CXLVIII. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

II. THE RÔLE OF THE FURFURALDEHYDE-YIELDING SUBSTANCES IN THE DECOMPOSITION OF STRAWS.

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INTRODUCTORY.

THAT the decomposition of cellulosic materials, whether in the soil, the manure heap, or in composts, is a microbiological process has long been recognised, but it sometimes appears to be overlooked that this process is not a single action, but the resultant of the metabolic activities of many organisms, the microflora varying according to the conditions obtaining. Although the morphological characters of many organisms participating in these processes have been described, in but few cases have their particular biochemical actions on the plant constituents been determined. In this work it is proposed to study first the general process of decomposition, and then the biochemical activities of some of the more important organisms which are found.

It is obvious that the microbiological decomposition of a particular plant tissue depends on the availability of its chemical components to the organisms present. If it is to be decomposed rapidly there must be present available carbonaceous material both to meet the energy requirements, and to build up the necessary protoplasm. At the same time there must be a sufficiency of nitrogen to meet the structural demands of the micro-organisms in building up biological protein. Mature plant materials tend usually to contain rather a low percentage of nitrogenous substances, and many investigators have shown that the amount of nitrogen available controls the rate and degree of decomposition by micro-organisms, provided that the carbonaceous constituents of the material are at all available to the organisms concerned. The nitrogen is immobilised temporarily by conversion into protein and allied nitrogenous compounds, in the protoplasm of the organisms. In this immobilisation of nitrogen, and, to a lesser extent, the immobilisation of other necessary inorganic ions, lies the explanation of the injurious effect of the addition of straw to the soil on the growth of the crop following. Hutchinson and Richards [1921], in their work on the production of artificial manure from vegetable materials, recognised that simple nitrogenous substances were essential for this decomposition, and determined the quantity necessary for a satisfactory

rot under normal aerobic conditions. They found that for 100 g. of straw about 0.7 g. of nitrogen in a simple form was necessary for rapid decomposition. Moreover they found that the addition of an excess of nitrogen retarded very considerably the rate and extent of this process, 2 % being apparently the upper limit. In their patent for the making of artificial manure (Adco) Richards and Hutchinson [1924] claimed that materials "which contain an adequate total quantity of carbohydrate (for example, 30 % upwards) such as starch or pentosans, and preferably not too high a proportion of ligno-cellulose" were capable of undergoing decomposition in this way. This patent appears to contain the first record of an attempt to account for the decomposability of any tissue in terms of the substances composing it.

Hebert [1892] had examined the order of decomposition of the constituents of straw and stated that vasculose (lignin) was the most resistant. This was borne out by Dvöřak [1912], who found that mature and old plants decomposed less readily than young plants owing to the resistant lignocellulose in the former. In connection with his work it must be remembered that the nitrogen content of the young plants is as a rule much higher than in the same plant at maturity.

Accepting therefore the importance of nitrogen in this process, the problem of decomposition of vegetable materials appears to be bound up with the chemical composition of the plant, and the availability of its constituents to the microflora present. This aspect of the question was investigated by Rege [1927] (see p. 1378).

Waksman and Tenney [1926, 1927, 1, 2, 1928], in investigating the formation of humus, come to the conclusion that it is the lignin fraction which is the most resistant constituent of plant tissues, and the one which accumulates during the process of rotting. The soluble materials, sugars, starch, etc., the cellulose, and pentosans are progressively removed.

SCHEME OF WORK.

The investigations to be described are centred round the furfuraldehyde-yielding constituents, and the relative importance of these substances in the rotting of plant materials in the presence of sufficient available nitrogen. Since it is possible to differentiate between the different furfuraldehyde-yielding substances as described in the preceding paper, an examination is made of the theory put forward by Rege [1927] of the rôle of these substances, grouped together by him as "pentosans." In all the experiments described herein the rotting was effected by a mixed natural flora. More critical studies are in progress in which the biochemical effects of single organisms isolated from the natural flora are being determined.

EXPERIMENTAL.

The materials used in these preliminary investigations were straws, since these are known to rot comparatively easily under normal conditions. Two

were selected, oat straw, which decomposes readily, and rye straw which appears in practice to be somewhat more resistant. Detailed analyses of each of these are given in the preceding communication [Norman, 1929]. So that the rotting might be as complete as possible the optimum conditions were studied and adopted. The straws were chaffed for convenience in handling and to ensure proper wetting. Equal amounts of straw of known moisture content were weighed out and moistened thoroughly by spraying, being then bottled. Available nitrogen was added in the form of ammonium carbonate solution, to the extent of 1 g. nitrogen to 100 g. straw. The bottles were then laid down in an incubator at 35°, and frequently turned, the contents being stirred up periodically for the first few days to ensure even distribution of the nitrogen and uniform wetting. It is important that the straw be kept thoroughly wet throughout the process, additional water being supplied by a spray when necessary. Water-logging, however, must at all costs be avoided, since anaerobic conditions then set in, and the whole course of the process is altered.

In the case of the oat straw fermentations, an inoculum from old well-rotted manure was added to each, but later it was found that this is quite unnecessary since chaffed straw itself commonly carries an extensive and efficient microflora. The rye straw fermentations therefore did not receive any inoculum.

Every four days a bottle was taken for the dry weight determination. The following analyses were carried out on the dried residue in each case, the methods employed being precisely as described in the preceding paper [Norman, 1929].

- (i) Ash.
- (ii) Total furfuraldehyde yield.
- (iii) Cellulose (Cross and Bevan).
- (iv) Furfuraldehyde from Cross and Bevan cellulose.
- (v) Calcium pectate yield.
- (vi) Carbon dioxide yield (uronic acids).

Mycelial hyphae were observed by the sixth day, remaining visible till about the twentieth day. In the case of large bottles containing a considerable quantity of straw larger forms, such as *Coprinus*, sometimes appeared at the end of five or six weeks. In no case, however, did this occur in the smaller bottles as used for analysis.

The analytical figures obtained for Series I (oat straw) and Series II (rye straw) are given in Tables I and II; they are all calculated on the basis of 100 g. of the original straw employed.

It was shown in the preceding paper that it is possible to obtain from these figures considerable information as to the quantity of the various furfuraldehyde-yielding substances present. The groups upon which light is thrown and the method by which they are arrived at are as follows.

- (i) *Total uronic acid anhydride.*
 $\text{CO}_2 \text{ yield} \times 4.$
- (ii) *Uronic acid anhydride in pectin.*
 $\text{Calcium pectate yield} \times \frac{70}{100}.$
- (iii) *Non-pectin uronic acid, i.e. in hemicelluloses*
 Difference of above.
- (iv) *Furfuraldehyde from total uronic acid (D).*
 $\text{Total uronic acid} \times \frac{16.66}{100}.$
- (v) *Furfuraldehyde from pectin (C).*
 $\text{Calcium pectate yield} \times \frac{19.8}{100}.$
- (vi) *Furfuraldehyde from uronic acid in pectin (E).*
 $\text{Uronic acid in pectin} \times \frac{16.66}{100}.$
- (vii) *Furfuraldehyde from pentose in pectin.*
 $(C - E).$
- (viii) *Furfuraldehyde from pentose groups in hemicelluloses.*
 $[A - \{B + D + (C - E)\}]$
 where A = total furfuraldehyde yield,
 B = furfuraldehyde from C. and B. cellulose.
- (ix) *Xylan associated with C. and B. cellulose.*
 $\text{Furfuraldehyde in C. and B. cellulose} \times 1.55.$
- (x) "Pure" cellulose.
 (C. and B. cellulose — associated xylan.)

N.B. This factor 1.55 converts furfuraldehyde to anhydroxylose, and is applied in the case of the two straws under investigation since it has been shown that it is xylan that is present. In other materials it may not be xylan, and in consequence another factor will have to be found.

Table I. *Decomposition of oat straw at 35°.*

(Expressed on 100 g. original dry straw.)

Day	Dry matter	Organic matter	Total furfurald. yield (A)	C. and B. cellulose	Furfurald. yield from C. and B. cellulose (B)	Calcium pectate yield	CO ₂ yield	Lignin by 72 % H ₂ SO ₄
—	100.0	92.93	16.08	53.14	5.99	1.10	1.21	18.5
4	92.3	85.11	12.29	52.13	6.00	1.16	1.20	—
8	85.5	78.41	9.96	39.63	4.51	1.43	1.14	—
12	76.5	69.13	9.16	34.37	4.06	1.52	1.03	—
16	72.5	65.43	8.86	31.09	3.90	1.53	1.06	—
20	69.2	62.16	9.37	28.54	3.83	1.56	0.97	—
24	66.3	59.19	9.24	27.04	3.77	1.66	0.89	—
28	65.4	58.28	8.92	25.21	3.57	1.80	0.89	—
32	64.7	57.66	7.67	25.02	3.47	1.70	0.92	—
36	64.0	56.98	6.77	23.70	3.29	1.77	0.92	—
40	63.5	56.45	6.51	22.58	2.95	1.76	0.87	—
56	61.0	53.92	6.12	21.67	2.71	1.76	0.65	—
85	55.9	48.85	5.59	19.91	2.29	1.73	0.65	—

Table II. *Decomposition of rye straw at 35°.*

(Expressed on 100 g. original dry straw.)

Day	Dry matter	Organic matter	Total furfurald. yield (A)	C. and B. cellulose	Furfurald. yield from C. and B. cellulose (B)	Calcium pectate yield	CO ₂ yield	Lignin by 72 % H ₂ SO ₄
—	100.0	96.54	17.05	55.27	5.64	0.34	1.15	19.5
4	96.9	93.46	15.07	54.09	5.68	0.34	1.08	—
8	79.1	75.61	10.73	40.86	5.56	0.40	0.79	—
12	72.4	68.92	10.02	36.89	5.02	0.43	0.81	—
16	68.5	65.00	8.38	32.56	3.89	0.50	0.76	—
20	66.2	62.73	7.69	31.41	3.72	0.54	0.72	—
24	63.1	59.67	7.21	29.48	3.35	0.60	0.66	—
28	60.4	56.94	7.63	28.77	3.35	0.70	0.71	—
33	59.7	56.20	7.36	27.58	3.20	0.73	0.69	—
36	59.3	55.84	7.26	27.32	3.21	0.77	0.70	—
40	58.4	54.96	7.26	26.77	3.18	0.80	0.70	—
48	56.9	53.35	7.22	26.41	3.22	0.80	0.71	—
84	50.5	47.01	5.75	23.17	2.67	0.82	0.51	—

Table III. *Decomposition of oat straw at 35°.*

Furfuraldehyde-yielding constituents and cellulose.

Day	Total uronic acid anhydride	Uronic acid in pectin	Non-pectin uronic acid	Furfurald. from total uronic acid (D)	Furfurald. from pectin (C)	Furfurald. from uronic acid in pectin (E)	Furfurald. from pentose in pectin (C-E)	Furfurald. from pentose groups in hemi-celluloses [A - {B + D} + (C - E)]	Xylan associated with C. and B. cellulose	"Pure" cellulose by difference
—	4.84	0.77	4.07	0.81	0.21	0.13	0.08	9.2	9.28	43.86
4	4.80	0.81	3.99	0.80	0.23	0.14	0.09	5.4	9.3	42.83
8	4.56	1.00	3.56	0.76	0.28	0.17	0.11	4.6	7.0	38.63
12	4.12	1.06	3.00	0.68	0.29	0.18	0.11	4.4	6.3	31.07
16	4.24	1.07	3.17	0.71	0.30	0.18	0.12	4.1	6.1	27.59
20	3.88	1.09	2.79	0.65	0.31	0.18	0.13	4.8	6.0	23.54
24	3.56	1.16	2.40	0.59	0.32	0.19	0.13	4.7	5.9	21.14
28	3.56	1.26	2.30	0.59	0.35	0.21	0.14	4.6	5.6	19.61
32	3.68	1.19	2.50	0.61	0.33	0.20	0.13	3.5	5.4	19.62
36	3.68	1.23	2.45	0.61	0.34	0.21	0.13	2.8	5.1	18.60
40	3.48	1.23	2.25	0.58	0.34	0.20	0.14	2.8	4.6	17.98
56	2.60	1.23	1.37	0.43	0.34	0.20	0.14	2.8	4.2	17.47
85	2.60	1.21	1.39	0.43	0.33	0.20	0.13	2.7	3.6	16.31

Table IV. *Decomposition of rye straw at 35°.*

Furfuraldehyde-yielding constituents and cellulose.

Day	Total uronic acid anhydride	Uronic acid in pectin	Non-pectin uronic acid	Furfurald. from total uronic acid (D)	Furfurald. from pectin (C)	Furfurald. from uronic acid in pectin (E)	Furfurald. from pentose in pectin (C-E)	Furfurald. from pentose groups in hemi-celluloses [A - {B + D} + (C - E)]	Xylan associated with C. and B. cellulose	"Pure" cellulose by difference
—	4.60	0.24	4.36	0.77	0.07	0.04	0.03	10.6	8.7	46.54
4	4.32	0.24	4.08	0.72	0.07	0.04	0.03	8.6	8.8	45.30
8	3.16	0.28	2.88	0.53	0.08	0.05	0.03	4.6	8.6	32.24
12	3.24	0.30	2.94	0.53	0.09	0.05	0.04	4.4	7.8	29.11
16	3.04	0.35	2.69	0.51	0.10	0.06	0.04	3.9	6.0	26.60
20	2.88	0.38	2.50	0.48	0.11	0.06	0.05	3.4	5.7	25.66
24	2.64	0.42	2.22	0.44	0.12	0.07	0.05	3.4	5.2	24.29
28	2.84	0.49	2.35	0.47	0.14	0.08	0.06	3.7	5.2	23.58
33	2.76	0.51	2.25	0.46	0.14	0.08	0.06	3.6	5.0	22.62
36	2.80	0.54	2.26	0.47	0.15	0.09	0.06	3.5	5.0	22.35
40	2.80	0.56	2.24	0.47	0.16	0.09	0.07	3.6	4.9	21.84
48	2.84	0.56	2.28	0.47	0.16	0.09	0.07	3.5	5.0	21.42
84	2.28	0.57	1.71	0.38	0.16	0.10	0.06	2.6	4.1	19.03

These various calculations were made on the results of each series, and are quoted in Tables III and IV.

All figures of special significance are graphed below. In each case time is plotted against absolute loss, not percentage loss, on the amount of the constituent originally present in 100 g. dry straw. Figs. 1 and 4 deal with the loss of major constituents in oats and rye respectively, 2 and 5 the fall in furfuraldehyde yields, and 3 and 6 the loss in uronic contents.

DISCUSSION OF RESULTS.

For the sake of clarity it will be advisable to confine this discussion first to the figures obtained for the decomposition of oat straw, as given in Tables I and III and Fig. 1.

The most prominent feature of the decomposition of this straw is not so much the very rapid loss of organic matter, amounting to one-third of the whole in 24 days, but the loss in this time of 50 % of the cellulose of the straw, equivalent in quantity to two-thirds of the lost organic matter. At the close of the period of 2 months there is a loss of 42 % of the organic matter, the loss of cellulose over the same period being 60 % of that originally present. The cellulose lost accounts for nearly 70 % of the lost organic matter. It is perfectly clear from this, and from other observations recorded that the most important feature of this type of decomposition is the loss of cellulose. There may be other constituents which sustain a greater percentage loss, but nevertheless, the actual bulk loss of cellulose is by far the greatest.

On Fig. 1 there is plotted in dotted line the values for fall in furfuraldehyde yield due to pentose groups in the hemicelluloses. In the previous paper [Norman, 1929] it was shown that the hemicelluloses originally present in oats yielded from their pentose groups 40.4 % of furfuraldehyde so that the 9.2 % of furfuraldehyde from this source in 100 g. of straw represents a hemicellulose content of 22.8 %. It is not, however, justifiable to apply this factor for obtaining hemicellulose content to the analyses throughout the decomposition, since it is certain that it is only a mean figure from a mixture of various individual hemicelluloses, some of which may be more readily available than others, and consequently more quickly removed, thereby causing that factor to vary somewhat. However, considering for this purpose the furfuraldehyde yield to be an approximate measure of hemicellulose content, it is observed that there is over the whole period of 2 months a loss of nearly 70 % of these substances. The significant point, however, is that more than 40 % is removed in the first 4 days and a further 10 % in the next period of 4 days.

The removal of the xylan associated with cellulose appears to be slow and fairly constant in rate. In 2 months just over 70 % of the total amount present is lost. The rate is presumably controlled by the rate of removal of the cellulose.

Similar features are observed in the decomposition of rye straw as given

Fig. 1. Decomposition of oat straw at 35°. (Expressed on 100 g. original straw.)

Fig. 2. Decomposition of oat straw at 35°. Fall in furfuraldehyde yields. (Expressed on 100 g. original straw.)

Fig. 3. Decomposition of oat straw at 35°. Loss of uronic acid anhydride. (Expressed on 100 g. original straw.)

Fig. 4. Decomposition of rye straw at 35°. (Expressed on 100 g. original straw.)

Fig. 5. Decomposition of rye straw at 35°. Fall in furfuraldehyde yields. (Expressed on 100 g. original straw.)

Fig. 6. Decomposition of rye straw at 35°. Loss of uronic acid anhydride. (Expressed on 100 g. original straw.)

in Tables II and IV and Fig. 4. The early stages in this case are not so rapid as in oat straw since it is a much more difficult matter to get proper wetting of rye straw. The loss over a period of 2 months is slightly greater than the loss of oat straw in the same period, being about 43 %. This is somewhat surprising in view of the fact that rye straw is always considered to be more resistant than oat straw, which is regarded as the cereal straw that is most easily decomposed. Again the rapid loss of cellulose is noteworthy. In 2 months 54 % of that originally present is lost, and of this 70 % is removed during the first 12 days.

The loss of hemicelluloses as measured by the furfuraldehyde due to their pentose units is also extremely rapid in the early stages, just as occurred in the case of oats. A loss of 57 % is observed in the first 8 days, the total over 2 months being only 69 %.

The xylan associated with cellulose appears to be removed at a fairly constant rate. Over the period of 2 months a loss of about 45 % of the total amount is sustained. This is considerably less than is observed in the case of oat straw.

To sum up then, the most obvious feature in each case is the rapid loss of the major portion of the hemicelluloses, followed by an equally rapid loss of cellulose. The hemicelluloses, however, appear to remain at much the same level after the first 12 or 14 days, while the cellulose continues progressively to decrease. There is some evidence that this former observation may possibly be more apparent than real.

To regard the hemicellulose present in any plant material as a homogeneous substance is to be in error. As has been shown in the case of these straws, there are two or more types present, separable by differences in physical behaviour. These two types, called A and B, are probably themselves mixtures also. All that it is possible to say is that they are built up on the same general plan, from pentoses, hexoses, and uronic acids, and possess some physical properties in common. Of the method of linkage nothing is known. Since the hemicelluloses of a plant tissue are non-homogeneous chemically it is unjustifiable to regard them as of equal biological value. Not only must the availability vary considerably, but some organisms seem incapable of breaking down certain pentose sugars and pentose groupings. Further there is more than a possibility that certain groups only of the hemicelluloses might be removed leaving new intermediate residues. The microbiological decomposition of this type of substance is an unexplored field.

In Fig. 2 various furfuraldehyde yields are plotted for the decomposition of oat straw. The fall of total furfuraldehyde yield is extremely rapid during the first 8 or 12 days as has been seen. After that period there is, however, a small but none the less definite increase, indicated by a depression in the curve for fall of furfuraldehyde yields. The whole of the furfuraldehyde lost during the first 4 days is seen to be due to the loss of pentose groups in the hemicelluloses. The increase of furfuraldehyde-yielding substance which occurs

later is shown also to be due to pentose groups apparently formed by the organisms from some other substance more rapidly than they are removed. The same features are visible in Fig. 5 for rye, though they are there not quite so well marked. These observations make it clear that the curve representing furfuraldehyde loss from pentose units applies to loss of original hemicelluloses only in the opening stages. The pentose units present after that period must consist of those of the original hemicelluloses as yet unattacked together with pentose units produced by biological action on some other grouping. In the later stages it is not improbable that the furfuraldehyde due to pentose units is almost entirely due to hemicelluloses or hemicellulose residues which are biologically not readily available, the accumulation of transitionally formed pentose units being removed as the rate of decomposition slackens.

The source of these transitory substances cannot be definitely stated, though in all probability they arise from the cellulose, of the route of the biological decomposition of which little is known. It is unreasonable to suppose that such a complex substance as cellulose must be broken down swiftly and completely to carbon dioxide and water without the appearance of any intermediate product. Most theories of cellulose fermentation [see Buchner and Meisenheimer, 1908] postulate a breakdown to cellobiose, glucose and thence to lactic acid from which various acids and aldehydes are formed. There is, however, an absence of evidence for such a route. Cellulase must be regarded not as a single enzyme but as a team of enzymes. Possibly in a rapid decomposition such as this, the cellulose may be destroyed in part symbiotically. There is some evidence supporting the production of some intermediate substance in cellulosic decomposition. The aerobic organism *Spirochaeta cytophaga* described by Hutchinson and Clayton [1919] decomposed cellulose readily with the formation of fatty acids, a yellow pigment, and a mucilaginous substance, non-reducing in character. The physical behaviour of this substance, together with the fact that it was definitely not of a dextrin nature, caused them to suggest that it resembled the pectic substances. In point of fact their evidence fits more closely the hemicellulose group, but it is possible that there is present a mixture of several degradation products. This paper contains the first clear reference to the production of intermediary substances in the decomposition of cellulose. Indirect evidence exists pointing to the same conclusion, but throwing no light on the nature of the substances concerned. In the decomposition of woods, as the cellulose is removed, there is a marked increase in alkali-soluble constituents. Although alkali-solubility seems always to be determined in studies on wood, no record exists of an attempt to determine the nature of the portion removed.

It may be, to take an extreme view, that all the hemicelluloses found at the close of active rotting are not constituents of the original material, but that they have been formed as intermediary compounds in the breakdown. Possibly even the furfuraldehyde does not arise from transitional pentoses at all but from some other grouping yielding furfuraldehyde under the conditions

of that estimation. If this is the case it would be expected that these transitional substances would disappear if the decomposition is greatly prolonged. However the losses of furfuraldehyde-yielding constituents under such conditions are only slow, and comparable with the loss of cellulose. It seems more satisfactory to view this residue of furfuraldehyde-yielding constituents as biologically less available either for constitutional or physical reasons. An investigation of the nature of these substances remaining at the end of several months is being undertaken with a view to throwing light on this point. Attempts will also be made to isolate any intermediary compounds which may be present earlier in the decomposition.

Other features of interest in the decomposition are shown in Figs. 3 and 6 in which the losses of uronic acid anhydride contents are plotted. In each case the total loss of uronic acid is steady and slow. The variations which are observed are within the limits of error of this determination. The most interesting point, perhaps, is that the pectin of the straw does not appear to be removed during the decomposition. In fact the figures would seem to indicate that there is a definite synthesis. Whether pectin itself is produced, or some other substance which is estimated as pectin under the conditions of that determination, it is not possible to say. The percentage content in oat straw rose from 1.1 to 1.7 in just a month, and seemed to remain constant at that. In rye which contains considerably less, the final figure is 0.8. Corrections in furfuraldehyde yield have been made on the assumption that it is pectin that is formed, but whether this is justifiable or not it is impossible to say. The corrections applied for this in the case of rye straw are hardly significant, but are applied to demonstrate the method. There is some evidence that the pectin is destroyed if the reaction of the rot becomes acid—an abnormal condition. Certainly the organisms present appear to decompose pure pectin actively, but not if the medium is kept neutral by the addition of chalk.

The loss of non-pectin uronic acid is presumably due to the degradation of the uronic groups in the hemicelluloses. That it is as small as it is would seem to support the theory mentioned above that there is a considerable residue of unavailable hemicellulose. If that is not so, then the uronic acid portion of the molecule must itself be less readily decomposed than the remainder, and intermediate compounds of high uronic acid content must be left.

GENERAL DISCUSSION.

From the results cited here, and from an analysis of those given by Rege [1927] and Waksman [1926, 1, 2; Waksman and Tenney, 1926; 1927, 2; 1928] it becomes clear that the theory of decomposition of cellulosic material as put forward by Rege is no longer tenable. Since the removal of cellulose is the most important feature of the process, and since the major loss of carbonaceous material is loss of cellulose, the cellulose must obviously be regarded as the most important food material, playing the chief part in the conversion of inorganic nitrogen to microbial protein. Rege's contention

that the large loss of cellulose is apparent, not real, cannot be justified by his own figures. It is true that the chlorination method of estimation of cellulose is not entirely satisfactory, but the error can only be small. Waksman and Tenney [1927, 1] who used a cuprammonium method for this estimation give figures in every way paralleled by those from the chlorination method. Observing the rapid decomposition of hemicelluloses which takes place in the first few days, and having proved that these substances are, unlike cellulose, readily available to the organisms in pure culture, Rege assumes that their presence is necessary as food, and that they therefore control the decomposability of the material. He is undeniably correct in regarding lignin as most resistant to biological attack and therefore the lignin content as a factor militating against decomposition. Rege connects the two factors, hemicellulose and lignin, and states that it is possible to predict the decomposability of any material by determining these two substances. If the ratio of food or energy factor, as he termed the hemicelluloses, to the inhibitory or lignin factor is greater than unity the material is easily decomposed, or if less than 0.5 it is very resistant. Such a theory ignores entirely the cellulose which forms a large part of plant tissues, and, as has been shown, forms the chief source of carbonaceous material for the organisms decomposing it. Moreover it fails to take into account mechanical and physical differences in structure of the tissues concerned. It is obvious that it is not possible to insert a factor into any formula to cover this variable quantity, but nevertheless it must be recognised as existing. Lignin being of a resistant nature acts as a physical barrier to the organism in search of food, and therefore the quantity present is an important factor, but no less important is its distribution, which cannot obviously be ascertained by any chemical means. The nearest one can get to a general statement is to say that the presence of a considerable quantity of cellulose and available hemicelluloses favours the decomposition of a tissue, while the presence of increasing quantities of lignin retards it.

The advisability of fixing a numerical relationship between these two is a matter upon which it is difficult to come to a decision. If it is recognised that structural and physical conditions are variables which it is not possible to assess, and that any decision on the basis of chemical determinations is subject to this other factor, then there can be no serious objection to its employment. With the above reservation it may be stated that if the ratio of cellulose and hemicelluloses to lignin is well over 3 the material will decompose fairly readily. If, however, it is in the neighbourhood of 2 decomposition will be very slight or non-existent. The following figures may be quoted.

Material	C. and B. cellulose + hemicelluloses	Lignin	Ratio
Oat straw	$53.14 + 22.8 = 75.94$	18.5	4.1
Rye straw	$55.27 + 33.4 = 88.67$	19.5	4.5
Deal	$66.24 + 4.3 = 70.54$	27.6	2.5

The figures for the two straws indicate clearly the importance of structural and physical considerations as stressed above. It might be supposed that rye straw decomposed more easily than oat straw, which in point of fact is not

the case. Oat straw decomposes with great ease, while rye straw in practice presents considerable difficulty, owing to its stiff waterproof nature.

Attempts have been made to provide further support for this theory by varying artificially the proportions of these groups in a natural material. That the hemicelluloses are not of pre-eminent importance is stressed by the work just to be described. Rye straw, which had been extracted to remove the hemicelluloses as completely as possible, was rotted down rapidly, though cellulose and lignin only were present. It is true that the lignin content was also lowered by the alkali treatment, but nevertheless, in accordance with the theory put forward by Rege [1927], the material should have been undecomposed.

EXPERIMENTAL.

Chaffed rye straw, as used for the above investigations, was extracted with hot water, and then four times for 4 hours each time with 4 % sodium hydroxide. It was very thoroughly washed and allowed nearly to dry, being broken up thoroughly. Equal portions were weighed out into bottles and moistened with a mixed mineral salt solution, since it was supposed that the alkali treatment would have removed much of this. Inorganic nitrogen was added as before, as ammonium carbonate, and an inoculum from rotting rye straw. The bottles were incubated at 35° and one taken for analysis every 8 days. The analytical figures obtained are given in Tables V and VI and illustrated in Figs. 7 and 8.

Table V. *Decomposition at 35° of rye straw extracted to remove hemicellulose and pectin.*

(Expressed on 100 g. rotted material.)

Day	Dry matter	Ash	Total furfurald. yield	C. and B. cellulose	Furfurald. yield on 100 g. C. and B. cellulose	Furfurald. yield on cellulose present	Lignin by 72 % H ₂ SO ₄
—	100.00	1.48	5.73	90.01	5.57	5.01	9.3
8	89.02	1.66	4.05	87.44	3.67	3.21	—
16	82.71	1.79	3.90	83.90	3.75	3.15	—
24	76.49	1.92	3.50	82.68	3.25	2.79	—
32	73.99	2.00	3.30	80.04	3.48	2.79	—
48	68.85	2.15	3.20	75.97	3.10	2.36	—

Table VI. *Decomposition at 35° of rye straw extracted to remove hemicellulose and pectin.*

(Expressed on 100 g. dry extracted straw.)

Day	Dry matter	Organic matter	Total furfurald. yield	C. and B. cellulose	Furfurald. yield on C. and B. cellulose	Furfurald. from pentose groups in hemicellulose	Xylan associated with C. and B. cellulose	"Pure" cellulose by difference
—	100.00	98.52	5.73	90.01	5.01	0.72	7.77	82.24
8	89.02	87.54	3.60	77.84	2.86	0.74	4.46	74.38
16	82.71	81.23	3.22	69.40	2.60	0.62	4.06	65.34
24	76.49	75.01	2.68	63.25	2.13	0.55	3.34	59.91
32	73.99	72.51	2.44	59.24	2.06	0.38	3.22	56.02
48	68.85	67.37	2.20	52.30	1.62	0.58	2.53	49.77

The result of the preliminary extraction, admittedly a severe one, was to remove all but a trace of free hemicellulose, and to lower the content of lignin from 19.5 % to 9.3 %. At the same time the xylan associated with the Cross and Bevan cellulose product was also reduced considerably.

In spite of the absence of free hemicelluloses, the material decomposed quite rapidly, practically 40 % of the cellulose being removed in 7 weeks. It will be seen from Fig. 7 that the loss of cellulose coincides almost exactly with the loss of organic matter throughout, despite the fact that the xylan

Fig. 7. Decomposition of extracted rye straw at 35°. (Expressed on 100 g. extracted straw.)

Fig. 8. Decomposition of extracted rye straw at 35°. Fall in furfuraldehyde yields. (Expressed on 100 g. extracted straw.)

associated with cellulose is also being removed. This gives additional evidence for the production from cellulose of some intermediate substance, for the apparent loss of cellulose and xylan at the end of 7 weeks is 37.5 g. expressed on 100 g. of extracted straw, while the total loss of organic matter over the same period is only 31 g. Fig. 8, which gives the fall in furfuraldehyde yields from the various groups, does not seem to indicate that there is any large production of transitional pentose or furfuraldehyde-yielding substances, although there is undoubtedly some.

To sum up, then, the decomposition seemed to take a perfectly normal course despite the absence of hemicelluloses. The organisms were able to obtain what energy they required from the cellulose, which was accordingly broken down rapidly. The decomposition was no doubt aided by the reduction of lignin, since a greater surface of cellulose would be exposed to attack. The final product, though light in colour, was not unlike well rotted manure.

CONCLUSIONS.

The biological decomposition of plant materials is the resultant of the activities of many organisms. Carbon compounds are required mainly as a source of energy for the organisms, though in part also for structural purposes. The most important carbonaceous substance in the tissue appears to be cellulose, and since it is rapidly removed under normal conditions it must be regarded as the chief source of energy for the micro-organisms. The hemicelluloses, of which there is usually a much lower percentage, are also utilised, and particularly so during the opening stages of the decomposition. Lignin has been shown by many workers to be an unavailable source of energy to ordinary micro-organisms. Being resistant it forms a barrier preventing them from reaching available sources of energy, and the ratio of available carbonaceous material to lignin must be some measure of the probability of decomposition of any material. The rapid early loss of hemicelluloses has probably two contributory causes. In the first place the hemicelluloses are presumably encrusting substances on the walls of the cell, which in any case would have to be removed in part before the cellulose could be attacked. Secondly, it is probably true to say that some hemicelluloses or some groups in the hemicellulose molecule are more easily available to the organisms than is cellulose. Together with the soluble components of the tissue, such as sugars and starches, they play an important part in the provision of easily obtainable energy, and in consequence promote the rapid growth and multiplication of the organisms in the early stages, thereby paving the way for the general attack on the cellulose. Only to the extent of hastening the incidence of the rapid breakdown of cellulose can the hemicelluloses be said to control the decomposition.

The somewhat obscure furfuraldehyde-yielding substance associated with the cellulose seems to play no very prominent part in this process. It appears to be decomposed but slowly and to a limited extent. The rate of its decomposition is probably determined by the rate of removal of the cellulose with which it is in such close association. It must, however, be regarded as an available and not a resistant constituent.

It seems therefore that the hemicelluloses must be deposed from the place of pre-eminent importance assigned to them by Rege [1927] in this type of decomposition, and must be viewed as of equal importance as other available carbonaceous sources of energy, save possibly in the opening stages.

Note on the prediction of the decomposability of any mature plant material.

The prediction of the decomposability of any plant material solely from the results of chemical analyses cannot be wholly satisfactory, in view of the differences in structure which cannot be taken into account in any formula. Nevertheless, despite this drawback, some rough prediction is of considerable value when considering the decomposition of lesser known plant materials. Particularly is it so in the case of the production of artificial manure (Adco) by the method of Richards and Hutchinson [1924]. The relationship which Rege [1927] indicated between "pentosans" and lignin has frequently been employed successfully to predict the decomposition of new materials for this purpose. Although, as has been shown earlier, the basis upon which his predictions are made is not entirely correct, their success is due to the inverse relationship between "pentosans" and lignin, which appears to hold in most plant materials.

Any alternative method need only be approximate but must be relatively simple to carry out. The following is suggested, with some reserve, as a more satisfactory basis for prediction. It involves only the analyses which were necessary for the Rege method. The determinations which are necessary are lignin, total furfuraldehyde yield, Cross and Bevan cellulose, and furfuraldehyde on Cross and Bevan cellulose product. The food or energy factor, as Rege described it, is obtained by adding to the Cross and Bevan cellulose a figure for free hemicelluloses. An approximation for this last may be obtained by multiplying the free furfuraldehyde by 2. This is purely arbitrary, but probably more satisfactory than the Kröber factor, which is too low. In any case the factor 2 errs on the low side for most hemicelluloses, so that under-estimation is more probable than over-estimation. This combined figure for available material is then divided by the figure for resistant material, or

Table VII. *Table demonstrating the use of a ratio to predict the decomposability of mature plant materials.*

(Figures expressed as g. per 100 g. dry matter.)

Material	C. and B. cellulose [A]	Total furfurald. yield [E]	Furfurald. from		Lignin [C]	Ratio: $\frac{A+2B}{C}$	Decom- posability
			C. and B. cellulose [D]	"Free" furfurald. [E - D = B]			
Ext. rye straw	90.01	5.73	5.01	0.72	9.3	9.8	Rapid
Rice straw	45.88	14.5	5.2	9.3	10.31	6.3	"
Oat straw A	51.7	16.8	7.5	9.3	14.18	5.0	"
Wheat straw	52.1	18.3	8.9	9.4	14.57	4.9	"
Barley straw	49.4	16.6	6.6	10.0	16.16	4.3	"
Oat straw B	53.14	16.08	5.99	10.09	18.5	4.0	"
Rye straw	55.27	17.05	5.64	11.41	19.5	4.0	"
Rushes	51.3	16.3	7.4	8.9	17.71	3.9	Slow
Maize straw	43.0	15.2	6.2	9.0	20.34	3.0	"
Willow wood	49.46	13.7	3.8	9.9	24.70	2.8	Very slow
Poplar wood	66.3	12.1	6.4	5.7	28.44	2.7	"
Ash wood	44.64	13.9	2.6	11.3	26.01	2.6	"
Birch wood	44.53	15.2	1.6	13.6	28.27	2.5	"
White ash wood	53.4	13.1	5.4	7.7	28.38	2.4	"
Pine wood	57.4	5.2	3.4	1.8	26.65	2.3	Nil
Fir wood	43.44	6.7	1.7	5.0	29.17	1.8	"

lignin content, the quotient being a measure of the decomposability of the material. It is

$$\frac{[(\text{Cross and Bevan cellulose} + 2 (\text{total furfuraldehyde} - \text{cellulosic furfuraldehyde}))]}{\text{Lignin}}$$

The figures for a number of plant materials are given in Table VII. A few have been calculated from Rege [1927] and a few of the woods from König and Becker [1919].

The last column headed "Decomposability" gives the result of actual trials except in the case of one or two woods. The connection between the observed decomposability and the ratio is clear. If the ratio is above 4 the material decomposes rapidly, and below 3 only very slowly. It must be remembered that this ratio is solely an index of probability of decomposition under optimum conditions, and bears no relationship to the suitability of the material for making artificial manure or to the quality of any product which might be obtained. The amount and availability of the organic matter decomposed is important in that it controls the immobilisation of nitrogen and other inorganic ions to a less extent, and so indirectly, the quality of the manure. The ratio given above, however, is not a measure of the former, which can only be obtained by trial.

SUMMARY.

1. The decomposition of straw under optimum conditions by a mixed natural flora in the presence of sufficient available nitrogen is investigated. The course of the decomposition is followed by frequent and detailed analyses, particular attention being paid to the various types of furfuraldehyde-yielding substances.

2. The most prominent feature of the decomposition is the rapid loss of cellulose, which accounts for the major part of the loss of organic matter. The cellulose therefore forms the chief source of carbonaceous food or energy material for the organisms.

3. The hemicelluloses appear to decompose very rapidly in the first few days, and then to remain at much the same level while the cellulose is still decreasing. This is presumably due to the fact that as hemicelluloses are chemically non-homogeneous, their biological availability may vary very considerably, differing in this from unlignified cellulose. Two reasons may be advanced for the early loss of hemicellulose. Firstly, as they are encrusting substances on the cell wall, they must need to be removed, in part at least, before the cellulose can be attacked. In the second place, some hemicelluloses or some component groups of the hemicellulose molecule are easily available and these together with the soluble plant substances must play an important part in promoting the rapid multiplication of the organism.

4. The xylan associated with the Cross and Bevan cellulose fraction in this straw is not unavailable but is removed only slowly, the rate presumably being controlled by the loss of the cellulose with which it is in such intimate association.

5. There is definite evidence for the production of some intermediary substance which yields furfuraldehyde. That this is produced transitionally in the degradation of the cellulose is probable, though the support for this theory is scanty. The extent of the production of this substance is difficult to assess.

6. The loss of uronic acid from the straw is steady but small, and not paralleled by the apparent loss of hemicellulose. It seems likely that the uronic group is biologically less available, and that the residual hemicelluloses must contain intermediate degradation compounds of uronic content above normal.

7. The small amount of pectin present does not seem to be removed during normal decomposition, but only if acid conditions set in. There is some evidence that there is even a synthesis of pectin, or of some compound estimated as pectin.

8. Subject to structural and physical variations, it is agreed that the decomposition of plant materials in the presence of assimilable nitrogen is favoured by the food or energy factor, and retarded by the resistant factor. The food or energy factor must be regarded as being composed in mature materials of cellulose together with the available hemicelluloses, and the resistant factor as being the lignin fraction.

9. That the hemicelluloses are not of pre-eminent importance has been further supported by the decomposition of rye straw so extracted as to be practically hemicellulose-free.

10. A simple method for the prediction of probability of decomposition of any plant material is given, and its limitations outlined.

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