CXXII. ON THE EARLY STAGES OF MICROBIOLOGICAL DECAY AND HUMIFICATION OF VEGETABLE TISSUES.

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IN a previous communication [Thaysen, Bakes and Bunker, 1925] it was shown that the humification of vegetable tissues in peat bogs affects both the carbohydrate and the lignin constituents of the tissues. It was possible to demonstrate that the humus recoverable from peat is composed of two definite substances, one showing the characteristics of humus obtainable from carbohydrates after treatment with acid, and another functioning on chlorination as humus prepared from quinol or lignin.

A sample of humus behaving on chlorination as a typical carbohydrate humus was obtained also from a piece of ancient Egyptian linen cloth from the tomb of Tut-ankh-amen. The amount of humus collected from this source was of the order to justify the conclusion that it had been formed from the cellulose of the linen fibres through "ageing."

This was thought to indicate that the cellulose of vegetable tissues decaying in a peat bog might suffer a similar conversion through "ageing" and that the fraction of peat humus which had been shown to be identical with a carbohydrate humus in its behaviour on chlorination had been derived from cellulose.

In the course of certain investigations carried out on behalf of the Fuel Research Board, an opportunity arose of following the progress of decay of vegetable tissues, and it was thought of interest to ascertain the quantitative relationship of the two fractions previously recognised as present in natural humus, at various stages of the progress of humification, and to establish, if possible, the constituent of the vegetable tissues which served as raw material for the carbohydrate fraction.

The material which was subjected to humification was chopped oat straw. To secure humification its moisture content was increased to 50 % by addition of the requisite amount of water. In this water had been previously dissolved sufficient ammonium carbonate to raise the nitrogen content of the damped straw to 2 %.

After wetting, the straw was packed in boxes sufficiently large to hold 20 lbs. each. The sides of these boxes were made of wire netting to facilitate access of air to the material. The boxes were incubated at 22–25°. An inoculant

was not added to the straw as previous experiments had shown that the microflora of oat straw is capable of producing the high temperatures which appear to be, if not essential, at least highly favourable for the humification to proceed rapidly.

Any loss of moisture suffered by the straw heaps was made good every third to fourth day.

The temperature of the packed straw rose rapidly and had reached a figure of 32° on the fifth day after incubation. On the seventh day a thermometer inserted in the heaps registered 49·6°. This temperature remained unchanged during the following 6 days and then gradually declined to 35°, a figure which was reached on the fortieth day. The lowest temperature recorded was 27·5° after 62 days' incubation of the straw.

A detailed bacteriological analysis of the decaying straw was carried out on the sixth day when the temperature of the straw had reached 46°. The number of micro-organisms developing on ordinary agar at 37° at this time amounted to 4900 millions per g. of damp straw, while 1800 millions developed on wort agar from the same quantity of straw. The microflora was very varied, though consisting essentially of aerobic bacilli of the groups of *Bac. mesentericus*, *Bac. dendroides* and *Bac. mycoides*. Various types of *Micrococcus* were observed and in addition *Bact. Zopfii*, *Bact. fluorescens liquefaciens*, *Bact. prodigiosum*, *Bact. herbicola aureum*, a number of actinomycetes and species of *Aspergillus* and *Penicillium*. The daily microscopic examination of the decaying straw revealed, as had already been observed by Miehe [1911], that the non-spore-forming types made their appearance first and were followed by various spore-formers when the temperatures had risen to a height.

On the twenty-fifth day an extensive growth of a species of *Coprinus* developed on the straw, the mycelium of which spread throughout the mass of the straw in the course of 2 days. Subsequently numerous fructifications of this fungus appeared on the surface of the straw.

By the forty-fourth day the microbiological activity appeared to have greatly diminished and most of the heat-resisting types could be observed only in the form of their spores. By inoculating dilutions of a suspension of the material into medium containing xylan and cellulose it was established that micro-organisms capable of decomposing both hemicelluloses and cellulose were present in the decaying straw.

There must have been more than 100 millions of hemicellulose-fermenting bacteria present per g. of straw since a dilution of 1 in 100 million of the straw showed growth in the xylan medium after 4 days' incubation at 37°. The types developing were not the aerobic spore-bearing rods capable of fermenting hemicelluloses (*Bac. mesentericus*, etc.).

The media containing cellulose which had been inoculated with 1×10^{-6} g. of decaying straw showed growth and destruction of the cellulose after incubation at 65°. Incubated at 37°, growth and cellulose decomposition did not occur from dilutions higher than 0·001 g. of straw. This observation

indicates that a destruction of cellulose in the fermenting straw proceeded most vigorously from about the sixth to the twenty-ninth day, while the temperature of the straw remained above 37°.

The experiments by which it was hoped to throw light on the relative importance of the hemicelluloses and the cellulose for the formation of humus in decaying straw included the following determinations at repeated intervals.

(1) *The alkali solubility of the straw.* Here 4 g. of dried decaying straw were extracted under a reflux condenser with 200 cc. of 3 % sodium hydroxide. The residue was collected on weighed filter paper, washed, dried and weighed.

(2) *Hemicelluloses* (pentosans, estimated by Tollen's method [de Chalmot and Tollens, 1891; Günther *et al.*, 1890, 1891]).

(3) *The cellulose* (estimated by the method of Cross and Bevan [1903]).

(4) *Humic substances.* The filtrate from the alkali solubility determination was acidified with 30 cc. of concentrated hydrochloric acid, the precipitate left overnight and filtered through weighed paper, washed, dried and weighed.

The results of these determinations are compiled in Table I.

Table I*.

Sample	Days of fermentation before removal of sample	Temperature of heap on removal of sample ° C.	Alkali solubility of sample %	Hemicellulose content of sample %	Cellulose content of sample %	Humus content of sample† %
Original straw	—	25	48.5	31.3	39.86	22.4
Decaying straw	14	49.6	49.53	25.7	39.86	23.4
"	28	40.5	56.66	18.97	35.94	26.26
"	64	27.5	64.95	14.44	27.94	30.9
"	82	27.5	67.84	11.07	23.78	31.55
"	102	27.5	71.14	10.1	16.38	32.8
"	146	27.5	70.6	7.93	—	32.3

* In interpreting the results of Table I it should be kept in mind that no attempt could be made to adjust the figures given for any losses suffered by the straw through the evolution of gases. The recorded data represent the percentages found in the sample as available on the day of collecting and after drying. The figures therefore under rather than over-estimate any changes which have occurred.

† It has been mentioned already that the figures given in this column were based on the weight of the precipitate collected on acidifying the extracts obtained by boiling the straw sample with a 3 % sodium hydroxide solution. They do not therefore give a true picture of the humus content of the samples since other substances, notably the hemicelluloses and lignin constituents, are brought into solution and reprecipitated under the conditions of experiment.

The most rapid increase in humus production took place between the first and the sixty-fourth day of fermentation, at a time when the hemicellulose content of the fermenting straw had decreased from 31.3 to 14.44 % and the cellulose from 39.86 to 27.94 %.

With a view to ascertaining the relative proportion of carbohydrate and lignin fractions in the humus during this and the immediately succeeding period, samples of humus were collected at various stages and subjected to chlorination by Eller's technique [Eller *et al.*, 1923].

The separation of the chlorinated fractions by extraction with ether showed, as indicated below (Table II), that the proportions of the two fractions varied appreciably with the progress of the humification.

Table II.

Days' fermentation before collection of humus from straw	Carbohydrate fraction in humus (%)	Lignin fraction in humus (%)
28	28.0	72.0
49	40.7	59.3
82	48.6	51.4
102	38.4	61.6
122	36.82	63.18

During the early period of humification of the oat-straw there was thus a steady rise in the percentage of the carbohydrate fraction, a rise which reached its maximum after about 80 to 85 days' fermentation. The extent of the subsequent decline in the percentage of this fraction could not be followed beyond the 122nd day of fermentation. However, it is interesting to note in this connection that a sample of silage which had remained packed in a silo for 3 years showed a humus content of 31.0 %, composed of 38.6 % carbohydrate humus and 61.4 % of lignin humus. And in a sample of peat humus examined previously [Thaysen *et al.*, 1925] and estimated to have taken 40 years to form, the carbohydrate fraction still amounted to 39.28 % of the total.

Whether the observed decrease in the lignin fraction of the humus was due to an actual destruction of lignin during decay or to an increase in the carbohydrate fraction beyond that indicated in Table II may be deduced from a consideration of the figures recorded in this and in Table I.

On the twenty-eighth day of fermentation there were present in 100 g. of the decaying straw 26.26 g. of humus containing approximately 18.9 g. of lignin fraction. On the eighty-second day of fermentation the humus of 100 g. of decaying straw had increased to 31.55 g. containing 16.22 g. of lignin fraction. During the whole of this period of intensive humification therefore the lignin fraction had remained practically constant, while the carbohydrate fraction had doubled—from 7.3 g. in the humus of straw after 28 days' fermentation to 15.33 g. in the humus of straw after 82 days.

At the time of the presence of these 15.33 g. of carbohydrate humus in the decaying straw a loss of 20.33 % of hemicelluloses and of 16.08 % of cellulose had been suffered by the original straw. With no information in existence on the efficiency with which carbohydrates are humified under natural conditions it was impossible to say whether the losses of hemicelluloses (pentosans) or those of cellulose were sufficient to account for the formation of these 15.33 g. of carbohydrate humus.

Light can be thrown on this question, however, by examining the position at an earlier stage of the decay, after 28 days of fermentation.

In the sample of straw taken after 28 days there were present in 100 g. of straw 7.36 g. of carbohydrate humus. At this stage the straw had lost 12.33 % of hemicelluloses (pentosans), an amount which conceivably might have been sufficient to yield 7.36 g. of humus. It is unfortunate that evidence cannot be brought forward in support of this. During the same period of fermentation the straw had lost only 3.92 % of cellulose, an amount which even under the most favourable conditions could not have yielded 7.36 g. of humus per 100 g. of straw. It is evident therefore, that, whether responsible for the production of the whole of the carbohydrate humus or not, the hemicelluloses (pentosans) must have taken part in its formation. This conclusion is in agreement with the views of Stoklasa [1898] and of Omelianski [1905] who regard pentosans as particularly important for the increase in the fertility of the soil.

The interesting question of the fate of the carbohydrate fraction of natural humic substances during the process of coal formation unfortunately did not come within the scope of the present enquiry. In a few preliminary experiments it was possible to show that the regenerated humus of three samples of bituminous coal gave chlorine derivatives containing on an average 35.36 % of chlorine. These derivatives, which were of a deeper orange appearance than those of humus derived from recently decayed plant tissues, were entirely soluble in ether. They did not therefore contain typical carbohydrate fractions. The fact that their chlorine content was of the order of that of a mixture of carbohydrate and of lignin humus may perhaps be taken as an indication that the solubility of the whole of the derivatives in ether cannot be regarded as sufficient support for the conclusion that a carbohydrate fraction was absent in these cases.

The regeneration of the humus of the three samples of bituminous coal was carried out by a method recommended by Dr Francis of the Safety in Mines Research Board, Sheffield, in a private communication.

One of the coal samples labelled "Ellistown Main Brieze" was received from the Fuel Research Station, East Greenwich, the two others were samples of ordinary household coal obtained from local coal merchants.

The regenerated humus from the Ellistown sample amounted to 69.44 % of the coal. The corresponding figures for the two other coals were somewhat higher owing to the lower ash content of these coals.

While the above investigation was in progress, a sample of mummified tissues, consisting of a human vertebra with adhering muscular tissues was received by Mr H. J. Bunker of this laboratory from Mr A. Lucas, formerly Director of the Chemical Department of the Egyptian Government, and an opportunity was taken to establish the nature of, and to examine the changes which had occurred in, these tissues.

The material dated from about 200 B.C. and was stated to have become completely humified. On examination its muscular tissues were found to possess an alkali solubility of 82.1 %, while its humus content amounted to 33.8 %.

On chlorinating, the humus took up 22.81 % of chlorine. The chlorine derivative was very similar in appearance to the chlorine derivative of the carbohydrate fraction of vegetable humus. It resembled it also in being almost insoluble in ether.

An ultimate analysis showed the chlorinated humus from mummified muscular tissues to contain 14.16 % of C; 3.62 % of H and 22.81 % of Cl corresponding to a formula of $C_{21}H_{21}O_{13}Cl_4$, the formula of the chlorine component of the carbohydrate fraction of vegetable humus.

SUMMARY.

The early stages of humification of plant tissues (oat-straw) as induced by micro-organisms have been studied. It has been shown that the hemicelluloses (pentosans) of the raw material are at least partly responsible for the appearance of the carbohydrate fraction of the humus.

In three samples of bituminous coal no definite proof could be obtained of the presence of a carbohydrate fraction in the regenerated humus, though the percentage of halogen in the chlorine derivatives of these humus samples was of the order characteristic of a mixture of carbohydrate and lignin humus.

The muscular tissues adhering to a mummified human vertebra dating from 200 B.C. had been converted into a humus with the properties of a typical carbohydrate humus.

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