LXXV. THE BIOLOGICAL OXIDATION OF CARBOHYDRATES.

V. THE DECOMPOSITION OF CELLULOSE IN THE ACTIVATED SLUDGE PROCESS AND IN PERCOLATING FILTERS.

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(Received January 28th, 1936.)

THE greater part of the dispersed matter in domestic sewage liquors has been shown by Mills [1931] to be present in the non-colloidal state. Cellulose is an important constituent of the suspended matter. Before sewage is purified by biological methods it is generally pre-treated by sedimentation to remove as much of the suspended solids as possible. Nevertheless a considerable proportion of the suspended substances is not separated by the pre-treatment and so passes on for purification by biological methods. The experiments described in this paper were designed to follow the course of decomposition of cellulose in two processes commonly employed for purifying sewage, *viz.* the activated sludge process and treatment in percolating filters.

EXPERIMENTS WITH ACTIVATED SLUDGE.

Tests were first made to see if an activated sludge, which would settle rapidly and leave a clear supernatant liquor, could be produced by aerating suspensions of cellulose in water to which ammonium carbonate had been added; these tests were not successful. A similar result followed the addition of suspensions of cellulose to an activated sludge derived from sewage and used successfully for the oxidation of solutions of sucrose. The method which proved to give the best, though by no means perfectly satisfactory, results was to add the suspensions of cellulose to an activated sludge built up from sewage solids. The defects in this method were that activated sludge made from sewage contains much extraneous matter and is difficult to sample accurately; the first defect was overcome by building up the activated sludge from the solids washed out of a percolating filter used for treating domestic sewage. The small amount of cellulose in these solids was ignored. The second defect was partly remedied, when the activated sludge had been built up by prolonged aeration of an aqueous suspension of the filter solids, by shaking the sludge vigorously in a measuring cylinder, allowing the heavy mineral matter to settle for a few seconds and then pouring off the creamy, homogeneous sludge.

The method of experiment was to aerate the sludge *plus* a known amount of suspension of cellulose, made up to a constant volume, for a definite period. The sludge was allowed to settle and the supernatant liquid and sludge were analysed. As the rate of disappearance of cellulose itself could not be followed with any

degree of accuracy in the concentrations of cellulose employed, determinations of organic carbon were made and the carbon figures converted into amounts of cellulose by using a factor.

Analyses made. Determinations of organic carbon, total nitrogen, nitrogen as ammonia, nitrite and nitrate, and total solids were made on the supernatant liquors. Organic carbon was determined by Mills's method [1931] in the early stages of the experiment, absorbing the CO_2 in baryta solution contained in a Pettenkoffer tube, and back-titrating the baryta with standard acid. For the purposes of this experiment, however, the method of Robinson *et al.* [1929] was later found to be more suitable. The liquids were evaporated under reduced pressure to a small bulk and then digested with H_2SO_4 in a 300 ml. Kjeldahl flask fitted with a well ground-in head carrying two fused-in tubes. One tube projected about half-way into the bulbous part of the flask and was connected to a Saja air pump. The other tube, which did not project into the neck of the Kjeldahl flask, was connected to a Reiset tower fitted in a 250 ml. filter-flask. In the determination, organic carbon reduces the sulphuric acid and liberates sulphur dioxide, which is absorbed in standard iodine solution. In order to catch iodine volatilised during the passage of the gases through the iodine in the filter flask, the latter was connected to a trap containing a known volume of standard thiosulphate. Using a preparation of pure cellulose a quantity which in theory contained 10.63 mg. C was found to contain 10.29 and 10.97 mg. in duplicate determinations.

Total nitrogen was determined by the Kjeldahl method. When appreciable amounts of nitrite or nitrate were present, they were reduced with Devarda alloy and then distilled with magnesia into standard acid; organic nitrogen was determined in the residue. Ammoniacal nitrogen was determined by distillation with magnesia, nitrite colorimetrically by the *m*-phenylenediamine method.

In order to study the influence of an available form of nitrogen on the rate of disappearance of cellulose, two experiments A and B were made. In A, cellulose and nitrogen were always added to the sludge in the proportion of approximately 8 of C to 1 of N. In B the same amount of cellulose was added, but only one-tenth of the amount of N. These C/N ratios were used so that comparisons could be made with previous tests [Jenkins, 1933, 2] using sugar. The C/N ratios used over the whole experimental period of 153 days were 8.35/1 for Exp. A and 82.6/1 for Exp. B.

At intervals varying from 3 to 12 days, aeration was stopped and the liquid was allowed to settle for 1 hour. Supernatant liquid equal to 80 % of the total volume was then removed and replaced by an equal volume of water containing cellulose and nitrogen in the right proportion; aeration was then continued.

Solids washed out of a percolating filter, as previously mentioned, were used at the start of the experiments. These solids were aerated with a mixture of crude sewage and cellulose until a sludge which settled rapidly and left a clear supernatant liquid was produced. This sludge was used in the proportion of 200 ml. sludge to 800 ml. cellulose suspension. The cellulose used throughout these experiments was a special preparation kindly supplied by the Northfleet Paper Mills, Ltd., Gravesend, and had been made by carefully drying paper pulp wetted with dilute HCl: the dried pulp after extraction with dilute NaOH was washed free from salts. This preparation contained no fibre and remained as a paste for nearly a year without undergoing any change. The dried cellulose contained 44.4 % of carbon.

The compositions of the shaken liquids at the beginning of the experiment and after aerating for 7 days are given in Table I.

Table I. *Results of carbon and nitrogen determinations before and after aeration for 7 days.*

		(g. per litre.)					
		Organic carbon			Total nitrogen		
Approximate C/N ratio	Time	In sludge	In cel- lulose added	In sewage added	In sludge	In sewage added	In NH ₃ added
8/1 Exp. A	At start	2.27	0.45	0.04	0.331	0.028	0.047
8/1 Exp. A	After 7 days	Total 2.76			Total 0.406		
80/1 Exp. B	At start	2.27	0.45	0.04	0.331	0.028	0.005
80/1 Exp. B	After 7 days	Total 2.76			Total 0.364		
Distribution of N.							
		Total N	NH ₃ -N	NO ₃ -N	NO ₂ -N	Organic-N	
8/1 Exp. A	After 7 days	0.381	0.036	0.030	0.0004	0.309	
80/1 Exp. B	After 7 days	0.343	0.039	0	0	0.304	

A gain of carbon was found in Exp. A and a loss in Exp. B, but as both gain and loss were within the experimental error of the determination, it is assumed that the carbon content remained the same before and after aeration for the first 7 days. The liquids were allowed to settle after this period of aeration and a known volume of supernatant liquor was removed and analysed. This liquid was replaced in the manner previously described. An attempt was thus made to keep a complete carbon and nitrogen balance sheet. The numerous data are not shown in detail but the losses of carbon are given in Table II for each period of aeration. A negative sign means that an increase in the carbon content was found. Analytical and sampling errors are in all probability responsible for the apparent gains in carbon content. The net loss of carbon for the total period of 153 days is the sum of the positive and negative losses.

Table II.

Days aerated 0 = beginning of exp.	Exp. A g. carbon lost	Exp. B g. carbon lost
0- 7 = 7 days	-0.11	+0.05
7- 19 = 12 "	+0.51	+0.11
19- 24 = 5 "	-0.10	0.00
24- 27 = 3 "	+0.25	+0.32
27- 35 = 8 "	-0.21	-0.21
35- 38 = 3 "	+0.01	0.00
38- 89 = 51* "	+0.08	+0.07
89- 97 = 8 "	0.00	0.00
97-109 = 12 "	+1.24	+1.69
109-117 = 8 "	+0.20	+0.20
117-126 = 9 "	0.00	+0.18
126-133 = 7 "	+0.71	+0.30
133-140 = 7 "	+0.18	+0.05
140-145 = 5 "	+0.11	+0.17
145-153 = 8 "	-0.08	+0.20
Net loss	+2.79	+3.13

* An accident prevented analyses and additions of cellulose *etc.* being made from the 38th to the 89th day. The development of green algal growths during this period probably accounts for the small loss of carbon recorded.

Over the whole period the vessel which received additions of C and N in the ratio of 80/1 showed a somewhat greater loss of C than the one receiving more N. In all 5.17 g. of C as cellulose were added in each experiment, of which 54 % was removed in Exp. A and 60 % in Exp. B. But in view of the unaccountable

variation in the amount of C oxidised during similar periods of time, it cannot be stated with certainty that the differences between the total amounts of C oxidised in Exps. A and B are really significant. The conclusions can safely be drawn, however, that (1) cellulose in a fine state of division is removed from suspensions aerated for several days with a sludge derived from sewage; (2) the cellulose removed from suspension is oxidised, at least in part, during aeration; (3) a very small ration of nitrogen is required for the oxidation of cellulose by the activated sludge process. No advantage resulted from adding more N than was provided when the C/N ratios of the liquids added were about 80/1. It may be that the effect of adding little nitrogen in proportion to cellulose was to stimulate the activity of nitrogen-fixing organisms, as is known to occur in soil [Beijerinck, 1904], and in percolating filters fed with solutions of sugar and small amounts of nitrogen [Jenkins, 1933, 2]. However, the well-known effect of available nitrogen in stimulating the decomposition of cellulosic substances in the soil, or the activity of cellulose-decomposing organisms such as *Spirochaeta cytophaga* [Hutchinson and Clayton, 1919], does not appear to apply to the same extent in the case of cellulose breakdown in the activated sludge process.

The liquid in Exp. A generally contained a considerable proportion of nitrogen as ammonia at the end of the periods of aeration. When the concentration of ammonia at this stage was low, a correspondingly large amount of nitrogen was found as nitrate and sometimes as nitrite. In Exp. B ammonia was frequently present in small amounts. At times, however, the whole of the nitrogen added as ammonia was found after aeration to be in the organic form. Only on one occasion was a trace of nitrite detected and on another a trace of nitrate. As cellulose and nitrate co-existed in Exp. A without the nitrate disappearing it is clear that the assimilation of nitrate during the microbiological decomposition of cellulose takes place much more slowly in the activated sludge process than in the soil [Murray, 1921]. To illustrate the difference in concentrations of various compounds of nitrogen in Exps. A and B typical results of analysis are given in Table III.

Table III. Results showing the difference in concentration of nitrogenous compounds in Exps. A and B.

Period of experiment	(Parts per 100,000.)					
	Exp. A			Exp. B		
	Ammonia	Nitrite	Nitrate	Ammonia	Nitrite	Nitrate
19th day	4.67	0.03	2.15	1.76	0	0
After aeration from 19th to 24th day	0.03	0	3.20	0	0	0
27th day	2.93	0.02	0.77	0.31	0	0
After aeration from 27th to 35th day	0.12	0	1.43	0.09	0	0
89th day	4.23	—	0.14	0.34	—	0.01
After aeration from 89th to 97th day	0.47	0	0	0.14	0	0
117th day	4.91	0	0.09	0.50	0	0
After aeration from 117th to 126th day	2.05	0	2.50	0.15	0.06	0
133rd day	5.06	0	0.36	0.49	0	0
After aeration from 133rd to 140th day	1.73	0	2.31	0.03	0	0

BIOLOGICAL FILTRATION EXPERIMENTS.

The apparatus used for these experiments consisted of a device for maintaining cellulose in suspension and for releasing the suspensions at any desired rate, together with percolating filters. The stirring and dropping mechanism which was fitted into the 2 l. aspirator is shown in Fig. 1; the filters were made of glass sections filled with glass medium and were of the type described by Jenkins [1933, 1]. The apparatus worked satisfactorily at low propeller speeds, but at high speeds, *e.g.* when the propeller was not entirely covered with liquid, solid blocked the side tube.

Two filtration experiments, C and D, were made, each lasting for 200 days. The liquids supplied to the filters were of the composition shown in Table IV.

The filters were supplied with the appropriate liquid at only 0.5 l. per day for the first 20 days, equivalent to 50 gallons per day per cubic yard of filtering medium. Any residue in the aspirator containing the nutrient solution was not discarded but was added to the next day's charge. This accounts

Fig. 1. Driving gear A, assembled; B, Unscrewed.

Table IV. Composition of liquids used in Exps. C and D. Parts per 100,000.

	Filter C	Filter D
Cellulose	50	50
C in cellulose	22.2	22.2
N as NH_4HCO_3	2.5	0.25
P_2O_5 as K_2HPO_4	1.5	1.5
K_2O as K_2HPO_4	2.0	2.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2.5	2.5
CaCl_2 as $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	2.5	2.5
NaCl	0.25	0.25
Fe_2Cl_6	Trace	Trace
C/N ratio	8.8/1	88/1
p_{H} liquid	6.6	6.2
Approximate rate of flow	2l. daily	2l. daily

From 133rd day the concentrations of cellulose and salts to C and D were doubled.

Table V. C and N in solutions fed to Filters C and D, and in the corresponding effluents.

Filter C: C/N ratio 8.8/1; Filter D: C/N ratio 88/1.							
Parts organic C in 100,000 parts of nutrient liquids and final effluents.							
Day of exp.	20	26	35	117	131	141	153
In liquid to C	28.7	27.0	29.1	27.4	39.8	41.1	63.2
In effluent from C	4.3	5.6	16.1	4.3	4.3	12.1	5.7
In liquid to D	28.7	27.0	24.2	27.3	27.3	39.6	41.1
In effluent from D	6.75	6.3	9.9	5.1	5.1	1.4	4.9
Parts N as ammonia and nitrite in 100,000 parts of nutrient liquids and in effluents.							
	NH_3 HNO_2		NH_3	NH_3	NH_3	NH_3	NH_3 HNO_2
In liquid to C	2.50	0	2.4	2.25	2.30	2.30	5.74 0
In effluent from C	1.84	0.02	1.6	1.30	1.65	1.75	4.58 0.04
In liquid to D	0.25	0	0.24	0.20	0.24	0.24	0.53 0
In effluent from D	0	0	0	0	0	0	0.03 0

for the deviations in the concentration of carbon in the nutrient liquids given in Table V, from the amounts shown in Table IV.

A few determinations of carbon and nitrogen in various forms were made at different levels in the filters. The results are shown in Table VI.

Table VI. *Organic carbon and ammonia in liquids fed to Filters C and D and in the effluents from different sections.*

Filter C, C/N ratio 8.8/1; Filter D, C/N ratio 88/1. Parts per 100,000.

Day of exp.	Nutrient liquid	Filter C				Nutrient liquid	Filter D			
		Section					Section			
		1	2	3	4		1	2	3	4
		Organic carbon.								
35	29.1	16.0	6.9	6.4	16.1	24.2	14.5	7.2	9.9	—
117	27.4	6.7	—	5.2	4.9	25.5	5.7	—	3.6	3.9
131	39.8	3.8	—	3.8	4.3	27.3	3.6	—	3.8	5.1
141	41.1	6.3	—	17.0	12.1	39.6	20.1	—	1.8	1.4
153	63.2	12.2	10.9	8.7	5.7	41.1	9.3	4.3	2.9	4.9
N as Ammonia.										
35	2.25	2.0	1.5	1.25	1.30	0.2	0	0	0	0
117	2.3	2.0	—	1.75	1.65	0.24	0	—	0	0
131	2.3	1.75	—	1.75	1.75	0.24	0	—	0	0
141	4.0	—	4.0	3.0	3.0	0.4	Trace	—	0	0
153	5.74	5.04	4.91	4.16	4.58	0.53	0.03	0.03	0.03	—

At the end of the filtration experiments the composition and amount of the solids in all four sections of Filters C and D were determined. The solids were removed by shaking the tubular glass medium in the filters with hot water. The results are summarised in Table VII in which the appearance of the film is also noted.

Table VII. *Filter C, C/N ratio 8.8/1; Filter D, C/N ratio 88/1.*

Weight of solids, C, N and NH_3 , found in sections at end of experiment (g.).

	Filter C					Filter D				
	Section				In all sections	Section				In all sections
	1	2	3	4		1	2	3	4	
Total solids	3.03	1.83	1.08	0.47	6.41	13.00	7.60	3.41	1.93	25.94
Combustible solids	3.03	1.82	1.07	0.47	6.39	12.81	7.34	3.13	1.91	25.19
Ash	0	0.01	0.01	0	0.02	0.19	0.26	0.28	0.02	0.75
Organic C	1.34	0.78	0.45	0.24	2.81	5.38	3.33	1.25	0.75	10.71
Total N	0.050	0.044	0.031	0.023	0.148	0.081	0.071	0.061	0.057	0.270
$\text{NH}_3\text{-N}$	0.006	0.005	0.001	0.001	—	0.005	0.002	0.001	0	—
Organic N	0.044	0.049	0.030	0.022	0.145	0.076	0.069	0.060	0.057	0.262
Organic C/Org. N	30/1	16/1	15/1	11/1	—	71/1	48/1	21/1	13/1	—
	Section					Section				
	1	2	3	4		1	2	3	4	
Colour ivory-brown. Texture like original cellulose pulp: slimy. <i>Colembola</i> , worms, <i>Psychoda</i> flies and larvae present		Colour as 1. Less film than in 1. Worms and <i>Colembola</i> abundant	As 2, but less in amount	Little film, mostly worms and <i>Colembola</i>		Ivory colour. Thick algal growth on side of filter. Protozoa abundant	As 1, but less in amount	Ivory colour. Like cellulose in texture		Much algal growth

Discussion of filtration experiments.

With two exceptions the figures giving the amount of carbon in the effluents (Table V) show little difference between the two filters. It would be wrong to conclude from this fact that the filter receiving C and N in the ratio of 88/1 was as effective in decomposing cellulose as the other filter, receiving C and N in the ratio of 8.8/1. Whilst the amounts of carbon in the effluents were of the same order, the quantities which accumulated in the filters were quite different. The first section of Filter C was the only one which contained much growth or deposit. The first three sections of Filter D, however, were thick with an ivory-white deposit. The first section of this filter contained enough film to impede filtration in the later stages of the experiment. The amounts of total solids and organic carbon in the various sections agreed with the appearances of those sections. The figures show a descending order of solid matter and organic carbon in passing from section 1 to section 4. They also show that each section of Filter D had about four times as much solid matter as the corresponding section in Filter C. There was 6.5 % of ash in the solids from Filter C and only 2.8 % in those of Filter D. It is worth recording that part of the film often passed in flushes from the bottom of one section to the top of the section immediately below. This appeared liable to occur at any stage of the experiment, as the film lacked cohesion. The amounts of carbon in the effluents from different sections (Table VI) show that the greater part of the carbon removed was deposited in the first sections of the filters. Inspection indicated that the film in the lower sections in both filters originated from pieces broken away from the film in the upper sections and washed down.

The results of determinations of nitrogen in its various forms are of interest. Although Filter C received 10 times as much N as ammonium bicarbonate as Filter D, the amounts of organic nitrogen in the films at the end of the experiment were: 0.145 g. in C, and 0.262 g. in D. Assuming that the microbial C and N were present in the ratio of 10/1, the amounts of C in the two filters in this state would be 1.451 g. and 2.622 g. in Filters C and D respectively, leaving 1.358 and 8.079 g. C in the non-biological form in C and D respectively. A large proportion of this non-microbial C was probably undecomposed or only partly decomposed cellulose.

Filter D removed ammonia quantitatively from the nutrient liquid, except on the 153rd day. The amount of ammonia taken up by Filter C varied between twice and four times as much as that retained by D, yet, as already pointed out, the film in Filter D contained more N than Filter C. Most of the N taken from the nutrient liquid was removed by the first section, as was the case with carbon. The first section of Filter D extracted quantitatively all the N supplied, with the one exception mentioned. Nevertheless, a relatively large amount of N appeared in the remaining sections of this filter derived from the film in Section 1.

The information required to draw up a balance sheet between C and N supplied and the amounts of these elements found in the effluents and the filter is incomplete. By assuming that the analytical results obtained whenever samples were taken are typical of the behaviour of the filters between different times of sampling, the following approximate balance sheet may be drawn up.

	Filter C. C/N ratio 8.8/1		Filter D. C/N ratio 88/1	
	C	N	C	N
Added	56.3	6.62	56.3	0.66
Found in effluents	14.1	4.78	8.9	0.03
Found in filter	2.8	0.15	10.7	0.27
Unaccounted for	39.4	1.69	36.7	0.36

The accuracy of the figures for C and N unaccounted for depends upon the assumption, made in the previous paragraph, that the composition of the final effluents remained constant over fairly long periods. The variation in composition of the effluents suggests that normal fluctuations would not greatly affect the estimates of C and N lost. The C lost during filtration probably disappeared as CO_2 , since it did not appear in the effluents as soluble organic carbon. Although the amounts of carbon oxidised by Filters C and D may not be significantly different, the differences in the carbon content of the filters are undoubtedly associated with the differences in the nitrogen supplied to the filters. Most of the carbon in Filter D was probably undecomposed cellulose.

The nitrogen used in the filtration of suspensions of cellulose depends upon the amount of nitrogen supplied. In presence of a large excess of nitrogen, 39.4 g. C utilised 1.7 g. N; with a small supply of nitrogen 36.7 g. C used only 0.36 g. N. Under the conditions of these experiments the nitrogen requirement of a filter is not an absolute figure but is capable of variation within wide limits. With different conditions Hutchinson and Richards [1921] found in the case of decomposition of straw that favourable decomposition occurred with an initial C/N ratio of about 40/1.

SUMMARY.

Aqueous suspensions of cellulose, containing different amounts of N as NH_4HCO_3 , were treated by the activated sludge process in one set of experiments, and in glass percolating filters in another.

Activated sludge experiments. Approximately the same amount of cellulose was oxidised when cellulose and nitrogen were added in the C/N ratio of about 80/1 as when the C/N ratio was 8/1. The cellulose did not disappear at a steady rate, for on occasions more was lost during short periods than during longer periods of aeration.

The oxidation of NH_3 to nitrite and nitrate was inexplicably erratic when the C/N ratio was approximately 8/1 and bore no apparent relationship either to the amount of cellulose oxidised or to the period of aeration. Cellulose and ammonia were oxidised simultaneously. There was no evidence to show that the decomposition of carbohydrate preceded the oxidation of the nitrogen.

Filtration experiments. When cellulose and nitrogen were supplied to two filters in the ratio of about 8/1 and 80/1 respectively it appeared that about 10 % more decomposition occurred with the lower ratio; the difference may not be significant. The two filters differed in appearance at the end of the experiment. The one receiving cellulose and much N had enough active film to decompose 70 % of the C it received and allow liquid to percolate freely; the other, receiving the same amount of cellulose and less N, oxidised 65 % of the C, contained nearly four times as much film as the first filter and did not allow the free passage of liquid.

Of the N supplied to the filter receiving C and N in the ratio of 8/1, 72 % of the N was recovered as NH_3 in the effluent, 2 % in the filter and 26 % disappeared. The absence of N from the effluents from the filter receiving C and N in the ratio of about 80/1 showed that practically all was probably retained by the filter. However, out of the small amount of N supplied, only 45 % was accounted for in the film and in the effluents.

This work, which was carried out under the supervision of Mr E. H. Richards, was part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and the results are published by permission of the Department.

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