XXVII. STUDIES ON THE BACTERIAL DECOMPOSITION OF TEXTILE FIBRES.

III. THE OCCURRENCE OF HUMUS COMPOUNDS IN DETERIORATED FABRICS AND THE BEARING OF THEIR FORMATION ON THE ORIGIN OF PEAT AND COAL.

BY AAGE CHRISTIAN THAYSEN, WILLIAM EDGAR BAKES
AND HENRY JAMES BUNKER.

*From the Bacteriological Laboratory, Royal Naval Cordite Factory,
Holton Heath, Dorset.*

(Received January 7th, 1926.)

It is now universally conceded that coal owes its origin to the accumulation of vegetable *débris*, which, preliminary to actual carbonisation, underwent a process of decay identical with that occurring today in the formation of peat. This process was described by Fremy [1885] as a "fermentation tourbose," a fermentation process through which the vegetable tissues, or at least the bulk of them, became converted into an amorphous mass of humic substances while isolated tissues escaping decay remained embedded in the amorphous matrix. That Fremy must have regarded micro-organisms as responsible for the decomposition of the plant material is clear, though he gives no information on their nature. Nor does he establish to what extent the chemical units composing the plant tissues partake in the formation.

The decay of vegetable tissues which resulted in the formation of coal subsequent to their conversion into peat is outlined in somewhat greater detail by White and Thiessen [1913], who regard the process as due, at least during its preliminary stage, to the activity of fungi, bacteria playing the chief rôle later. According to White and Thiessen micro-organisms either disintegrate the accumulated plant material completely, in which case amorphous peats are produced, or cease to be active before this stage has been reached. In the latter case xyloid lignites or woody or fibrous peats are formed. The decomposition process is regarded by them as very protracted, in fact they consider that it is still in progress in most of the existing peat bogs even at depths which must have taken one thousand years and more to produce. As regards the effect of the decay on the constituent substances of the plant tissues, White and Thiessen remark that the more labile compounds are removed first, the more resistant later. Finally there remain behind only such parts of the plant material as resins and suberised tissues, which do not usually suffer destruction by micro-organisms. Though cellulose cannot be regarded

as a particularly resistant substance, and, therefore, might be thought to disappear during the process of peat formation, White and Thiessen emphasise the fact that most peats contain a large percentage of cellulose. Its presence as an integral part of peat and coal is accepted by most authorities. Recently, however, Fischer and Schrader [1922] have expressed the view that cellulose, except in insignificant quantities, must undergo complete destruction during the decomposition process which results in the formation of peat. They regard the lignins present as the sole organic constituent of the fuels mentioned, except for the comparatively small quantities of resins and waxes present in the decaying plant tissues.

Fischer and Schrader, and other writers who have dealt with the microbiological aspect of peat and coal formation, base their conclusions on theoretical considerations and advance no convincing experimental evidence in support of their views. This is particularly regrettable since these theories bear unmistakable signs of having been elaborated without sufficient knowledge of the limiting factors of microbiological and particularly of fungal activity.

As the question of the production and origin of humic substances has a bearing on problems on which the writers have been engaged for some time, they have had an opportunity of submitting the microbiological aspect of peat formation to renewed inquiry. The preliminary observations made have been collected in the following notes.

Considering first the *production of peat*, it may be claimed that the decomposition of the peat-yielding plant material takes place under water-logged conditions. From a microbiological point of view this is particularly noteworthy since an important group of micro-organisms, the fungi, thereby becomes eliminated as a serious participant in the microbiological reactions involved. Water-logged conditions are known to prevent the action of fungi on vegetable *débris* to such an extent that immersion in water has been recommended as an infallible means of preserving timber from rot [Snell, 1921]. Of the sparse representation of fungi among the microflora of a typical peat the following experiment bears proof.

The samples of peat were taken from a local bog under precautions sufficiently stringent to prevent subsequent contamination. The actual samples examined, 1 g. in each case, were removed aseptically from the interior of the original samples. Plated out on wort agar which Long and Harsch [1918] recommend for the cultivation of wood-destroying fungi, the samples showed the following numbers of fungi.

Table I.

Sample of peat				No. of fungi found in 1 g. peat
In the surface peat immediately under the vegetation				0
In the peat 1 ft. below the surface				60
"	2	"	"	30
"	3	"	"	6
"	4	"	"	9

In every case the number was so small as to be practically negligible. They show that fungi can have taken no part in the decomposition processes which resulted in the conversion of the vegetation of the bog into this particular peat. The observation agrees entirely with what is known to occur in other, and better investigated decomposition processes or fermentations of water-logged vegetable tissues, *e.g.* the ensilaging of green fodder [Jones and Gibbard, 1923].

It appears, therefore, that the microbiological processes involved in the formation of peat must remain restricted to the activity of bacteria and actinomycetes, groups which contain numerous cellulose-decomposing species and, the former at least, also some which are capable of fermenting lignin [Pringsheim and Fuchs, 1923].

An insight into the extent of the activity of these micro-organisms on water-logged vegetable tissues may be gained from a study of the process of ensilaging, which, as already mentioned, bears striking resemblances to that of peat formation except for the fact that it offers more favourable conditions for microbiological activity than does peat formation.

In broad outline, the action of micro-organisms in the silage heap is restricted to the first three or four weeks after the construction of the heap. The maximum number of bacteria is reached, according to Jones and Gibbard, after 14 days. Subsequently a rapid decrease sets in which results in a complete cessation of microbiological activity 2 to 3 weeks later. By then the silage has frequently acquired the appearance of a young peat, though it still shows the plant tissues composing it in a well-preserved condition. When ripe it is dark brown to blackish in colour and contains an appreciable quantity of humus [Russell, 1908]. In spite of the humification it still contains most of the chemical substances originally present in the raw material, particularly the cellulose and the pentosans on which its feeding value chiefly depends. The following analysis of a typical sample of dark brown sweet silage, taken from the interior of a heap which had been stored on a farm for three years, shows the approximate chemical composition of this fodder.

Table II.

Pentosans	17.54 %
Cellulose	27.36
Nitrogen	1.34
Humic compounds (soluble in 3 % NaOH)	26.00
Ash	20.60
Unaccounted for	7.16

In a series of peat samples it was possible to observe a similar decrease in microbiological activity before the plant tissues had been completely disintegrated. The data recorded below, which illustrate this, were obtained from the analysis of samples of peat taken from a typical peat bog, renowned in the district for its valuable black, well disintegrated peat. The bog is built up of a dense growth of plants, consisting chiefly of low shrubs which

frequently become partly submerged, and reaches a considerable depth, the exact measurement of which could not be established.

Samples of peat, approximately 500 g. each, were taken from this bog from the top immediately below the growing plants and at intervals of a foot down to and inclusive of 4 feet, representing an age ranging from a few years to 40 years approximately. At a depth of 3 and 4 feet the plant material composing the peat was fairly well disintegrated, but it still contained an appreciable proportion of whole tissues mainly in the form of roots. On one occasion a sample of undisintegrated wood with a cellulose content of 34.2 % was detected at a depth of 4 feet. This was found in a place where the upper layers of peat were extremely soft, almost semi-liquid.

The result of the bacteriological analysis (Table III), carried out a few hours after the removal of the samples from the bog, showed that the secondary microflora, consisting of non-cellulose-decomposing bacteria and developing on ordinary laboratory media, had reached its maximum activity in or above the first stratum examined, and that the numbers of this flora, and consequently its activity, were decreasing rapidly with increasing age of the peat.

Table III.

Sample of peat	No. of bacteria present in 1 g. peat		Glucose agar at 37°; anaerobic cultures
	Developing on agar at 37°	Developing on gelatin at 18°	
Surface peat immediately under the vegetation	19200	200	Nil
Peat 1 ft. below the surface layer	460	104	"
" 2 " "	90	100	"
" 3 " "	1500*	240*	"
" 4 " "	4	Nil	"

* It is possible that this sample had become infected during collection.

A similar decrease with increasing age was noticeable in the activity of those bacteria which decomposed cellulose. The data given in Table IV illustrate this.

Table IV.

Sample of peat	Filter paper incubated aeri- bically at 30° after inoculation became attacked after				Filter paper incubated anaero- bically at 35° after inoculation became attacked after			
	7 days	14 days	21 days	35 days	7 days	14 days	21 days	35 days
Surface peat immediately below the vegetation	+	+	+	+	-	-	+	+
Peat 1 ft. below the vegetation	-	+	+	+	-	-	-	+
" 2 " "	-	-	-	+	-	-	+	+
" 3 " "	-	+	+	+	-	-	+	+
" 4 " "	-	-	-	-	-	-	-	-

* It is possible that this sample had become contaminated during collection.

For the determination of the presence of cellulose-decomposing bacteria, about 1 g. of the fresh peat was inoculated into a medium consisting of filter paper, completely or partly submerged in a solution of peptone and inorganic

salts. This medium, which is very suitable for the cultivation of these bacteria, was recommended for the purpose by McBeth and Scales [1913].

In spite of the favourable conditions thus offered, no cellulose-fermenting bacteria could be developed from the peat taken at a depth of 4 feet, a sample which still contained a percentage of undisintegrated tissues, chiefly roots. Further, the number of cellulose-fermenting bacteria in the strata above must have been decreasing with increasing age of the peat judging by the delay shown by the filter paper in becoming attacked in these cases. That this decrease should have been particularly noticeable in the cultures kept aerobically is not surprising when it is considered that oxygen has little or no opportunity of gaining access to the lower depths of a peat bog where conditions therefore become unfavourable to aerobic types.

Though the observations recorded in Table IV do not justify the conclusion that peat taken from a depth of 4 feet cannot contain micro-organisms in sufficient numbers to indicate microbiological activity, they certainly point to bacterial activity in a peat bog being greatest immediately below the surface of the vegetation, that is, in the youngest layer, and to this activity decreasing as the peat gets older, coming to a standstill while the peat is still young, before all the plant tissues have been disintegrated and before their cellulose has been completely decomposed. Micro-organisms, therefore, cannot be responsible for the elimination of all the cellulose of the peat-forming vegetable tissues. The fact that cellulose-decomposing micro-organisms, fungi or bacteria, are present in, and have taken part in the decay of, peat-forming plants, does not in itself justify Fischer and Schrader's theory of lignin as the only or the chief source of peat and coal. But since cellulose as such eventually does disappear completely in peat, a fact which nobody desires to deny, there must be other agencies than micro-organisms through which this elimination becomes possible. A study of a sample of Egyptian linen cloth dating from the 18th Dynasty has, in the writers' opinion, given a clue to the elucidation of this question. The cloth in question was dark-brownish and very brittle. It had a high alkali solubility and the part of it which went into solution in a hot 3 % sodium hydroxide solution could be precipitated with acid yielding what appeared to be a humic compound. That the deterioration of the cloth had not been caused by micro-organisms was established by the swelling test [Fleming and Thaysen, 1921]. The further examination of the humus-like compound obtained from it yielded results of considerable interest. Following the method used by Eller and his collaborators [1923, 1, 2, 3], a chlorine derivative was prepared from the substance, which on analysis was found to contain 24.17 % of chlorine. It was almost insoluble in ether. In both of these respects it greatly resembled the humus compounds obtainable by the action of acids on carbohydrates. The chlorine derivative of these artificial humus compounds was found by Eller and his collaborators to contain 22.76 % of the halogen, as against 42.3 % in the chlorine derivative of natural humic compounds isolated from a sample of

typical lignite, and 44.5 % in that of an artificial humic compound prepared from quinol. These two highly chlorinated compounds were found by Eller to be readily soluble in ether and are regarded by him as identical substances, definitely distinct from the chlorine derivative of humic compounds prepared from carbohydrates.

That the humic compound collected from the ancient Egyptian fabric had been derived from the cellulose present could not, of course, be definitely proved, though this seemed more than probable since the fabric had been woven from pure linen fibres and had not been dyed.

It was felt that if it were possible to assume that ageing alone might have been responsible for the humification of the cellulose in this fabric and the formation of a compound very similar to, if not identical with, that obtainable from other hexoses, the possibility could not be excluded that similar processes might be responsible for the disappearance of cellulose from peat.

If this had been the case it was to be expected that the humic compounds of peat would consist of a mixture of at least two substances, one representing the cellulose and another the lignin constituents of the original plant tissues.

An attempt was made to determine experimentally whether this was the case. To do so four different peats of varying ages were extracted in the usual way and their chlorine derivatives prepared. These were found to contain the following percentages of the halogen.

Table V.

Humic compound from sample of peat		Percentage of chlorine in its chlorine derivative
Sample	I	36.8 %
"	II	36.8
"	III	36.9
"	IV	34.9

As was expected, the percentage of chlorine represented an intermediate stage between that of a typical artificial humus and a typical natural (or lignin) humus.

A treatment of these chlorine derivatives with ether in a Soxhlet extraction apparatus entirely confirmed the assumption of the presence of two different types of humus. By this extraction method it was possible in each case to separate the chlorine derivative into two parts, one soluble in ether, the other insoluble.

The subsequent analysis of the two component parts of these chlorine derivatives gave the following results which confirm the presence of two fractions in the peat humus.

Table VI.

Chlorine derivative of peat humus		Percentage of chlorine in the fraction	
		Insoluble in ether	Soluble in ether
Sample	I	21.9 %	40.7 %
"	II	20.6	40.8
"	III	21.6	40.35
"	IV	22.4	41.65

The ether-soluble portion was very similar as regards its chlorine content to that yielded by a typical natural humus; the insoluble portion even more similar to that of a carbohydrate humus than was the case with that isolated from the Egyptian fabric.

SUMMARY.

The experiments discussed in the preceding pages indicate that micro-biological activity is insufficient to eliminate all the cellulose present in plant tissues decaying to form peat.

It is shown that the humic compounds which may be obtained from typical peats consist of two different types, one of which yields a chlorine derivative identical with, or very similar to, that of "natural humus" (lignin humic compound), whilst the chlorine compound of the other is closely related to that of the artificial humus compounds obtainable either by the action of inorganic acids on carbohydrates or from cellulose fibres decayed through ageing.

It is suggested that the presence of this latter humus compound in peat may be due to the decomposition of cellulose not eliminated by microbiological activity.

The above experiments arose out of work carried out on behalf of the Fabrics Co-ordinating Research Committee of the Department of Scientific and Industrial Research. The authors desire to express their thanks to the Department of Scientific and Industrial Research and to the Admiralty for permission to publish the results obtained. They are also very much indebted to Mr A. Lucas, O.B.E., F.I.C., formerly Director of the Chemical Department of the Egyptian Government for having procured for them the mummy cloth examined.

REFERENCES.

- Eller (1923, 1). *Liebig's Ann.* **431**, 131.
 Eller, Meyer and Saenger (1923, 2). *Liebig's Ann.* **431**, 162.
 Eller, Herdieckerhof and Saenger (1923, 3). *Liebig's Ann.* **431**, 177.
 Fischer and Schrader (1922). *Entstehung und chemische Struktur der Kohle* (2nd edition, W. Girardet, Essen).
 Fleming and Thaysen (1921). *Biochem. J.* **15**, 407.
 Fremy (1885). *Encyclopédie de Chimie*, vol. II; Métalloïdes, p. 509.
 Jones and Gibbard (1923). *Abstr. Bact.* **7**, 20.
 Long and Harsch (1918). *J. Agric. Res.* **12**, 33.
 McBeth and Scales (1913). *U.S. Dept. Agric. Bur. Plant Ind., Bull. No. 266*.
 Pringsheim and Fuchs (1923). *Ber. deutsch. chem. Ges.* **56**, 2095.
 Russell (1908). *J. Agric. Sci.* **2**, 392.
 Snell (1921). *Paper*, **28**, 22.
 White and Thiessen (1913). *U.S. Bureau of Mines, Bull. No. 38*.