption, but it has been shown to be so in the case of the flax, the oat straw and the esparto grass.

Discussion of results.

I. "*Nitrogen factor.*"

In considering the results, the two sets of figures of importance in comparison are those for loss of dry matter, and the respective "nitrogen factors," at the end of 48 days, by which time the decompositions have become very slow. Series A, B and C fall together for comparison, since B consists of the flax shives removed from A in the operation of scutching after the retting process. Retting is, of course, a biological process by which the fibre bundles are loosened from the wood. In the first stage the soluble materials are fermented away by aerobic organisms, producing thereby anaerobic conditions in the tanks and paving the way for the main anaerobic fermentation of

Table III. *"Nitrogen factor" of various materials at several stages of decomposition.*

Series No.	Plant material	Time in days	Total N %	NH_3-N %	Organic N %	Loss of dry matter %	Organic N per 100 g. original	"Nitrogen factor"	Nitrogen equivalent
A	Flax straw (normal)	—	0·51	—	0·51	—	—	—	—
		8	1·46	0·15	1·31	19·54	1·05	0·54	2·76
		16	1·50	0·07	1·43	24·22	1·08	0·57	2·35
		24	1·68	0·17	1·51	31·10	1·04	0·53	1·70
		48	1·91	0·22	1·69	42·62	0·97	0·46	1·08
B	Flax shives (retted)	—	0·21	—	0·21	—	—	—	—
		8	0·54	0·19	0·35	5·14	0·33	0·12	2·33
		16	0·53	0·19	0·34	12·90	0·30	0·09	0·70
		24	0·70	0·31	0·39	16·76	0·32	0·11	0·66
		48	0·77	0·36	0·41	19·63	0·33	0·12	0·61
C	Flax fibre	—	0·55	—	0·55	—	—	—	—
		8	0·88	0·19	0·69	1·96	0·68	0·13	6·63
		16	1·28	0·29	0·99	9·16	0·89	0·34	3·71
		24	1·22	0·21	1·01	18·98	0·82	0·27	1·42
		48	1·48	0·27	1·21	28·29	0·87	0·32	1·13
D	Willow peelings	—	1·58	—	1·58	—	—	—	—
		8	2·67	0·32	2·37	2·77	2·30	0·72	26·0
		16	3·01	0·52	2·49	6·54	2·32	0·74	11·3
		24	3·05	0·32	2·73	14·20	2·34	0·76	5·3
		48	3·34	0·26	3·08	15·39	2·61	1·03	6·7
D2*	Willow peelings (without added nitrogen)	8	1·82	Nil	1·82	6·20	1·70	0·12	(1·93)
		16	2·01	Nil	2·01	12·22	1·76	0·18	(1·47)
		24	2·22	0·06	2·16	15·33	1·83	0·25	(1·63)
		48	2·21	0·05	2·16	16·93	1·79	0·21	(1·24)
E	Oat straw	—	0·30	—	0·30	—	—	—	—
		8	1·91	0·71	1·20	12·58	1·05	0·75	5·96
		16	1·56	0·40	1·16	24·90	0·97	0·67	2·69
		24	1·92	0·25	1·67	37·03	1·05	0·75	2·03
		48	2·54	0·36	2·18	50·44	1·10	0·80	1·38
F	Oat straw extracted with hot water	—	0·27	—	0·27	—	—	—	—
		8	1·65	0·86	0·79	2·05	0·77	0·50	24·4
		16	1·61	0·81	0·80	10·02	0·72	0·45	4·41
		24	1·53	0·77	0·76	13·82	0·66	0·39	2·82
		48	1·71	0·74	0·97	17·79	0·80	0·51	2·86
G	Oat straw extracted with 4 % NaOH	—	0·12	—	0·12	—	—	—	—
		8	1·70	1·51	0·19	8·86	0·17	0·05	0·45
		16	1·49	1·27	0·22	14·53	0·19	0·07	0·48
		24	1·18	0·82	0·36	16·84	0·30	0·18	1·07
		48	0·88	0·53	1·35	21·32	0·28	0·16	0·74
H	Oat straw extracted with 2 % HCl	—	0·34	—	0·34	—	—	—	—
		8	1·64	1·24	0·40	4·24	0·38	0·04	0·94
		16	1·67	1·29	0·38	6·76	0·35	0·01	0·14
		24	1·43	0·76	0·67	8·32	0·61	0·27	3·24
		48	1·11	0·44	0·67	9·14	0·61	0·27	2·95
I	Oat straw extracted with 4 % alc. NaOH	—	0·11	—	0·11	—	—	—	—
		8	1·26	0·88	0·38	11·24	0·34	0·23	2·05
		16	1·28	0·94	0·34	16·72	0·28	0·17	1·02
		24	1·47	0·97	0·50	19·26	0·40	0·29	1·31
		48	0·98	0·39	0·59	21·11	0·47	0·36	1·70
J	Barley straw extracted with 4 % NaOH	—	Nil	—	Nil	—	—	—	—
		8	1·22	0·95	0·27	15·16	0·23	0·23	1·52
		16	1·08	0·60	0·48	17·67	0·39	0·39	2·20
		24	1·20	0·56	0·64	18·81	0·52	0·52	2·76
		48	0·76	0·21	0·55	19·66	0·44	0·44	2·24
K	Esparto grass waste	—	0·95	—	0·95	—	—	—	—
		28	2·03	0·14	1·89	21·93	1·48	0·53	2·41
		56	2·05	0·07	1·98	25·65	1·47	0·52	2·03
L	Esparto grass waste extracted with 4 % alc. NaOH	—	0·23	—	0·23	—	—	—	—
		28	1·13	0·41	0·72	23·20	0·55	0·32	1·38

* Nitrogen absorbed as ammonia from incubator.

pectin which results in the separation of the bundles. In the flax shives, therefore, there must be an absence of soluble and easily fermentable material, and a close agreement in the contents of the main structural constituents, since these are unaffected in the retting process. The figures obtained on the rotted materials show a very marked difference, considerably larger than anticipated in view of the resemblance in composition. The straw suffered a loss of over 40 % and the shives less than 20 %, the nitrogen immobilised by the latter being only one-fourth of that locked up by the former. This would suggest that the absence of readily soluble and fermentable material tends to lower the "nitrogen factor." The flax fibre, which is completely deficient in soluble materials and consists mainly of cellulose, suffers a 30 % loss, at the same time immobilising 0·32 % of nitrogen. The fibre being non-lignified is in theory wholly available, but nevertheless decomposition is not extensive nor nitrogen immobilisation large. Possibly this is due to the fact that the range of active organisms is rather restricted since the medium is almost completely cellulosic.

The same general trend will be seen in the oat straw series, E–I. The loss of organic matter in the case of water-extracted straw is unaccountably low. The hemicellulose content of this material is high, and from the analytical figures a decomposition considerably more extensive would have been expected. In spite of this, however, the "nitrogen factor" is fairly high, being 0·5 as opposed to 0·8 in the case of normal straw. In Series G the straw was extracted extensively with 4 % NaOH, and this is seen to have the effect of removing the major part of the hemicelluloses, as evidenced by the low pentose and uronic acid figures, while in addition the lignin content is considerably lowered. The composition of the resulting material approaches that of the flax fibre, the differences being that it still contains pentose material intimately associated with the cellulose and is partially lignified. The absence of soluble materials and the presence of lignin together limit the extent of decomposition to a little over 20 % and the "nitrogen factor" to the low figure of 0·16. This may also be seen in Series H, in which all soluble and hydrolysable material was removed by boiling for a considerable period with 2 % HCl, a process which does not remove lignin to any extent. The final composition of this extracted material resembles that of certain woods. The total loss of organic matter did not reach 10 % but the "nitrogen factor" was a little higher than expected, being 0·27. In Series I, the oat straw was extracted with 4 % NaOH in 55 % alcohol, a treatment designed to remove much of the lignin without extracting the hemicelluloses. The lignin content was lowered from 18·5 to 8·2 %, but a portion of the hemicelluloses was also removed. In later work, the NaOH concentration was lowered to 2 % and the alcohol concentration increased to 60 %. The decomposition after this treatment was rather more than 20 % and the "nitrogen factor" 0·36. It was noticed that this treatment resulted in an unusual physical condition of the straw, and, however prolonged the washing with alcohol and cold water, the straw remained very gelatinous when wet.

Series J was a young barley straw cut 10 weeks after the appearance of the first tiller and exhaustively extracted with 4 % NaOH, a procedure which resulted in the production of a material giving analytical results very similar to those of the flax fibre. The figures on decomposition also resembled those of flax fibre, there being a 20 % decomposition and a "nitrogen factor" of 0·4.

To examine again the effect of extraction with alcoholic NaOH and the result of lowering the lignin content by this means, esparto grass waste was treated in this way. It will be seen in K and L that the loss of organic matter in the treated and untreated is about equal, but that the "nitrogen factor" is lowered in the former case from 0·5 to 0·3. That decomposition is not more extensive is no doubt due to the physical condition of the extracted material.

All the above mentioned materials contained an insufficient supply of nitrogen as evinced by their positive "nitrogen factors"; in the absence of added nitrogen their decomposition would have been slow or in some cases negligible in extent. The willow peelings, however, must be considered separately. In Table I a "nitrogen factor" of 1·33—the highest ever observed—was recorded for a sample of this material. This particular sample contained originally 1·9 % N, an amount so large that it would be expected to be more than sufficient for complete decomposition. In fact, it might be expected that losses of nitrogen would occur during the rotting process. Nevertheless, in the presence of added inorganic nitrogen an additional amount of 1·33 % per 100 g. was retained. A second sample, distinctly more woody in nature, was obtained, the analytical figures for which are given under D in Table II. It will be seen that the lignin estimation by the 72 % H_2SO_4 method gave 36·5 %. On decomposition in the presence of added ammonia, just over 15 % of organic matter was lost and 1·0 g. nitrogen immobilised per 100 g. In the absence of added nitrogen rather more organic matter was lost. There was, however, a slight pick-up of nitrogen from the ammonia lost by the other bottles in the incubator. This, however, does not obscure the salient point, which is that this material, though already containing ample nitrogen for decomposition will cause the immobilisation of a considerable additional amount without, however, undergoing a more extensive decomposition. In fact, the presence of ammonia seems even to have retarded decomposition in the early stages. It is clear that the protein or nitrogenous material of the willow peelings, although available, is less so than the ammonia, which is utilised preferentially.

Similar observations have been made in the case of certain other substances given in Table IV.

The retarding effect of an unnecessary addition of nitrogen in the form of ammonium carbonate is seen in the case of the maize straw decompositions, while actual losses, shown by a negative "nitrogen factor" occur in the case of the bean husks which have a very high initial nitrogen content.

The change in the "nitrogen factor" as the decomposition proceeds is of

Table IV. *"Nitrogen factors" of certain materials already containing sufficient nitrogen for decomposition.*

Plant material		Original nitrogen content %	Organic nitrogen content of rotted material %	Loss of organic matter %	"Nitrogen factor"
Rice straw (abnormal manurial treatment)	No N	0·31	0·59	25·36	0·13*
	With N	0·31	1·37	25·37	0·63
Maize straw (Brazil)	No N	1·02	1·61	36·7	—
	0·5 % N	1·02	1·79	31·53	0·20
	1·0 % N	1·02	1·86	28·31	0·31
	2·0 % N	1·02	2·21	26·20	0·28
Bean husks:					
Sample I	With N	3·00	6·48	44·08	0·62
Sample II: (*a*)	No N	2·99	4·80	48·31	−0·51
(*b*)	No N	2·99	4·64	47·16	−0·54
(*a*)	With N	2·99	5·40	42·54	0·11
(*b*)	With N	2·99	5·38	40·93	0·18

* Absorbed from ammonia in incubator.

some interest, since it emphasises the fact that the nitrogenous figures represent an equilibrium between immobilisation on the one hand and ammonification on the other. The equilibrium point may change during the course of a decomposition, for as the various plant constituents are removed and by-products formed, there is a sequence of active forms. Under the conditions of the experiments given above, relative stability would have been reached before the end of 48 days. A higher "nitrogen factor" may be recorded earlier as shown in Series A (flax straw) or in other cases there may be a fall followed by a rise.

II. *"Nitrogen equivalent."*

The "nitrogen factor" is an expression which contains no direct reference to the efficiency of the organism or organisms in decomposing the plant material in question, or inversely, to the availability of the material. Two plant materials may be found to have the same "nitrogen factor," say 0·5, but one may have lost in decomposition 50 % and the other only 20 %. Obviously the organisms in the former case were more efficient per unit of nitrogen, the plant material being readily available. If, however, the nitrogen requirements are re-calculated on a basis of equal amounts of organic material fermented away, a figure is obtained which measures the efficiency of the microbial tissue in decomposition. The term "nitrogen equivalent" is suggested for this factor, some of the theoretical aspects of the use of which have been described in Part IV of this series. In the example given above, the former material would have a "nitrogen equivalent" of 1·0 and the latter of 2·5, indicating the difference in availability of the two materials. The "nitrogen equivalent" may be defined as the nitrogen immobilised in the course of the removal of 100 g. of organic matter from any material. When determined at any given time of decomposition, this factor also is a summation of the

microbial activities up to that point. If the decomposition is effected by a mixed general flora, ammonifying organisms by liberating ammonia from dead microbial tissue undoubtedly reduce the total immobilised nitrogen and render both the "nitrogen equivalent" and the "nitrogen factor" apparent rather than absolute. If rapid ammonification takes place it is possible that a portion of the nitrogen may be active, that is, present as living protein in active microbial tissue more than once during the decomposition. This, however, does not wholly invalidate the use of the "nitrogen equivalent" for mixed flora decompositions, though it must be admitted that its chief value is for pure culture decompositions. As a standard for comparison the figures for Series E, oat straw, may be taken, the initial "nitrogen equivalent" being about 6, falling after 48 days to 1·4. The following figures for rice straw were calculated from those given by Rege [1927] and show the same trend in more detail.

Table V. *"Nitrogen equivalent" of rice straw in decomposition (Rege).*

Time in days	4	8	12	16	20	24	28	36	40
Loss of dry matter (%)	4·4	8·8	24·6	30·2	36·1	39·2	41·4	43·2	45·0
"Nitrogen factor"	0·12	0·53	0·88	0·90	0·92	1·02	0·93	0·91	0·90
"Nitrogen equivalent"	2·71	6·01	3·58	2·98	2·55	2·60	2·24	2·10	2·00

The standard "nitrogen equivalent" likely to be reached by mixed flora decomposition of a common straw is about 1·5, it being assumed that it has a "nitrogen factor" of 0·75 and suffers a loss of organic matter of 50 %.

The effect of extraction of the several plant materials on the "nitrogen equivalent" does not appear to be so regular as in the case of the "nitrogen factor." The removal of easily soluble materials in the case of the straw extracted with hot water caused a high initial "nitrogen equivalent" falling at the close to about 3, a figure distinctly higher than that for the normal straw. In the case of the flax shives the initial "nitrogen equivalent" is only a little over 2, falling ultimately to 0·6. There is similarly a considerable difference in trend between the highly cellulosic materials in Series C, G and J, indicating differences in availability which cannot be assessed by any method of chemical analysis.

Conclusions.

To determine the relationship, if any, between the chemical composition of a material and the quantity of inorganic nitrogen temporarily immobilised during decomposition was the purpose of these experiments, and in this only partial success has been achieved. It is possible to indicate approximately what the "nitrogen factor" is likely to be, knowing the general composition of the material, but any absolute prediction is not possible for at least two reasons. In the first place the mechanical and physical differences in structure cannot be determined or taken into account, but must affect considerably the biological availability of the tissue. In the second place, the nitrogen picture is obscured by the fact that it is only possible to determine the changes

in the quantity of added inorganic nitrogen immobilised. It is impossible to learn whether the natural proteins of the plant tissue are also utilised. The evidence in the case of Series D, willow peelings, would suggest that in the presence of a sufficiency of inorganic nitrogen, they are not utilised, but there is no means of being certain on this point. It is possible that plant proteins are as readily ammonified as microbial tissue and that in the presence of ammonifiers, the normal condition in the case of a mixed flora, the ammonia liberated would be utilised by other organisms. If this is the case, the actual total "active" nitrogen would be considerably in excess of the "nitrogen factor" and the latter would merely be the difference between the amount of nitrogen liberated from the plant proteins and the total nitrogen requirement of that plant material in decomposition. If that is so, then the amount and availability of the nitrogenous substances of the plant is of great significance in determining the "nitrogen factor." As "availability" is impossible to ascertain, any prediction on these grounds is of doubtful value.

It seems that a high "nitrogen factor" is likely to be given by a substance containing about 50 % Cross and Bevan cellulose, not more than 20 % lignin, a fairly high content of hemicelluloses as measured by free pentose content, and a low nitrogen content, provided that it also contains water-soluble material. The removal of the water-soluble material seems markedly to affect the "nitrogen factor," and plant wastes that have been submitted to extraction with water or removal of these substances by biological means, although otherwise suitable, are likely to have a "nitrogen factor" considerably lower. A highly cellulosic material low in nitrogen will have a "nitrogen factor" of 0·3 to 0·4, while high lignification depresses the factor, since decomposition is impeded.

Summary.

1. No direct relationship was found between the composition of plant materials and the amount of additional available nitrogen immobilised during decomposition ("nitrogen factor").

2. The nitrogen factor represents only the equilibrium between immobilisation on the one hand and ammonification on the other, and does not necessarily represent the whole of the nitrogen active in decomposition, since the plant proteins may also be attacked and utilised in part, or microbial nitrogen may be liberated and re-utilised.

3. Plant materials already containing sufficient or more than sufficient nitrogen for decomposition may nevertheless immobilise an additional amount, owing to preferential utilisation of the inorganic form.

4. If the nitrogen requirements of materials in decomposition are related on the basis of equal amounts of organic matter removed, a figure is obtained which expresses the efficiency of the organism or mixed flora effecting the decomposition. This "nitrogen equivalent," like the nitrogen factor is a summation of the microbial activities and is apparent rather than absolute.

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