LXIX. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

VII. THE NATURE OF THE RESIDUAL HEMI- CELLULOSES OF ROTTED STRAW.

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IN the first paper of this series [Norman, 1929, 1] a detailed method was described for the evaluation of the furfuraldehyde-yielding constituents of plant materials, and the nature of these substances in straws was determined. Later, by employing these methods on decomposing straws, the loss of such constituents was followed and their rôle in decomposition investigated [Norman, 1929, 2].

The direct estimation of hemicelluloses has not yet been satisfactorily achieved owing to their heterogeneous structure, and accurate evidence of their removal as a whole was not possible. It was only possible to follow with certainty the changes in the content of pentose and uronic acid units. The hexose units present are indeterminable directly. Acid hydrolysis methods, such as have been proposed by Waksman and Stevens [1930], are unsatisfactory and erratic, inasmuch as it has been shown by Link and Niemann [1930] that the uronic acids are unstable in presence of the concentration of hydrolysing agent employed and undergo decarboxylation with the formation of reducing substances of unknown constitution.

As far as it was possible to judge from the loss of the pentose units in the hemicelluloses, these substances undergo a very rapid and extensive early loss in the decomposition of such materials as oat or rye straw. It seemed that about 50 % of the total hemicelluloses was fermented away in the first week, but that after this point, while the cellulose was being rapidly removed, the hemicelluloses suffered only a small but steady additional loss up to the end of the two months, which was the period of the experiment. Analyses of well-rotted materials which have been made at other times have indicated that when decomposition is practically at a standstill only traces of hemicelluloses remain.

The hemicelluloses are a very indefinite class of polysaccharides, and their physical properties are such that no separation is entirely satisfactory. Hydrolysis studies suggest that the type of linkage involved is similar in different

fractions, and in hemicelluloses from different sources. Biological availability, however, does not depend only on linkage, but also on the nature of the units involved. The following sugars are commonly found—arabinose, xylose, glucose, galactose and mannose, together with glycuronic and galacturonic acids. These vary very much in their utilisation by micro-organisms, so that there is the possibility that certain groups may be preferentially removed, leaving intermediate residues which are attacked more slowly. In the studies already described, the losses of uronic acid from the hemicelluloses of oat straw were very different from those of the pentose units, while in the rye straw series they were rather similar. Attention was not drawn to this at the time. In view of the variations in availability between the constituent units of hemicelluloses it was thought possible that resistant groupings might remain, even at the close of an active fermentation, and an investigation was accordingly made of the nature and amount of the residual hemicellulose material of oat straw rotted under optimum conditions for many months.

EXPERIMENTAL.

One kg. of dry oat straw, well chaffed, was thoroughly moistened by spraying and placed in a large bottle to which available nitrogen in the form of ammonium carbonate was added at the rate of 1.0 g. N to 100 g. straw. The bottle was incubated at 35° and frequently turned to secure even distribution of the nitrogen and thorough and uniform wetting. From time to time additional water was added from a spray, water-logging, however, being avoided. Rapid decomposition set in, and the straw shrank in bulk and darkened in colour. By the end of nine months the straw had rotted to a black pulpy mass, without cellular structure. A portion was taken for analysis and the remainder extracted with 1500 cc. of water on a water-bath for 4 hours. The residue after filtration was re-extracted twice with 1000 cc. water for the same period. The combined filtrates were concentrated under reduced pressure and poured into three volumes of alcohol, yielding a light brown precipitate (precipitate I). The dark residue was then extracted with 1500 cc. of alcoholic sodium hydroxide (2 % NaOH in 60 % C_2H_5OH) for 4 hours. Two further treatments with alcoholic sodium hydroxide followed. The very dark filtrates obtained were combined, nearly neutralised with acetic acid, and concentrated under reduced pressure. When of small volume acetic acid was added, and a dark heavy precipitate was obtained (precipitate II). The residual straw was then treated twice with 500 cc. 2 % NaOH for 4 hours at 80°, followed by 4 % NaOH at 100° for 4 hours. The residue was washed many times with hot water, and dried. The combined alkaline extracts were made definitely acid with glacial acetic acid, whereupon a precipitate, grey-brown in colour, was obtained and was centrifuged out (precipitate III). To the acid-aqueous residue were added $2\frac{1}{2}$ volumes of alcohol, and the precipitate obtained was filtered off on fluted filter-papers (precipitate IV).

Purification of the fractions. Precipitate I was redissolved in water and reprecipitated first by acid alcohol and then by neutral alcohol, finally being dried in increasing concentrations of alcohol, and over P_2O_5 in a vacuum desiccator; yield <0.5 g. Precipitate II was washed several times with warm water and ether, and dried in a vacuum-oven; yield 8 g. Both precipitates III and IV were redissolved in alkali and precipitated by the addition of acid alcohol, finally being washed with alcohol and dried with increasing concentrations; yields, precipitate III <1.0 g.; precipitate IV <0.5 g.

The composition of the fractions. Owing to the smallness of the quantities available, the number of estimations which could be made was of course very limited. The pentose and uronic acid contents of the fractions were pre-eminently of interest and were determined in each case. Tests were made for mucic acid after oxidation with nitric acid, and, in the case of precipitate III, for arabinose by heating with diphenylhydrazine, in which case the result was positive.

The following results were obtained.

Precipitate	Ash %	Furfuraldehyde yield %	CO ₂ yield %	Mucic acid
I	*	5.94	8.33	—
II	*	—	Trace	—
III	1.74	17.08	3.73	+
IV	*	12.38	3.12	+

* Not determined.

DISCUSSION.

The water-soluble precipitate I from the rotted material bore no obvious relationship to any original constituent of the straw. The fact that on oxidation it yielded no trace of mucic acid precludes any possibility of relationship to pectin. It seems probable that this substance represents a microbial product akin to the bacterial gums. Hopkins, Peterson and Fred [1930, 1931] have recently reported that the water-soluble gum produced by strains of *Rhizobium* is a polysaccharide consisting of glucose and glycuronic acid. An osazone prepared from a very small quantity of the hydrolysed precipitate I resembled glucosazone; but the quantity was insufficient for characterisation and too much stress is not put on this point. The analytical figures indicate the presence of 33.3 % uronic acid anhydride, which accounts for 5.55 % furfuraldehyde. The balance of the furfuraldehyde, 0.4 %, might be due to pentose groupings or more probably to experimental error. It seems therefore that this substance contains about 66 % hexosan and 33 % uronic acid anhydride.

Precipitate II was found to yield no furfuraldehyde or CO₂ on treatment with HCl. Further it did not give reducing sugars on hydrolysis with dilute acid. This fraction probably contains lignin and "humic" matter, possibly the "hymatomelanic acid" described by Odén [1919]. It was not investigated further since it appeared not to be of polysaccharide nature.

Precipitates III and IV may be considered together. They are the fractions prepared in the same way as the A and B hemicellulose fractions of the straw, and may be compared with them.

	CO ₂ yield %	Uronic acid anhydride %	Total furfur- aldehyde %	Pentose furfur- aldehyde %	Anhydro- pentose calculated as anhydro- arabinose %	Anhydro- hexose by difference %
Precipitate III	3.8	15.2	17.4	14.9	27.6	57.2
Hemicellulose A	2.7	10.8	42.9	41.3	76.7	12.5
Precipitate IV	3.1	12.4	12.4	10.3	19.2	68.4
Hemicellulose B	7.9	31.8	43.3	38.0	68.0	—

The calculations of anhydro-hexose are made with some reserve, inasmuch as there is little evidence of the purity of fractions obtained in such small quantities. It is possible that they might be contaminated with substances of a humic nature, possessing similar properties, though it is likely that soluble substances of this class would be removed by the pre-treatment with alkali-alcohol. From the positive mucic acid tests the presence of galactose or galacturonic acid in both fractions may be inferred. In both cases the residual products were much lower in pentose content than the straw products. It is probably safe to conclude that these substances represent small unavailable fragments of the original hemicelluloses, rather than elaborated products, since no water-insoluble microbial constituents or products which contain uronic acids and mixed sugars are known. This investigation failed to reveal the presence of any considerable amount of biologically unavailable hemicellulose, or of any resistant grouping formed as a consequence of incomplete attack. Instead it emphasised the complete availability of the hemicelluloses, an availability quite remarkable. The kg. of dry straw originally contained about 230 g. of free hemicelluloses, of which, at the close of the fermentation less than 2 g. could be recovered. It provides clear evidence that the structural arrangement and architecture of the hemicelluloses in the cell-wall is not such that the attack of micro-organisms is in any way hindered or retarded, as it is in the case of cellulose, by the presence of a more resistant lignin barrier. The cellulose in the same period suffered a loss of only two-thirds.

Variations in the biological availability of the hemicelluloses, or of units or constituent groupings in them, cannot therefore be of much importance in the case of the decomposition of straws by a general mixed flora, though it is likely that in pure culture decompositions they would be more obvious. In the earlier stages of decomposition by a mixed flora it is possible that such differences might be detectable. Information on this point can only be obtained by the laborious method of preparation of the hemicellulose fractions at different periods during the decomposition.

SUMMARY.

1. The nature of the residual hemicelluloses of well-rotted straw has been investigated with a view to obtaining information on possible resistant groupings.

2. Only very small quantities were obtained, and there was no indication of variation in availability or the accumulation of less available groupings. The complete availability of these substances to biological attack is emphasised.

3. The results indicate that the distribution and arrangement of the hemicelluloses in the cell-wall are such that microbial attack is not hindered by the presence of any resistant barrier.

4. A water-soluble polysaccharide, probably of microbial origin, was also prepared. It contained 33 % uronic acid anhydride, and 66 % hexosan, and gave evidence of the presence of glucose units.

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