CCXIV. THE PRODUCTION OF MUCUS DURING THE DECOMPOSITION OF PLANT MATERIALS¹.

II. THE EFFECT OF CHANGES IN THE FLORA.

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THE production of stickiness during the process of decomposition of cellulosic materials has been attributed by Hutchinson and Clayton [1919] to the activity of soil organisms such as *Spirochaeta cytophaga*. Since the time of Mitscherlich [1850] bacteria were considered to be the chief agents in the natural decomposition of plant materials until König [1904] suggested the greater importance of fungi. McBeth and Scales [1913] and Scales [1915] found fungi to be capable of using cellulose as the sole source of energy. It has been shown by Waksman [1926] and later confirmed by Norman [1929] that in the presence of sufficient available nitrogen, cellulose is the chief constituent to be decomposed. Waksman [1924] and Rege [1927] have shown that fungi are mainly responsible for the decomposition especially during the early stages.

Norman [1931] has studied the nitrogen transformations and changes in the carbohydrate constituents with pure cultures of fungi but no such study has so far been made of the subsequent action of bacteria after fungal attack, or of the simultaneous action of a bacterium with that of a fungus. Apart from the study of stickiness produced during decomposition, it was thought worth while to follow the ammonification and nitrogen changes and the losses in carbohydrate constituents at different stages of decomposition.

The work described in this paper was carried out in order to test whether mucus is produced during decomposition of straw with pure cultures of organisms. Straw was therefore decomposed with selected fungi, fungi and bacteria and bacteria alone. Analyses of the different constituents were made in order to find any correlation between the rates of their decomposition and the formation of mucus. The amount of mucus produced was measured by the physical test described in Part I [1933].

Scheme of work.

(1) Oat straw was rotted with pure cultures of fungi at 35° with ammonium carbonate as source of nitrogen and analysed at intervals of 8, 16, 24 and 48 days.

(2) Straw was rotted with pure cultures of bacteria: (i) *Mycobacterium agreste*, (ii) *Spirochaeta cytophaga*, and analysed at intervals of 8, 16, 24 and 48 days to see if any bacterium which, like the latter, produces a gummy colony on an artificial medium would produce stickiness in straw.

(3) Straw was rotted first for 48 days with fungi alone: (a) and then inoculated with *M. agreste* and analysed on the 8th, 16th, 24th and 48th days after inoculation; (b) as (a) but with the substitution of *S. cytophaga* for *M. agreste*.

¹ This paper is an abridged form of part of a thesis approved for the Degree of Doctor of Philosophy in the University of London.

(4) Straw was rotted with a fungus progressively and then inoculated with *S. cytophaga* on the 8th, 16th, 24th and 48th days, and analysed on the 8th and 40th days after inoculation with the latter.

(5) Straw was rotted simultaneously with fungus and *S. cytophaga* and analysed on the 8th, 16th, 24th and 48th days.

Methods.

Weighed quantities of straw were bottled and sterilised in an autoclave under 115 lbs. pressure for 45 minutes on two consecutive days. The treatment is undoubtedly drastic yet it is necessary for complete sterilisation. Available nitrogen was supplied as sterile ammonium carbonate in the proportion of 1 g. per 100 g. straw and the moisture was adjusted at about 80 %. A heavy inoculum of a suspension of the spores of the required organism in sterile water was then added to each bottle. Platings were made at the end of the experiments to test the purity of the organism. Fungal contamination was easily noticeable in most cases, since the straw was characteristically coloured by the spores of the fungi.

The following fungi were tested: *Trichoderma lignorum*, *Acremonia olivacea*, *Aspergillus niger*, *A. terreus*, *A. nidulans*.

The fungi tested were obtained from the stock cultures of the Mycology Department (isolated from the Rothamsted soils). They were maintained on agar slants in Waksman's medium. Before inoculation they were always grown afresh and in all the experiments described below cultures one week old were used.

Estimations of ammonia-N, total N, extraction with 90 % alcohol and treatment with hydrogen peroxide were carried out as in Part I.

(1) *Pentose units* (total furfuraldehyde). The standard method of Krober and Tollens was employed. The phloroglucide precipitate was not extracted with alcohol.

(2) *Cellulose* was estimated by Jenkins's method [1930].

(3) *Furfuraldehyde in cellulose* was determined as in (1).

The loss of dry matter (D.M.) as a result of decomposition is usually not a true index of the amount of decomposition. It does not consider the synthesis of new complexes by micro-organisms, which would modify to a considerable extent the actual amount of material decomposed. The cellulose and furfuraldehyde determinations would indicate how much of the loss in organic matter is represented by the loss in cellulose and hemicelluloses.

In any mature plant material, the cellulose, the polysaccharide associated with cellulose, or as it is termed by Hawley and Norman [1932], the "cellulosan," and the hemicelluloses are the three chief groups of biologically available material. The cellulose as isolated either by the Cross and Bevan chlorination method or by the Jenkins hypochlorite method is always associated with cellulosan. This cellulosan generally consists of pentose units. In the case of oat straw it is xylan as proved by Norman [1929]. This xylan can be determined by distilling the cellulose with 12 % HCl. A figure for "true" cellulose can then be obtained by deducting the figure for xylan. It is found that the decompositions of cellulose and the xylan associated with it run parallel. A figure for pentose units in the hemicelluloses can be obtained by subtracting from the total yield of furfuraldehyde that from the xylan associated with cellulose.

For the sake of comparison the figures recorded below are calculated on a basis of 100 g. of dry straw and interpreted in terms of cellulose, xylan associated with cellulose and pentose in hemicelluloses.

EXPERIMENTAL.

The same sample of oat straw was used throughout these experiments with pure cultures of organisms.

Ash	...	...	...	...	...	...	9.04
Total N	...	...	...	...	...	...	0.35
Total furfuraldehyde	...	...	...	...	...	...	15.6
"True" cellulose	...	...	...	...	...	...	41.0
Furfuraldehyde on Jenkins's cellulose product	...	...	...	...	...	...	7.0
Xylan associated with cellulose	...	...	...	...	...	...	10.8
Furfuraldehyde due to pentose in polyuronides	...	...	...	...	...	...	8.6
Alcohol extract	...	...	...	...	...	...	3.46

Series I (Tables I and II).

Stickiness. No stickiness was observed with any fungi used for decomposition.

Carbohydrate constituents. Table I contains analyses of these constituents. *Acremonia* *sp.* appears to be the most active fungus as judged from the loss of dry matter at the end of 48 days. *T. lignorum* seems to be the least active. The losses obtained follow practically the same order as those recorded by Norman [1931]. In general the hemicelluloses suffer a loss of about 60 % during 48 days. The hemicelluloses appear to decompose rapidly in the first few days and then remain at much the same level while the cellulose is being decomposed. On the whole it is obvious that the major part of the lost carbonaceous material is accounted for by the cellulose which is a more abundant food and energy source for the micro-organisms than the hemicelluloses.

Table I. *Decomposition of straws by various fungi at 35° at different intervals.*

Expressed on 100 g. original straw.						
Fungus	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
<i>Acre. olivacespora</i>	8	10.9	6.37	36.90	4.1	2.23
	16	21.2	5.19	27.81	13.1	3.41
	24	28.1	4.56	23.65	17.3	4.04
	48	41.2	3.32	16.17	24.8	5.28
<i>A. nidulans</i>	8	9.2	7.04	38.85	2.1	1.56
	16	15.6	6.01	35.64	5.3	2.59
	24	31.5	3.87	22.42	18.5	4.73
	48	38.9	3.23	17.02	23.9	5.37
<i>A. terreus</i>	8	11.0	6.98	37.66	3.3	1.62
	16	15.9	5.22	31.23	9.7	3.38
	24	31.9	4.66	24.09	16.9	3.94
	48	34.6	4.01	19.88	21.1	4.59
<i>A. niger</i>	8	11.2	4.98	37.37	2.6	3.62
	16	15.8	5.38	34.27	7.1	3.22
	24	23.1	4.86	26.25	14.7	3.74
	48	34.1	3.67	23.13	17.8	4.93
<i>T. lignorum</i>	8	9.3	4.23	38.29	2.7	4.37
	16	16.0	5.08	35.08	5.9	2.80
	24	24.4	5.02	27.82	13.1	3.58
	48	29.0	4.17	23.36	16.6	4.43

Table II. *Nitrogen content of straws rotted by pure cultures of fungi at 35° at different intervals.*

Expressed on 100 g. original straw.						
Fungus	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>Acre. olivaeaspora</i>	8	10.9	1.37	0.98	0.63	5.7
	16	21.2	1.32	1.29	0.93	4.4
	24	28.1	1.24	1.21	0.85	3.0
	48	41.2	1.19	1.17	0.84	2.0
<i>A. nidulans</i>	8	9.2	1.14	0.62	0.27	2.9
	16	15.6	0.92	0.72	0.37	2.3
	24	31.5	0.94	0.92	0.56	1.8
	48	38.9	1.12	1.09	0.73	1.8
<i>A. terreus</i>	8	11.0	1.22	0.75	0.40	3.7
	16	15.9	1.12	0.99	0.64	4.0
	24	31.9	0.85	0.82	0.51	1.6
	48	34.6	1.02	1.01	0.66	1.9
<i>A. niger</i>	8	11.2	1.20	0.82	0.46	4.1
	16	15.8	1.11	1.03	0.65	4.1
	24	23.1	1.24	1.22	0.86	3.7
	48	34.1	1.02	1.02	0.86	2.5
<i>T. lignorum</i>	8	9.3	1.23	0.86	0.50	5.4
	16	16.0	1.09	0.93	0.65	4.8
	24	24.4	1.25	1.24	0.88	3.6
	48	29.0	1.25	1.24	1.01	3.4

Nitrogen immobilisation. From Table II it can be seen that more than 50 % of the inorganic nitrogen has been used up by the organisms except *A. nidulans* in the first 8 days. The power of an organism in building up the microbial protein can best be judged from the nitrogen factor [Richards and Norman, 1931]. *T. lignorum* seems to be the best from the point of view of nitrogen immobilisation and *A. nidulans* the poorest. Norman [1931] also recorded a low nitrogen factor for *A. nidulans* compared with *A. niger* and *A. terreus*. As compared by their nitrogen equivalents [Norman, 1931] *A. nidulans* seems to be the most efficient and *Trichoderma* the least. *A. niger* differs markedly from *A. nidulans* and *A. terreus* though belonging to the same genus.

Series II (Tables III, IV and V).

Stickiness. Both the organisms produce a negligible amount of stickiness. The figures for *S. cytophaga* are contrary to expectation. Since it is an organism which produces a gummy colony on agar media, it was supposed that it might also give a rotted product showing stickiness.

Table III. *Physical test, alcoholic extracts and the H₂O₂ treatment of straws rotted with pure cultures of bacteria at 35° at different intervals.*

Expressed on 100 g. dry manure.						
Bacterium	Time in days	Loss of D.M.*	Alcohol extract	H ₂ O ₂ extract	Loss of O.M. after H ₂ O ₂ treatment	Physical test (g.)
<i>S. cytophaga</i>	8	7.5	8.40	7.0	10.1	108.6
	16	12.2	6.87	10.1	11.4	108.2
	24	13.0	7.48	13.9	11.4	212.3
	48			Infected		
<i>M. agreste</i>	8	4.8	7.05	13.7	12.6	101.3
	16	8.5	7.41	12.1	11.4	0.0
	24	14.2	7.51	9.7	13.8	334.1
	48			Infected		

* On 100 g. original straw.

Extractions with alcohol and hydrogen peroxide (Table III). On an average the alcoholic extract is the same for both of the organisms with no further change in the degree of decomposition. There is an increase in the hydrogen peroxide extract with *S. cytophaga* whereas the order is reversed with *M. agreste*.

Carbohydrate constituents (Table IV). The decomposition is practically of the same order with both the organisms at the end of 24 days. The bottles for the 48th day were infected and hence rejected. The cellulose lost with *Mycobacterium* is nearly one and a half times that destroyed by *Spirochaeta*. This was

Table IV. *Decomposition of straw by bacteria at 35° at different intervals.*

Expressed on 100 g. original straw.						
Bacterium	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
<i>S. cytophaga</i>	8	7.5	5.60	38.82	2.1	3.00
	16	12.2	6.11	36.76	4.2	2.59
	24	13.0	5.31	36.29	4.7	3.29
	48			Infected		
<i>M. agreste</i>	8	4.8	6.06	39.14	1.8	2.54
	16	8.5	6.02	36.43	4.5	2.58
	24	14.2	5.95	34.31	6.6	2.65
	48			Infected		

entirely unexpected, since the former is not ordinarily regarded as being a cellulose-decomposing organism and does not develop on a cellulose-agar plate, but, as pointed out by Norman [1930], the ability to utilise cellulose in the presence of other available carbohydrates is more common than is generally supposed. On the other hand *Spirochaeta* destroys hemicelluloses to a considerably greater extent than does *Mycobacterium*. Both organisms compare poorly with fungi in destructive action as regards organic matter.

Nitrogen immobilisation. The nitrogen figures are recorded in Table V. *Mycobacterium* builds up more microbial tissue than does *Spirochaeta* as seen

Table V. *Nitrogen content of straw rotted with pure cultures of bacteria at 35° at different intervals.*

Expressed on 100 g. original straw.						
Bacterium	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>S. cytophaga</i>	8	7.5	1.268	0.75	0.39	5.2
	16	12.2	1.000	0.64	0.29	2.3
	24	13.0	0.957	0.80	0.46	3.5
				Infected		
<i>M. agreste</i>	8	4.8	1.290	0.93	0.58	11.8
	16	8.5	1.040	0.66	0.30	3.5
	24	14.2	1.180	0.97	0.60	4.2
				Infected		

from the nitrogen factor. Compared with fungi, bacteria appear to be much slower in building up microbial protein. *Spirochaeta* consumes more carbohydrate per g. of nitrogen than *Mycobacterium* as shown by the nitrogen equivalent.

Table VI. *Decomposition of straw rotted first with pure cultures of fungi for 48 days and then inoculated with M. agreste and analysed at different intervals.*

Expressed on 100 g. original straw.						
Fungus	Days after inoculation	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
<i>T. lignorum</i>	8	30.1	3.52	24.74	16.2	5.08
	16	44.5	2.34	12.92	28.0	6.26
	24	49.1	2.46	11.86	29.1	6.14
	48	39.0*	3.61	21.14	19.8	5.00
<i>A. niger</i>	8	35.7	3.74	20.20	20.8	4.86
	16	42.0	3.04	15.99	25.0	4.56
	24	36.0	3.63	17.52	23.4	4.97
	48	41.0	3.06	17.70	23.3	4.74
<i>A. nidulans</i>	8	25.0	4.39	29.53	11.4	4.21
	16	28.0	4.26	27.38	12.6	4.34
	24	36.0	2.94	18.98	22.0	5.66
	48	38.7	3.79	20.96	20.0	4.81
<i>Acre. olivaeaspora</i>	8	32.4	4.06	25.18	15.8	4.54
	16	43.3	3.14	16.48	24.5	5.46
	24	43.4	2.94	15.59	25.4	5.66
	48	44.5	3.60	18.32	22.6	5.00
<i>A. terreus</i>	8	31.2	3.57	23.12	17.8	5.03
	16	36.9	3.55	19.00	22.0	5.03
	24	37.5	3.91	21.19	19.8	4.69
	48	39.7	3.42	19.81	21.2	5.18

* Short of moisture.

Table VII. *Nitrogen content of straw rotted first with pure cultures of fungi for 48 days and then inoculated with M. agreste and analysed at different intervals.*

Expressed on 100 g. original straw.						
Fungus	Days after inoculation	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>T. lignorum</i>	8	30.1	1.01	1.00	0.58	3.3
	16	44.5	1.22	1.21	0.70	1.5
	24	49.1	1.14	1.10	0.77	1.5
	48	39.0*	0.91	0.88	0.55	1.4
<i>A. niger</i>	8	35.7	1.08	1.06	0.72	2.0
	16	42.0	1.25	1.19	0.84	2.0
	24	38.0	0.95	0.94	0.67	1.7
	48	41.0	0.92	0.90	0.54	1.3
<i>A. nidulans</i>	8	25.0	0.97	0.95	0.62	2.5
	16	28.4	0.97	0.96	0.60	2.1
	24	36.9	1.16	1.14	0.80	2.1
	48	38.7	0.97	0.95	0.60	1.5
<i>Acre. olivaeaspora</i>	8	32.4	1.00	0.98	0.70	2.1
	16	43.3	1.25	1.19	0.84	1.9
	24	43.4	1.19	1.13	0.82	1.9
	48	44.5	1.10	1.08	0.73	1.6
<i>A. terreus</i>	8	31.2	1.06	1.05	0.69	2.2
	16	36.9	0.97	0.96	0.59	1.6
	24	37.5	0.87	0.86	0.51	1.3
	48	39.7	0.95	0.88	0.53	1.3

* Short of moisture.

Series III (a) (Tables VI and VII).

Stickiness. The rots showed no stickiness, indicating that *M. agreste* is unable to synthesise sticky material even after acting upon the elaborated fungal tissue.

Carbohydrate constituents (Table VI). *M. agreste* following upon *Trichoderma* gives the best decomposition and a loss of 49.1 % organic matter in only 24 days. The losses with the *Aspergilli* are practically of the same order. The rate of decomposition in the first eight days after inoculation with all the fungi is greater than in any subsequent periods. The average loss of cellulose with *Mycobacterium* following upon the five different fungi ranges from 20 to 23 %. Further decomposition does not affect the pentose unassociated with cellulose showing that all the available pentoses have already been removed by the fungi.

Nitrogen immobilisation (Table VII). With *Mycobacterium* following on each of the fungi, there is a drop in the nitrogen factor after the 16th day in general, which is due to the ammonification or as it is termed by Jensen [1929] the "mineralisation" of a portion of the fungal protein.

Series III (b) (Tables VIII, IX and X).

Stickiness. The results for stickiness are given in Table VIII and are highly significant. No stickiness was found with fungi on the 48th day, but by the subsequent action of *S. cytophaga* for 8 days only, the pull required to separate

Table VIII. *Physical test, alcohol extracts and the H₂O₂ treatment of straws rotted first with pure cultures of fungi and then inoculated with S. cytophaga and analysed at different intervals.*

Expressed on 100 g. dry manure.						Physical test (g.)
Fungus	Days after inoculation	Loss of D.M.	Alcohol extract	H ₂ O ₂ extract	Loss of O.M. with H ₂ O ₂	
<i>T. lignorum</i>	8	39.2	10.50	13.1	22.0	3495
	16	39.7	8.50	12.1	22.3	6435
	24	42.6	8.87	12.8	23.0	3958
	48	57.7	12.20	29.0	26.0	6550
<i>A. niger</i>	8	41.4	9.90	13.3	24.7	6305
	16	45.0	8.90	17.5	25.6	4242
	24	46.0	10.20	26.6	24.7	7134
	48	48.3	9.20	24.7	25.0	6145
<i>A. nidulans</i>	8	35.8	8.80	11.7	22.8	5197
	16	41.2	8.60	16.8	26.2	6796
	24	43.4	9.04	13.8	30.0	5869
	48	43.5	9.05	22.8	22.0	5290
<i>Acre. olivaceospora</i>	8	43.1	9.14	13.3	24.0	4425
	16	43.2	8.02	13.8	23.2	7866
	24	45.0	8.56	21.0	25.0	4995
	48	46.3	7.64	15.0	25.0	6904
<i>A. terreus</i>	8	37.5	7.64	14.2	23.7	6439
	16	41.9	7.90	11.8	26.4	5970
	24	45.4	7.64	15.0	25.0	8386
	48	51.3	7.82	26.4	25.0	6442

The final p_H of all the samples was between 7.0 and 7.5.

the two plates was at once raised by from 3 to 6 kg. varying with the nature of the fungus. This obviously means that *Spirochaeta* has synthesised some organic matter which is responsible for the stickiness. Since it was observed in Series II that *Spirochaeta* alone could hardly produce any stickiness, one is naturally led

to believe that the sticky material has some relation to the previous action of the fungus. Again, *Mycobacterium* did not produce any stickiness even after a previous fungal decomposition, suggesting that the production of stickiness has also some relation to the nature of the bacterium taking part in the decomposition.

Extractions by alcohol and hydrogen peroxide (Table VIII). The averages for the alcohol extracts differ in each case, the maximum being 10 % with *Spirochaeta* following on *Trichoderma* and 7.7 % on *A. terreus*. These figures are interesting when compared with stickiness. The maximum stickiness obtained is with the latter combination, whereas the former gives the least. On the whole the alcoholic extracts are greater than those with *Mycobacterium*. The higher solubility of the sticky material in alcohol indicates its possible non-gummy and non-dextrin nature. The losses of organic matter on treatment with peroxide are in general proportional to the losses in dry matter.

Carbohydrate constituents (Table IX). Greatest decomposition is obtained by *Spirochaeta* following on *Trichoderma* and *A. terreus* and amounts to 51.7 and 51.3 % respectively. In these two cases there is a big jump in the loss of dry

Table IX. *Decomposition of straws rotted first with pure cultures of fungi for 48 days and then inoculated with S. cytophaga and analysed at different intervals.*

Fungus	Days after inoculation	Expressed on 100 g. original straw.			
		Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
<i>T. lignorum</i>	8	39.2	3.16	14.61	5.24
	16	39.7	3.06	18.26	5.54
	24	42.6	3.17	14.89	5.43
	48	51.7	2.21	10.58	6.39
<i>A. niger</i>	8	41.4	2.88	14.57	5.72
	16	45.0	2.44	13.78	6.16
	24	46.0	2.56	13.52	6.04
	48	48.3	2.39	12.03	6.21
<i>A. nidulans</i>	8	35.8	3.88	18.46	4.72
	16	41.2	2.89	13.65	5.71
	24	43.4	3.09	16.28	5.51
	48	43.5	2.94	13.89	5.66
<i>Acre. olivarespora</i>	8	43.1	2.86	13.79	5.74
	16	43.2	3.41	15.85	5.19
	24	45.0	2.66	13.42	5.94
	48	46.3	2.20	13.06	6.40
<i>A. terreus</i>	8	37.5	3.15	16.45	5.45
	16	41.9	2.86	14.61	5.74
	24	45.4	2.20	13.74	6.40
	48	51.3	2.06	12.58	6.54

matter between the 24th and 48th days after the inoculation with *Spirochaeta*, but with the remainder the decomposition appears to be steady. The average loss of cellulose is about 26 % in each case. The average loss of pentose not associated with cellulose is also practically the same. These losses are not significantly higher than those given by fungi alone at the end of 48 days, indicating that no available hemicelluloses are left after fungus action.

Nitrogen immobilisation (Table X). Here again there is a drop in the nitrogen factor as the decomposition proceeds. This fall is more marked with *Spirochaeta* than with *Mycobacterium*. The former appears more efficient than the latter per unit of nitrogen as judged by the nitrogen equivalent.

Table X. *Nitrogen content of straws rotted first with fungi for 48 days and then inoculated with S. cytophaga and analysed at different intervals.*

Expressed on 100 g. original straw.

Fungus	Days after inoculation	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>T. lignorum</i>	8	39.2	1.26	1.24	0.89	2.2
	16	39.7	1.13	1.11	0.77	1.8
	24	42.6	1.10	1.08	0.73	1.7
	48	51.7	1.12	1.09	0.74	1.4
<i>A. niger</i>	8	41.4	1.35	1.30	0.96	2.3
	16	45.0	1.24	1.18	0.82	1.8
	24	46.0	1.26	1.18	0.83	1.8
	48	48.3	1.20	1.11	0.76	1.5
<i>A. nidulans</i>	8	35.8	1.22	1.20	0.91	2.5
	16	41.2	1.39	1.31	0.84	2.0
	24	43.4	1.14	1.12	0.77	1.7
	48	43.5	1.16	1.13	0.80	1.8
<i>Acre. olivaeaspora</i>	8	43.1	1.31	1.23	0.95	2.1
	16	43.2	1.15	1.11	0.78	1.8
	24	45.0	1.13	1.10	0.76	1.7
	48	46.3	1.25	1.23	0.82	1.7
<i>A. terreus</i>	8	37.5	1.34	1.27	0.99	2.6
	16	41.9	1.28	1.25	0.85	2.0
	24	45.4	1.23	1.19	0.84	1.7
	48	51.3	1.20	1.17	0.81	1.6

Series IV (Tables XI and XII).

Stickiness (Table XI). Fungi alone produce no stickiness, but it develops as soon as *S. cytophaga* is inoculated even when the fungus had a start of only a week. This further emphasises the necessity of the presence of fungal tissue for

Table XI. *Decomposition of straw rotted first with fungus for different periods and then inoculated with S. cytophaga and analysed on the 8th and 40th days after inoculation.*

Expressed on 100 g. original straw.

Organism	Days of fungus action	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents	Physical test (g.)
<i>T. lignorum</i>	8	27.8	4.27	23.52	17.4	3.33	3771
+	16	36.5	2.93	16.60	24.4	5.67	4452
<i>S. cytophaga</i>	24	37.6	3.36	14.10	26.9	5.24	4735
for 8 days	48	48.5	2.18	9.30	31.7	6.42	4143
<i>T. lignorum</i>	8	46.2	2.74	10.24	30.7	5.86	5196
+	16	47.9	2.32	9.45	31.5	6.28	1998
<i>S. cytophaga</i>	24	48.9	1.76	10.03	30.9	6.94	1880
for 40 days	48	54.9	1.36	7.97	33.0	7.24	2077

the production of stickiness. The stickiness was practically constant at about 4275 g. when *Spirochaeta* acted for only 8 days whereas it varied considerably when it acted for 40 days.

Carbohydrate constituents (Table XI). By comparing the figures in Series I for losses in dry matter with those of *Trichoderma* alone in Table I an estimate can be obtained as to the further decomposition due to *Spirochaeta* in conjunction with the fungus. For instance, the fungus working alone decomposed 16 % of dry matter in 16 days, whereas if working in association with the bacterium for the second 8 days, *i.e.* a total of 16 days, a decomposition of 27 % was obtained.

Obviously this extra loss must be ascribed to the action of the bacterium. For 48 days with the fungus and 8 days with the bacterium the decomposition amounted to 48 %, which is very close to that obtained with the fungus for 16 days and the bacterium for 40 days. The loss of dry matter for 48 days with the fungus and 40 days with the bacterium is about 55 %, which compares well with 51 % obtained in Series III (b) for a total period of 96 days. The losses in carbohydrate constituents run parallel with the losses in dry matter.

Nitrogen immobilisation (Table XII). The increase in ammonification with the period of incubation explains the drop in the nitrogen factor. *Spirochaeta*

Table XII. *Nitrogen content of straws rotted first with fungus for different periods and then analysed on the 8th and 40th days after inoculation with S. cytophaga.*

Expressed on 100 g. original straw.						
Organism	Days of fungus action	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>T. lignorum</i>	8	27.8	1.49	1.45	1.09	3.9
+	16	36.5	1.36	1.21	0.86	2.3
<i>S. cytophaga</i>	24	37.6	1.32	1.18	0.82	2.2
for 8 days	48	48.5	1.29	1.23	0.89	1.8
<i>T. lignorum</i>	8	46.2	1.33	1.30	0.94	2.0
+	16	47.9	1.38	1.34	0.98	2.0
<i>S. cytophaga</i>	24	48.9	1.30	1.23	0.88	1.8
for 40 days	48	54.9	1.21	1.16	0.81	1.5

has accordingly decomposed the fungus protein for its nitrogen requirements; the excess of ammonia is volatilised, thus accounting for the nitrogen losses.

Series V (Tables XIII and XIV).

Stickiness. The figures for stickiness indicate that the joint action of the two organisms from the very start is unfavourable for its production. The average of 240 g. for stickiness is far below the figures recorded where the fungus had acted alone for at least a week. The low value for stickiness may be accounted for by the failure of the fungus to build up the microbial tissue which appears to be of prime importance in the production of stickiness.

Carbohydrate constituents (Table XIII). The rate of decomposition appears to be very steady, giving a maximum of about 28 % which is approximately that of *Trichoderma* alone for 48 days. In fact, greater decomposition was expected

Table XIII. *Decomposition of straw with fungus and S. cytophaga together for different intervals.*

Expressed on 100 g. original straw.							
Organism	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents	Physical test (g.)
<i>T. lignorum</i>	8	6.9	6.69	37.93	3.0	1.91	139
+	16	11.9	6.52	35.41	5.5	2.08	163
<i>S. cytophaga</i>	24	14.5	5.02	33.77	7.2	3.58	395
together	48	27.9	4.68	20.62	20.3	3.92	266

considering the cellulose-decomposing nature of both the organisms. This low decomposition may partly be accounted for by the apparent failure of the fungus to grow in presence of *Spirochaeta*. The cellulose loss is in proportion to the loss of dry matter. A loss of 13 % dry matter between the 24th and 48th days

corresponds with 13 % loss of cellulose during the same period. The greater part of the loss of organic matter is thus accounted for by the loss in cellulose. There is hardly any increase in the loss of pentose between 24 and 48 days, no doubt due to the inactivity of the fungus.

Table XIV. *Nitrogen content of straws rotted with fungus and S. cytophaga together for different intervals.*

Expressed on 100 g. original straw.						
Organism	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
<i>T. lignorum</i>	8	6.9	1.37	0.95	0.60	8.6
+	16	11.9	1.28	1.00	0.64	5.4
<i>S. cytophaga</i>	24	14.5	1.04	0.84	0.48	3.3
together	48	27.9	1.30	1.27	0.92	3.3

Nitrogen immobilisation (Table XIV). The organic nitrogen is lower than that obtained with the fungus alone. This may be explained by the inactivity of the fungus. The low nitrogen factor in the early stages may be due to the poor synthesis of fungal tissue in the presence of the bacterium.

DISCUSSION AND CONCLUSIONS.

It appears from these decomposition studies that the production of stickiness in straws depends upon the presence of fungal tissue and the nature of the bacterium. Since *S. cytophaga* has a comparatively low optimum temperature for development, it appears that under the high temperature conditions of fermentation in a manure heap some other organisms must be the main causes of production of stickiness. Stickiness seems to have no correlation with the disappearance of any particular carbohydrate constituent during decomposition.

Although *S. cytophaga* produces gum on a synthetic medium, it fails to produce any stickiness while working upon straws, but in the presence of fungal tissue *S. cytophaga* produces stickiness. It is possible therefore that there is a fundamental difference between the sticky material synthesised by *S. cytophaga* while working upon straw previously decomposed by fungi and the gum it produces on an artificial medium which contains no fungal tissue. Obviously some sticky material other than the bacterial gum is synthesised during decomposition of straw by the interaction between the fungal tissue and the bacterium.

The simultaneous inoculation of fungus and *S. cytophaga* on sterile straw was not very successful either from the point of view of production of stickiness or of general decomposition. The fungus developed with difficulty and two further inoculations were necessary. This may be due either to competition for food material between the fungus and the bacterium or the retarding effect of the end-products of the bacterial action on the growth of the fungus. No counts were made to test this possibility, but it has been observed by Rege [1927] and others that fungi which bring about the initial decomposition of straw eventually disappear and are replaced by bacteria.

SUMMARY.

(1) A number of common soil fungi and two cellulose-decomposing bacteria in pure culture and in different associations have been tested with reference to the production of stickiness and general decomposition.

(2) These fungi and bacteria, while working independently of each other, do not produce stickiness irrespective of the nature of the bacteria.

(3) Fungus decomposition followed by the action of *Mycobacterium agreste* does not produce stickiness.

(4) Fungus decomposition followed by the action of *Spirochaeta cytophaga* produces stickiness.

(5) Progressive decomposition with a fungus and subsequent inoculation with *S. cytophaga* at different stages produce stickiness even if the period of action of the fungus was brief.

(6) Simultaneous inoculations of fungus and *S. cytophaga* produce very little stickiness.

(7) The amount of decomposition effected, the losses in carbohydrate constituents and the nitrogen immobilisation in each case were determined. All the substances studied were removed approximately in proportion to the apparent losses of dry matter.

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