XVI. THE BIOLOGICAL OXIDATION OF CARBOHYDRATES.

IV. THE PHOSPHORUS REQUIREMENTS OF PERCOLATING FILTERS.

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THE purification of sewage and certain trade effluents can be effected in percolating filters by allowing the polluting liquids to trickle through a well-aerated bed of material such as clinker, gravel *etc.*, suitably graded as regards size of particles. The first stage in the purification may be the physical retention of some of the polluting matter within the filter, but ultimately purification depends upon the breakdown and oxidation by living agencies of dissolved or precipitated organic matter. Although the biological nature of the process has long been recognised, insufficient attention has been given to a study of the conditions necessary to maintain the organisms responsible for purification in a state of maximum activity. A study of this kind should include experiments on the effect of changes in concentration of compounds of nitrogen, phosphorus and potassium on the rate of oxidation of the organic matter. Sewages of domestic origin are invariably rich in nitrogenous compounds, while they also contain fats, carbohydrates and salts of potassium and phosphorus. It is improbable, therefore, that the organisms responsible for the oxidation of domestic sewage liquors will be without any essential food material. Some waste waters containing organic matter, such as those from beet-sugar factories, may, however, have insufficient quantities of certain materials to ensure optimum rate of purification in percolating filters.

In a study of the process of biological oxidation of effluents from beet-sugar factories, Richards and Cutler [1933] have shown that the rate of oxidation of the carbohydrates in solution is influenced by the amount of available nitrogen. In view of the lack of data regarding the relationship between the supply of compounds of phosphorus and the rate of oxidation of organic matter, particularly carbohydrates, in percolating filters an investigation has been made with the objects of examining (1) the effect of phosphate on the rate and extent of oxidation of sucrose; (2) the relationship between the quantity of phosphate and the removal of nitrogen from solution; (3) the availability to micro-organisms of organic compounds of phosphorus; (4) the influence of phosphate supply on the development of an active biological film on the filter media. The results are described in the present paper. Similar work published by Barritt [1931] while these experiments were in progress will be referred to later.

EXPERIMENTAL.

Filters constructed from glass tubes containing glass medium made from broken bottles were used for the first set of tests and the results are described in Exp. 1. After the filters had been supplied for 28 days with solution con-

taining 0.1 % sucrose, pools of liquid formed on the surface of one filter owing to a heavy growth of film in the upper portion. Choking was avoided in a second series of experiments by using glass tubes as medium instead of broken glass. Details of construction of the filters have already been described [Jenkins, 1933, 1]. The results of this second set of tests are described as Exp. 2. In Exp. 3 the results are given of a large-scale trial at a beet-sugar factory. The main purpose of the experiment was to test the effect of inoculating selected organisms on to a filter. The liquids filtered were effluents from the beet-pulp presses of the factory after treatment in a sedimentation tank to remove suspended solid matter. During the period of sedimentation some fermentation occurred and the liquids became acid.

Method of analysis. Sugars, nitrogen as ammonia, nitrite and nitrate were determined by methods described previously [Jenkins, 1933, 2]. Phosphorus in the inorganic and organic forms was determined colorimetrically by the Denigès method as modified by Parker and Fudge [1927]. In the determination of phosphorus in organic combination, however, 1 ml. of 10 % calcium acetate solution was used instead of $\text{Mg}(\text{NO}_3)_2$ for igniting with the residue left after evaporation to dryness.

Experiment 1.

Each filter consisted of four glass cylinders with perforated metal plates at the lower ends. The sections were joined together by rubber bands and could easily be disconnected when samples of the effluent from any of the upper sections were required. The filtering medium was broken glass graded $\frac{1}{16}$ – $\frac{1}{4}$ in. Solutions of nutrient substances in the concentrations given below were filtered at a rate of 2 litres per day (100 gallons per cubic yard of filtering medium per day, abbreviated g.y.d.). These solutions each contained 5 parts N per 100,000 and

Solutions filtered from beginning of experiment until the 22nd day.

Filter	A_1 g.	B_1 g.	C_1 g.
Sucrose	1.000	1.000	1.000
NH_4Cl	0.191	0.191	0.191
$\text{MgSO}_4, 7\text{H}_2\text{O}$	0.050	0.050	0.050
$\text{CaCl}_2, 6\text{H}_2\text{O}$	0.050	0.050	0.050
NaCl	0.005	0.005	0.005
K_2HPO_4	Nil	0.00736	0.0736
Fe_2Cl_6	Trace	Trace	Trace
Distilled water to	1 litre	1 litre	1 litre

the concentrations of P_2O_5 were 0, 0.3 and 3.0 parts per 100,000 to filters A_1 , B_1 and C_1 , respectively. From the 23rd day until the end of the experiment on the 150th day 0.05 g. K_2SO_4 per litre was added to the solution to ensure an excess of potassium. Ammonium bicarbonate replaced ammonium chloride as the source of nitrogen from the 68th day until the end of the experiment for a reason given later, but the amount of N added remained unchanged.

The results of the experiment, showing the amounts of sucrose, P_2O_5 and N left in the effluents are given in Table I.

Oxidation of sugar. A small proportion of the sugar in the solution fed to filter A_1 appeared to be oxidised during passage through the filter, but the amount did not exceed 18 % of the sugar in the nutrient solution. The filtering medium was apparently devoid of organic film but microbiological examinations made by Miss Dixon proved that a few protozoa were present. It is concluded

Table I. *Effect of phosphate on biological oxidation of sucrose and removal of nitrogen.*

Exp. 1. Glass filters and glass medium ($\frac{1}{8}$ in. to $\frac{1}{4}$ in. graded glass).

Filter A_1 received sucrose equivalent to 105.3 parts glucose per 100,000 of solution, and no P_2O_5 .

Filter B_1 received the same amount of sucrose as A_1 , and 0.3 part P_2O_5 per 100,000.

Filter C_1 received the same amount of sucrose as A_1 , and 3.0 parts P_2O_5 per 100,000.

Day of exp.	Parts per 100,000.		Parts per 100,000.			Parts per 100,000.		
	P ₂ O ₅ in effluent from filter		Sucrose in effluent from filter calculated as glucose. Amount supplied, 105.3 parts			Nitrogen in effluent from filter. N supplied, 5 parts		
	B_1 (0.3 supplied)	C_1 (3.0 supplied)	A_1	B_1	C_1	A_1	B_1	C_1
14	0.0	1.25	86.0	3.6	0.4	4.800	3.530	1.430
19	0.012	1.50	95.9	19.6	1.9	4.809	3.907	3.170
27	0.0	2.50	—	0.9	1.1	4.539	3.998	4.556
36	0.002	1.50	100.4	2.6	0.7	4.916	3.041	1.176
43	0.0	1.39	—	2.3	0.3	4.460	3.442	2.675

therefore that in filter A_1 the absence of phosphate almost inhibited the growth of film so that not more than 18 % of a 0.1 % solution of sugar could be oxidised.

The addition of 0.3 part P_2O_5 per 100,000 as potassium phosphate to filter B_1 had a marked effect on the amount of sugar oxidised. When the first analyses were made after the filters had been in operation for 13 days 96.6 % of the sugar was being oxidised and over the first 43 days of the experiment the average amount decomposed was 94.5 %. Although the percentage oxidation of sugar was high the amount of film in B_1 was not considerable and consisted of a thin slime covering the glass medium in the uppermost sections, with much less in the three lower sections. The greatest contribution towards the total amount of sugar oxidised was made by section 1, which alone accounted for 89.7 % out of a total average oxidation of 94.5 %.

Filter C_1 received 3 parts P_2O_5 per 100,000 as K_2HPO_4 and gave a daily average oxidation of sugar of 99.2 % over the first 43 days. It should be pointed out that differences of a few per cent. are of greater significance when the purification figure approaches 100 % than at, say, 50. The amount of work, measured by extra filter space, required to increase a percentage purification from 50 to 60 is much less than that required to produce the same increment from 90 to 100. Thus section 1 of C_1 oxidised 98 % of the total sugar but a small amount of sugar was still present in the final effluent.

In filter C_1 the growth of film covered the surface of the medium and by the end of the first month the solution dropping on to the filter did not pass through immediately but gathered in pools on the surface and drained through the filter at a slower rate than was desired. This condition of ponding led to the initiation of the second series of experiments, the results of which are described later.

Removal of phosphorus and nitrogen. When no phosphate was supplied the amount of sugar removed from solution was, as previously stated, small and the amount of nitrogen removed was also small. Out of a total of 5 parts N supplied as NH_4Cl an average of only 0.3 part was taken up over a period of 43 days (Table I). An addition of 0.3 part P_2O_5 to B_1 increased the average removal of N over the 43 days to 1.416 parts. The quantity of P_2O_5 added in this case apparently did not provide enough phosphorus to cause all the dissolved sugar to be oxidised since some sugar remained after all the phosphorus

had been removed from solution. Not more than a trace of phosphorus was ever found in the final effluent from B_1 . The figures obtained with filter C_1 , however, showed that 3 parts P_2O_5 per 100,000 provided more than enough phosphorus. Practically all the sugar supplied was oxidised while only 1.63 parts of the P_2O_5 were removed. The fall in concentration of nitrogen in C_1 was 2.4 parts from an initial supply of 5 parts N.

Although it seemed unlikely that intermediate compounds such as hexosephosphate would be present in the effluents, as these substances do not ordinarily pass out of the living cell into the substrate, tests were made for the presence of soluble organic compounds of phosphorus in the effluents from the filters. The effluents were analysed for inorganic and organic forms of phosphorus as soon as sufficiently large volumes had been collected. In consequence the solutions were on an average 2 hours old at the time of analysis. The p_H values of the effluents of filters B_1 and C_1 were about 3.0. Under these conditions soluble organic compounds of phosphorus were invariably absent, although it is almost certain that at times organic phosphorus would be contained in the small amounts of film in suspension occasionally washed out of the filters. The effluents were settled for a few minutes so as to exclude visible suspended matter from the samples used for the determination of phosphorus.

In order to determine the amount of organic phosphorus which could be recovered in a typical hexosephosphate by the Parker and Fudge modification [1927] of the Denigès method, an examination was made of a sample of barium hexosephosphate, kindly supplied by Prof. A. Harden. The preparation contained 9.5 % moisture and the phosphorus content of the dry substance was approximately: total P_2O_5 , 22.7 %; organic P_2O_5 , 20.6 %; inorganic P_2O_5 , 2.1 %. Solutions of 10 mg. of the barium compound per litre were made up so that there were present: total P_2O_5 , 2.05 parts per million; organic, 1.87 parts; inorganic, 0.18 part. The corresponding figures as determined colorimetrically by the modified Denigès method were 2.1, 1.9, and 0.2 respectively, showing that it was reliable for the determination of the organic phosphorus in hexosephosphates. The p_H value of the distilled water in which the solutions were made up varied between 5.5 and 6.0. After storage for several hours it was observed that the amount of inorganic phosphorus in the hexosephosphate solutions had increased. Thus on one occasion 0.86 part inorganic P_2O_5 per million was found after the solution had stood for 8 hours, while on another occasion all the hexosephosphate had been completely hydrolysed after standing 24 hours at room temperature. It seems that very dilute aqueous solutions of hexosephosphate undergo hydrolysis on storage; the solutions were made up in distilled water but no precautions were taken to ensure that they were sterile. An alternative colorimetric method described by Zinzadé [1932] was tried, since the colour standards are more permanent than in the Denigès method, but the hexosephosphate appeared to be hydrolysed to inorganic phosphorus during analysis, owing to the greater concentration of mineral acid used by Zinzadé.

It has already been mentioned that the p_H values of the final effluents from filters B_1 and C_1 were about 3.0. When the source of nitrogen added was changed on the 68th day from ammonium chloride to ammonium bicarbonate, the p_H values of the solutions were either unchanged or increased on filtration through B_1 and C_1 . The p_H values of the nutrient solutions supplied to the filters were 5.5 for B_1 and about 6.0 for C_1 . Barritt [1931] filtered 0.2 % solutions of sucrose *plus* ammonium sulphate through sectional filters and found that the p_H of the liquid changed from an initial value of 7.0 to 5.5 in the

second upper section and to 7.1 in the sixth and final section; the reduction and subsequent increase in p_H were attributed to the production and oxidation of organic acids. The following considerations indicate that the observed differences in the p_H values of the solutions supplied to the filter and of the final effluents in Exp. 1 (Table II) were due mainly to the removal of nitrogen from

Table II. *Effect of source of nitrogen on formation of acid.*

Filters fed with sucrose solution and N as NH_4Cl (made up in distilled water) from start of experiment until the 68th day: after this they received N as NH_4HCO_3 .

A_1 received no P_2O_5 .

B_1 received 0.3 part P_2O_5 per 100,000.

C_1 received 3.0 parts P_2O_5 per 100,000.

N removed expressed as parts per 100,000.

Day of exp.	Nutrient solution p_H	1st section		2nd section		3rd section		4th section		Filter
		p_H	N re-moved	p_H	N re-moved	p_H	N re-moved	p_H	N re-moved	
14	5.4	5.1	—	5.1	—	4.8	—	4.6	0.2	A_1
	5.8	3.6	—	3.2	—	2.9	—	2.9	1.47	B_1
	6.6	2.9	—	2.9	—	2.9	—	2.9	3.57	C_1
19	5.6	—	—	—	—	—	—	4.4	0.19	A_1
	5.4	—	—	—	—	—	—	3.2	1.09	B_1
	6.0	—	—	—	—	—	—	3.0	1.83	C_1
26	5.4	5.2	0.37	5.2	0.00	5.4	0.00	5.6	0.00	A_1
	5.0	3.0	1.59	3.0	0.08	3.0	0.00	3.0	0.34	B_1
	4.7	—	3.38	3.0	0.08	3.0	0.09	3.0	0.28	C_1
43	6.0	6.0	0.56	5.1	0.04	5.3	0.49	5.5	0.38	A_1
	5.6	3.0	1.54	3.2	0.00	3.2	0.00	3.3	0.89	B_1
	6.2	3.2	2.07	3.2	0.00	3.1	0.34	3.1	1.02	C_1
70	7.4	—	—	—	—	—	—	7.2	—	A_1
	7.3	—	—	—	—	—	—	6.9	—	B_1
	7.1	—	—	—	—	—	—	6.0	—	C_1
75	7.4	—	—	—	—	—	—	7.3	—	A_1
	7.3	—	—	—	—	—	—	6.8	—	B_1
	7.1	—	—	—	—	—	—	6.4	—	C_1

ammonium chloride and the liberation of an equivalent quantity of free HCl. This was not neutralised or absorbed by the glass medium and therefore passed out with the effluent. For example, on the 19th day of the experiment the amounts of nitrogen expressed in parts per 100,000 removed by filters A_1 , B_1 and C_1 were 0.19, 1.09 and 1.83 respectively. Assuming that equivalent amounts of free HCl were liberated, the strength of acid in the effluents would be 0.137, 0.781 and 1.308 N/1000 for A_1 , B_1 and C_1 respectively. Sufficient acid would thus be produced to cause the drop in p_H value from 5.6 in the nutrient solution to 5.0 in the final effluent of A_1 , from 5.4 to 3.2 in B_1 and from 6.0 to 2.8 in C_1 . If chlorine ions, equivalent to the nitrogen assimilated, were taken up by the filter this argument would be untenable. Determinations of Cl were made therefore with silver nitrate and the following results were obtained:

Cl in nutrient solutions and effluents from filters.

Parts per 100,000.					
Cl in nutrient solution	Filter ...	...	A_1	B_1	C_1
" effluent of section 1	1		14.29	14.29	14.29
" "	2		14.26	14.26	14.87
" "	3		14.26	14.57	14.57
" "	4		14.27	14.72	14.72
" "			15.17	15.02	14.26

These results show that there was no uptake of Cl by the filters commensurate with the removal of nitrogen. If the decreases in p_H recorded with filters B_1 and C_1 were caused entirely by organic acids ionised to the same degree as acetic acid, then the concentration of acid required would be greater than 0.1 %. As the original amount of sugar was only 0.1 % a larger quantity of acetic acid could not be present. Moreover when determinations of the relative amounts of organic matter in the nutrient solutions and in the effluents were made by a test in which both sugar and organic acids are oxidised, *viz.* the biochemical oxygen demand, known as the "Dissolved oxygen absorbed test" in the Ministry of Health Methods [1929], results as follows were obtained:

Biochemical oxygen demand (B.O.D.) in 5 and 20 days.

Average results of five analyses made at different times on the liquids filtered and the effluents from the sections.

Parts per 100,000.										
Filter	Crude liquor filtered		Effluent of section 1		Effluent of section 2		Effluent of section 3		Effluent of section 4	
	5 days	20 days	5 days	20 days	5 days	20 days	5 days	20 days	5 days	20 days
A_1	69.2	103.6	71.1	93.6	63.4	78.7	61.1	99.4	56.3	95.3
B_1	64.1	105.3	46.2	73.2	39.1	55.0	45.1	69.2	33.1	55.4
C_1	69.6	96.2	26.4	41.9	25.4	45.1	15.4	28.4	8.6	18.3

A 0.1 % solution of sugar, acetic or lactic acid has a maximum B.O.D. figure of about 112, which is approached in 20 days. Even if the whole of the B.O.D. were due to one of these organic acids there would still be insufficient acid to account for the observed fall in p_H value during filtration of the sugar solutions. This evidence indicates that the differences in p_H values of the original solution and filter effluents are caused by mineral acid set free from the ammonium salt, a conclusion not necessarily conflicting with that of Barritt, if the differences between the two experimental conditions are considered. The 0.2 % sugar which Barritt used may have given a growth of film in which acid production proceeded more quickly than oxidation of sugar and acid.

Experiment 2.

This experiment was undertaken because (a) two of the filters used in Exp. 1 showed signs of choking before the completion of the work, (b) the development of highly acid effluents consequent on the use of ammonium chloride as a source of nitrogen may have interfered with the development of organic film and nitrification processes. To provide a larger air space in the glass filters used for Exp. 2 the broken glass medium was replaced by tubes, 1 cm. diameter and 1.5 cm. long. The sectional filters employed were A_2 , B_2 and C_2 and they received solutions of the same composition as were supplied to A_1 , B_1 and C_1 respectively in Exp. 1, except that (1) nitrogen was supplied as ammonium bicarbonate, (2) the nutrient solution filtered in A_2 contained 0.05 g. K_2SO_4 per litre. A rate of flow of 2 litres per day, equivalent to 100 g.y.d., was maintained and the experiment was continued for 73 days.

Development of film. At the end of the experimental period of 73 days there was no film visible on any of the glass tubes in filter A_2 ; the filter did not appear to retain liquid as long as B_2 or C_2 . The film in B_2 increased slowly in amount as the filter aged and apparently had not reached maximum activity at the

termination of the experiment. Filter C_2 had a white gelatinous growth on the surface within a fortnight and after this the film spread downwards through the first and second sections. At the end of the experiment the first section had a thick, pale yellow, gelatinous film but it was not choked, and liquid could be filtered at twice the desired rate without surface ponding. Section 2 in filter C_2 contained much film, though this was less in amount than in section 1, while there was considerably less film in section 3 than in section 2. In section 4 the small quantity of film present consisted of a thin slime on the glass tubes. It was observed in the early stages of the experiment that much of the film in sections 2 and 3 was formed by growths in the section above being washed into the one below, forming centres of inoculation from which the film spread to other parts of the filter.

Table III. *Effect of supply of phosphate on sucrose oxidation and removal of nitrogen.*

Exp. 2. Glass filters and tubular glass medium: Rate of flow = 100 g.y.d.

Filter A_2 received sucrose equivalent to 105.3 parts glucose per 100,000 and no P_2O_5 .

Filter B_2 received sucrose equivalent to 105.3 parts glucose per 100,000 *plus* 0.3 part P_2O_5 .

Filter C_2 received sucrose equivalent to 105.3 parts glucose per 100,000 *plus* 3.0 parts P_2O_5 .

2 litres of nutrient solution supplied daily = 100 g.y.d.

Day of exp.	P ₂ O ₅ in final effluent		Sugar in final effluents: glucose equivalent			Nitrogen in final effluents (5 parts N supplied)		
	B ₂ (0.3 supplied)	C ₂ (3.0 supplied)	A ₂	B ₂	C ₂	A ₂	B ₂	C ₂
26	0	2.00	96.8	67.1	2.0	4.98	4.07	2.20
33	0	—	107.0	61.5	2.0	—	—	—
44	—	1.00	111.8	58.0	0.4	3.73	4.13	2.67
58	0	0.75	108.5	50.3	0.8	4.79	3.75	2.98
60	0	1.00	103.2	57.8	0.9	4.81	3.98	2.36
66*	0.002	2.60	96.3	33.7	1.5	5.07	4.47	3.70
71	—	—	106.4	58.4	7.1	4.36	3.75	3.51
73	0	1.38	90.0	34.0	4.6	4.94	3.68	1.87

* Filter C_2 received 4 litres daily from 61st day.

Oxidation of sugar (Table III). The amounts of sugar oxidised in the three filters A_2 , B_2 and C_2 at different stages of the experiment are shown in Fig. 1.

Fig. 1. Effect of phosphorus supplied on oxidation of sucrose

The curves indicate a definite relationship between the supply of phosphorus and the amounts of sugar oxidised, provided adequate additions of other essential

mineral salts are made to the nutrient solutions. In the absence of added phosphorus filter A_2 rarely oxidised more than 14 % of the sugar, and the average amount oxidised over a period of 73 days was only 2.6 %. Filter B_2 , however, with 0.3 part P_2O_5 per 100,000 decomposed 36 % of the carbohydrates after a period of 26 days. The percentage oxidation increased steadily to 50 after 50 days and on the 73rd day, at the end of the experiment, filter B_2 was oxidising 68 % of the sugar. The trend of the curve for B_2 suggests that the amount of sugar oxidised by this filter would have continued to increase if the experiment had been prolonged. The results show that the smallness of the amount of phosphorus supplied restricted the growth of a population sufficiently active to oxidise all the sugar. As the filter accumulated phosphorus and the film developed, the amount of organic matter oxidised increased. Two of the many factors which influence biological oxidation in percolating filters are thus seen to be the supply of phosphorus and the time factor. When the results obtained with filters B_1 and B_2 are compared a striking difference is observed in the amounts of sugar oxidised. After receiving 0.1 % solution of sugar daily for 43 days (Table I) filter B_1 oxidised 98 % daily whereas B_2 oxidised only 43 % after operating for the same period (Fig. 1). This comparison emphasises another important factor in filtration, *viz.* the relationship between the size of filtering medium and the efficiency of the filter. Apart from the change made in the source of nitrogen the only difference between B_1 and B_2 was that B_1 contained small pieces of solid glass while B_2 was filled with larger pieces of tubular glass. This difference in size of medium affects the phosphorus requirements of the filters in the following manner. Sufficient phosphorus must be provided to B_1 to build a film able to retain the solution passing through the filter long enough for the sugar to be decomposed. With the small grade of medium in B_1 a limited growth of organisms was sufficient to join up the particles of medium and so increase the time of contact of the filter. The quantity of phosphorus used for B_1 , when supplied to B_2 , gave about the same growth, but here the film only covered the surface of the glass tubes and did not join them together so as to make a continuous growth. Spaces were thus left in the filter through which sugar passed and escaped oxidation. With filter C_2 , which received 3 parts P_2O_5 per 100,000, the amount of sugar decomposed reached 98 % in the first 26 days and later continued to improve until the oxidation attained was almost quantitative. Even when the rate of flow was doubled on the 60th day of the experiment over 93 % of the carbohydrate underwent oxidation.

The effect of supply of phosphorus on the contribution made by the different parts of the filter towards the total oxidation of the carbohydrate was found by dismantling the filters at various intervals and analysing the effluents of the different sections of the three filters. The results appear in Table IV. The samples were taken successively from the different sections over a period of 16 hours, beginning at section 4 and working upwards. Consequently the samples did not correspond exactly, and probably for this reason, the analytical results do not always show a regular decrease in sugar content from the inlet to the outlet of the filter. The figures in Table IV show that the effect of withholding phosphate from filter A_2 was to inhibit inversion and oxidation of sugar. Traces of invert sugar were generally present in the effluents from the first section of A_2 and in these cases the same quantity persisted almost unchanged in the effluents from lower sections. These small amounts of invert sugar may have been due to analytical errors in the Hagedorn-Jensen method as carried out by the author [Jenkins, 1933, 2]. With filter B_2 a high proportion of invert sugar always appeared in the effluent from section 1 with a tendency for the

Table IV. *Experiments with glass filters filled with tubular glass medium.*Solution fed to filter A_2 : Sucrose equal to 105.3 parts glucose; 5 parts N as NH_4HCO_3 ; no P_2O_5 .Solution fed to filter B_2 : Sucrose equal to 105.3 parts glucose; 5 parts N as NH_4HCO_3 ; 0.3 part P_2O_5 : per 100,000.Solution fed to filter C_2 : Sucrose equal to 105.3 parts glucose; 5 parts N as NH_4HCO_3 ; 3.0 parts P_2O_5 : per 100,000.

Total sugar and invert sugar (given as glucose) in effluents from sections.

Parts per 100,000.

Day of exp.	Section 1		Section 2		Section 3		Section 4	
	Total sugar	Invert sugar	Total sugar	Invert sugar	Total sugar	Invert sugar	Total sugar	Invert sugar
Filter A_2 . 2 litres solution fed daily.								
15	93.2	3.1	90.4	3.0	88.8	2.8	88.2	2.8
26	103.5	0.5	101.5	0.8	100.3	0.5	96.8	0.4
30	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	107.0	0.7
44	110.3	0.5	111.8	0.5	111.8	0.8	111.8	0.8
58	—	—	—	—	—	—	108.5	1.2
60	101.0	1.0	107.2	1.0	103.2	1.0	—	—
66	—	—	—	—	—	—	96.3	1.5
71	—	—	—	—	—	—	106.4	3.3
73	—	—	—	—	—	—	90.0	1.7
Filter B_2 . 2 litres solution fed daily.								
15	81.2	17.1	63.5	16.2	69.7	15.8	65.0	14.8
26	59.2	29.9	89.3	35.4	78.0	36.4	67.1	30.7
30	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	61.5	56.4
44	74.0	25.2	80.0	30.1	65.0	37.3	58.0	50.1
58	—	—	—	—	—	—	50.3	26.0
60	49.0	23.4	58.0	24.8	48.7	27.9	57.8	33.7
66	—	—	—	—	—	—	33.7	24.0
71	—	—	—	—	—	—	58.4	26.6
73	—	—	—	—	—	—	34.0	25.3
Filter C_2 . 2 litres solution fed daily until 60th day; after that 4 litres fed daily.								
15	44.5	16.8	48.9	14.2	46.4	14.6	40.6	12.9
26	22.1	6.8	5.8	4.0	1.4	1.0	2.0	0.6
30	44.4	26.1	31.9	25.4	29.1	20.0	18.7	18.7
33	—	—	—	—	—	—	2.0	2.0
44	3.5	1.6	1.2	0.7	0.9	0.3	0.4	0.3
58	—	—	—	—	—	—	0.8	0.8
60	3.4	0.8	0.8	0.4	0.3	0.3	0.9	0.6
66	—	—	—	—	—	—	1.5	1.5
71	—	—	—	—	—	—	7.1	3.2
73	—	—	—	—	—	—	4.6	1.1

amount of invert sugar to increase on passage down the filter. On two occasions the sugar in the final effluent was largely the inverted product, there being 56.4 parts invert sugar in a total sugar content of 61.5 parts on the 33rd day and 50.1 out of a total of 58.0 on the 44th day. The view has previously been advanced [Jenkins, 1933, 2] that the occurrence of a large proportion of invert sugar in the effluents of a filter receiving solutions of sucrose is a sign of an immature film, and that increase in efficiency of oxidation brings about a decrease in the proportion of invert sugar in the total sugar. The observations made above on the composition of the effluents from the different sections support this view, since B_2 never reached maturity during the 73 days of the experiment. Although the film in B_2 was unable to oxidise the carbohydrates that passed through it, the filter was yet able to invert a large proportion of

the sugar it received. The film in section 2 of filter B_2 was small in amount and considerably less in the two lower sections. All these sections however were able at times to bring about the inversion of appreciable quantities of sugar in the absence of measurable amounts of inorganic phosphate in the effluents (less than 0.002 P_2O_5 per 100,000) but were unable to carry the decomposition much beyond this stage. The addition of 3 parts P_2O_5 per 100,000 to the sugar solution fed to filter C_2 resulted in the growth of much film in section 1 and less in section 2. Only a very small amount of growth took place in sections 3 and 4, owing to the low concentration of sugar in the solutions reaching these sections. As previously observed, some of the film in section 2 was washed down from the upper part of the filter.

A large proportion of the sugar which passed through filter C_2 was hydrolysed to invert sugar: on the 30th day all the sugar contained in the effluent of section 4 appeared in this form. The concentration of sugar in effluent 4, filter C_2 , was negligible after the 30th day as long as the rate of 100 g.y.d. was maintained.

Phosphorus and nitrogen removed. The quantities of phosphorus and nitrogen taken up by the filters are given in Table V. The figures show that with one

Table V. *Phosphate and nitrogen in effluents at different levels of the filters.*

Exp. 2. Glass filters and tubular glass medium: rate of flow = 100 g.y.d.

Filter A_2 received sucrose equivalent to 105.3 parts glucose per 100,000 and no P_2O_5 .

Filter B_2 received sucrose equivalent to 105.3 parts glucose per 100,000 plus 0.3 part P_2O_5 .

Filter C_2 received sucrose equivalent to 105.3 parts glucose per 100,000 plus 3.0 parts P_2O_5 .

	Parts per 100,000.									
	26th day of Exp.					60th day of Exp.				
	P_2O_5		Nitrogen			P_2O_5		Nitrogen		
	B_2	C_2	B_2	C_2		B_2	C_2	A_2	B_2	C_2
Nutrient solution contained	0.3	3.00	5.00	5.00		0.30	3.00	5.00	5.00	5.00
Effluent of section 1 contained	Trace	2.00	4.04	2.23		0.00	1.00	4.92	4.36	3.40
" 2 "		1.87	4.07	2.09		0.00	0.76	4.81	4.28	2.88
" 3 "	0	1.50	4.07	1.92		0.00	0.88	4.81	4.17	2.77
" 4 "	0	2.00	4.07	2.20		0.00	1.00	4.81	3.98	2.36

exception the phosphate added to B_2 was removed quantitatively by this filter. Reference to Tables IV and V shows that section 1 of B_2 was responsible for the removal of the whole of the phosphate and for the oxidation of most of the sucrose. The absence of phosphorus from the solutions of sugar leaving this section accounts for the poor development of film in the remaining sections of this filter. Varying amounts of nitrogen were taken up by B_2 during the early stages of the experiment but later small amounts were removed continuously. An average of 1.02 parts N per 100,000 was removed from the solutions, which before filtration contained 5 parts. Table V shows that most of the nitrogen was absorbed by section 1 of B_2 and only small amounts by lower sections. The solutions leaving section 1 therefore contained sugar and ammonium salts but practically no phosphorus. On the other hand, filter C_2 received more than enough phosphorus for its requirements (Table V), an average of 1.54 parts P_2O_5 being taken from a supply of 3 parts. This uptake of phosphorus enabled C_2 to oxidise almost completely 2 litres of a solution of 0.1 % sucrose every day for the first 60 days and 4 litres daily after the 60th day of the experiment.

The average amounts of nitrogen retained by the filters are obtainable from the data given in Table V and show that the uptake of this element depends upon the quantity of phosphorus available for the growth of the film. Thus in the absence of phosphorus only 6.6 % of the N supplied to A_2 was removed: B_2 and C_2 , which received 0.3 part and 3 parts P_2O_5 respectively, took up 20.5 and 44.9 % of N respectively.

Figures for the total amounts of C, N and P removed from solution by filters B_2 and C_2 during the experiment are presented in Table VI. The difference

Table VI. *Carbon, nitrogen and phosphorus removed from solutions.*

Parts per 100,000.								
Filter B_2 Experimental period						Filter C_2 Experimental period		
First 44 days			Last 29 days			73 days		
C	N	P	C	N	P	C	N	P
17.2	1.83	0.13	23.4	1.07	0.13	41.1	2.08	0.64
Ratio C/N/P 9.4/1/0.071			21.9/1/0.12			19.8/1/0.31		

in the ratios of C, N and P in B_2 during the first 44 days and the last 29 days means that more carbon was oxidised for a given supply of N in the later stages of the experiment than in the earliest part. Although the C/N/P ratios for the last 29 days of B_2 and the whole period for C_2 are similar in respect of C and N yet the actual amounts of C and N oxidised and removed differed considerably in the two filters. The suggestion was made in a previous paper [Jenkins, 1933, 3] that nitrogen would be used most economically in a filter oxidising sugar if the initial C/N ratio were kept at about 30/1. A C/N ratio of 19.8/1 was obtained with C_2 because an excess of N was supplied. In the presence of less N the same amount of sugar would probably have been oxidised and C and N removed in a wider ratio. According to the figures given above, the minimum proportion of phosphorus required for the efficient utilisation of nitrogen would lie between 0.12 and 0.31 part available P (0.28 and 0.71 parts respectively P_2O_5) per part of nitrogen.

As previously mentioned, the formation of acid in percolating filters was found to arise from the use of NH_4Cl as the source of nitrogen. In Exp. 2 slight variations were found in the p_H values of the solutions undergoing filtration (Table VII) but reductions in p_H of the order observed when ammonium chloride

Table VII. *Average p_H values of nutrient solutions and effluent.*

Filter	Nutrient	Section effluents			
		1	2	3	4
A_2	7.8	7.8	7.8	7.9	7.8
B_2	7.3	7.2	7.3	7.3	7.1
C_2	7.1	7.6	7.2	7.3	7.0

was the source of N were not detected. There is thus no evidence that intermediate organic acids were formed to any considerable extent during the aerobic oxidation of 0.1 % sugar by the mixed flora of the percolating filter operated under the conditions observed in these experiments.

Experiments 3 (a) and 3 (b).

The availability of compounds of phosphorus in percolating filters.

Exp. 3 (a). The apparatus used for this experiment was the large scale filtration plant previously described by Richards and Cutler [1933]. The liquid treated by the biological filters was the effluent from the beet-pulp presses of a beet-sugar factory, diluted and partly fermented, so that it gave approximately the following results on analysis:

						Parts per 100,000
Oxygen absorbed in 4 hours from permanganate ...						122
Biochemical oxygen demand in 5 days						109
Biochemical oxygen demand in 20 days						156
Sucrose	...	...	...	...	...	68
Acetic acid	...	...	...	...	...	20
Lactic acid	...	...	...	...	...	20
Solids in suspension (4 % ash)... ..						42
P ₂ O ₅ , all organic						0.85
Nitrogen in organic combination						2.5
Nitrogen as ammonia						0.01

p_H, 5.1

The suspended matter consisted of cellulose and fibre derived from the sugar-beet root, while the organic nitrogen was mostly of protein origin and less readily available to micro-organisms than ammonia. The organic compounds of phosphorus were probably hexosephosphate, nucleoproteins and phospholipins derived from organisms and the cell contents of the beet. Liquid of the average composition given above was distributed at a rate of 50 g.y.d. over two filters. As the chief object of the experiment was to test the effect of inoculation on the rate of oxidation of the constituents of fermented press water, one of the filters was inoculated with organisms selected by Mr D. W. Cutler and Miss L. C. Crump, while the other filter was not inoculated. Despite this difference the final effluents of both filters were almost identical in chemical composition. Hence they have been considered as duplicates for the purpose of this experiment: the figures given are the averages of the analytical data from the inoculated and uninoculated filters. During the first 20 days the organic compounds of nitrogen and phosphorus present in the liquids filtered varied according to the composition of the factory press water, but known quantities of ammonia or potassium phosphate were added. The liquids filtered during this period could be classified as follows:

(1) For the first 7 days the liquids contained nitrogen as ammonia and in organic combination as well as 0.85 part P₂O₅ per 100,000 as organic phosphorus supplied by the pulp liquor.

(2) From the 8th day to 13th day the liquids contained nitrogen as ammonia and in organic combination besides phosphorus in organic combination and as added K₂HPO₄.

(3) From the 14th day to 18th day the substances in (2) were present, though the amounts of N and inorganic phosphorus were less.

(4) After the 20th day the liquids contained organic nitrogen, phosphorus in the organic and inorganic states and traces of ammonia.

The results of operating the filters under the conditions given above are summarised in Table VIII. The figures for percentage purification represent the averages of the amounts of oxidation of organic matter in the two filters as measured by the B.O.D. test. It must be pointed out that the conclusions

Table VIII. *Results of large-scale experiments.*

		Parts per 100,000.								
Day of Exp.	Rate g.y.d.	P ₂ O ₅ in liquid filtered		P ₂ O ₅ recovered in effluent.	N in liquid filtered		N recovered in effluent		Oxida- tion of organic matter %	
		Inor- ganic	Organic. Average figure		Ammonia N	Organic N	Ammonia N	Organic N		
0-7	50 for 10 hrs. daily	0	0.85	0	3.00	1.85	2.50	1.12	25	
8		3.0	"	0.30	3.00	1.85*	2.00	—	28	
10		"	"	0.50	3.00*	2.48*	(Total N = 1.49)		55	
11		"	"	0.50	3.00*	2.38*	(Total N = 1.03)		81	
12		"	"	0.41	3.00*	1.60*	(Total N = 0.78)		75	
13	50	"	"	0.56	2.5	3.76	0.07	1.16	86	
14		1.5	"	0.38	1.5	3.34	0.05	1.49	86	
15		50	"	"	0.02	2.25	2.00	0.03	1.21	85
16		50	"	"	0.02	1.00	2.16	0.01	1.25	78
18		100	"	"	0.22	—	—	—	—	66
20		100	"	"	0.75	0.08	2.80	0.03	—	51

* Approximate.

drawn should be regarded as tentative or as supporting those already given in the early part of the paper, for the reason that liquors of different composition were treated in the same filter in its early stages and not simultaneously in different filters. Hence comparisons are available only between the results of filtering liquids of different composition at different periods in a filter undergoing development.

If the results given above are considered with respect to the changes made in operating the filters the following conclusions may be drawn:

(1) *1st-7th days.* In the presence of organic phosphorus and nitrogen in organic and inorganic combination not more than 25 % of the organic matter was oxidised on filtration and only 17 and 39 % respectively of the inorganic and organic nitrogen supplied were removed by the filters. The amount of organic phosphorus removed from the liquid was not determined, but as an approximation it is assumed that the quantity was proportional to the uptake of organic nitrogen.

(2) *8th-13th days.* The response to the addition of an inorganic source of phosphorus on the 8th and on successive days was immediate. Inorganic phosphorus to the extent of 90 % was removed in the first 2 days and an average retention of 85 % was recorded over the period 8th to 13th days. The removal of inorganic nitrogen by the filters increased progressively from 33 % on the 8th day to 98 % on the 13th day, while the percentage purification increased from 28 to 86 in this period. This increase in percentage purification probably included the improvement in efficiency which generally accompanies the ageing or maturing of a filter. Cutler and Crump (unpublished work) found that the bulk of film rapidly increased in both filters between the 8th and 13th days.

(3) *14th-18th days.* A reduction in the amount of added phosphorus resulted in a greater percentage retention of P₂O₅, which increased to about 98. As long as ammonia was present, nitrogen in this form was removed almost quantitatively. The average oxidation of organic matter amounted to about 80 %.

(4) *20th day et seq.* When ammoniacal nitrogen was withheld the removal of inorganic phosphorus decreased and a rapid fall in the percentage oxidation followed. In the continued absence of inorganic nitrogen the percentage purification of organic matter remained at a low figure, in spite of the presence of an inorganic compound of phosphorus.

The figures above therefore show that (a) the phosphorus in the organic compounds contained in the press water of a sugar-beet factory is not so readily available to the micro-organisms in a percolating filter, even in the presence of sufficient available nitrogen, as an inorganic supply of phosphorus such as K_2HPO_4 ; (b) in the presence of potassium phosphate, nitrogen as ammonia is removed by the micro-organisms of a filter in preference to the organic compounds of nitrogen present in press water, with consequent improvement in the percentage purification of organic matter by the filter; (c) the removal of phosphorus as dissolved phosphate by a filter is slow and incomplete if the liquids filtered do not contain nitrogen in a form which can be rapidly utilised by the micro-organisms responsible for the activity of the filter. Press water appears to be deficient in this form of nitrogen.

Exp. 3 (b). The last conclusion, (c), received substantial corroboration from the results of an experiment briefly described below.

Two small laboratory filters were filled with gravel and supplied with solutions of the same composition as those used for *Exp. 3 (a)*, at a rate of 100 g.y.d. Sufficient K_2HPO_4 was added to the solution fed to one filter to provide 1.5 parts P_2O_5 per 100,000: no such addition was made to the liquid supplied to the control filter. The crude liquor of both filters contained approximately 0.85 part P_2O_5 as organic phosphorus during the experimental period of 26 days. Analyses were made on 21 days and the following average results for percentage purification were obtained:

Average percentage purification of diluted press water.

Control filter received press water containing 0.85 part organic P_2O_5 per 100,000.

Phosphate filter received press water containing 0.85 part organic P_2O_5 and 1.5 parts inorganic P_2O_5 per 100,000 added as K_2HPO_4 .

Oxygen absorbed from N/8 $KMnO_4$ % purification		B.O.D. in 5 days % purification		B.O.D. in 20 days % purification		% removal of total sugar	
Control	Phosphate	Control	Phosphate	Control	Phosphate	Control	Phosphate
46	52	44	38	36	37	53	37

In any one of the above tests the differences between the averages of the percentage purifications for the control and the phosphate filter are not significant.

The experiment therefore shows the oxidation of the organic matter in fermented press water by a percolating filter to be unaided by the addition of an available supply of phosphorus, if only those organic compounds of nitrogen normally found in press water are present.

DISCUSSION OF RESULTS.

The experiments described in this paper demonstrate that the influence of phosphorus on the rate of oxidation by a percolating filter of carbohydrates and the organic matter present in the press water of a beet-sugar factory is dependent on several factors, *viz.* (a) size of grade of filtering material, (b) the amount and nature of the compounds of phosphorus present, (c) the nature of the compounds of nitrogen present.

In order to build up a film able to oxidise a given quantity of carbohydrate at a given rate of flow a filter filled with $\frac{1}{16}$ to $\frac{1}{4}$ in. medium requires a smaller amount of available phosphorus than a filter with a coarse grade of material

such as glass tubes 1.5 cm. long and 1 cm. bore. The primary function of the phosphorus is to supply the nutrient necessary for the growth of enough film to increase the time of passage of the dissolved organic matter through the filter. The results of Exps. 1 and 2 bring out the difference caused by filtering solutions of the same composition through filters filled with different grades of material. Filter B_1 , with a small size of medium, received 0.3 part P_2O_5 per 100,000 and decomposed daily 98 % of a 0.1 % solution of sucrose after the filter had been in operation for one month, while B_2 , which contained a larger grade of medium, but received the same amounts of phosphorus and sugar, oxidised less than 50 % of the sugar after operating for the same period. Filters with too fine a grade of medium suffer from the disadvantage of choking whenever vigorous growth of film occurs. Thus, increasing the supply of phosphorus to 3 parts P_2O_5 per 100,000 in Exps. 1 and 2 led to the choking of the fine medium in filter C_1 , whereas the coarse medium in filter C_2 showed no signs of ponding although it received solution of the same composition as that passed through C_1 . This observation might be of importance in actual practice where the ponding of filters increases operating difficulties and lowers the efficiency of the filter.

When 0.1 % solutions of sucrose containing 0.3 part P_2O_5 per 100,000 were filtered the phosphorus was, with one exception, quantitatively absorbed by the filter. An increase to 3 parts P_2O_5 in the liquids undergoing filtration led to a removal of about 1.5 parts P_2O_5 and the remainder passed out as inorganic phosphate with the effluent. Organic compounds of phosphorus were never detected in the effluents from any sections of the filters, although by the method used [Parker and Fudge, 1927] it was possible to determine as little as 0.002 part P_2O_5 as hexosephosphate per 100,000 of solution. Barritt [1931] treated a 0.2 % solution of sucrose, containing about 23 parts P_2O_5 per 100,000 as mixed potassium phosphates, in a percolating filter and found that the whole of the phosphate was not taken up by the filter. Even when one-sixth of this quantity of phosphate was used, *viz.* about 4.0 parts P_2O_5 per 100,000, he observed that inorganic compounds of phosphorus still appeared in the effluents. The results given in this paper would suggest that the occurrence of phosphate in the effluents from Barritt's filters was the result of supplying the filter with more phosphate than it could remove from solution. No evidence was found to indicate that hexosephosphate appeared in the effluents and became hydrolysed during analysis. Harden [1923] supposes that hexosephosphates are formed within the living cell first by the diffusion of sugar from the substrate through the cell wall, followed by the union of sugar with the phosphate already present in the cell. This compound is then split up into simple products and the phosphate which is regenerated is again able to unite with sugar.

The experiments on the filtration of press water from a beet-sugar factory show that hitherto unsuspected differences may exist as regards the availability of compounds of phosphorus in a percolating filter. In previous work reported by Richards and Cutler [1933] an ample supply of available nitrogen was recognised as essential for the rapid oxidation of dissolved organic impurities. The organic compounds of nitrogen and phosphate in press water probably have varying degrees of availability. Such compounds are removed mechanically by the filter before being assimilated by micro-organisms. The rate of oxidation of the organic matter passing through the filter is limited by the rate at which the nitrogen becomes available and this rate does not seem to be increased by supplying compounds of phosphorus which are readily utilisable by micro-organisms. If, however, nitrogen exists in the filter in an easily assimilable form such as

ammonia, the rate of oxidation of organic matter is considerably increased by the supply of an available form of phosphorus.

Solutions of sugar and ammonium chloride suffered a considerable reduction in p_H on filtration, from a figure of 5.4 in the nutrient solution to 3.2 in one effluent, and from 6.0 to 2.8 in another. As previously mentioned, tests for the amount of dissolved organic matter showed that the decrease in p_H was too great to be accounted for solely by the production of organic acids from the sugar: negative results were obtained on testing for simple organic acids in the final effluents. It was also observed that the concentration of chloride in the solution undergoing filtration remained practically constant throughout its passage down the filter. Furthermore the p_H of the nutrient solution was not reduced appreciably when ammonium bicarbonate was substituted for ammonium chloride. These facts all indicate that the acid production in a percolating filter operated under the conditions described herein is chiefly derived from the mineral acid liberated by the removal of ammonium ion from the chloride or sulphate. Barritt [1931] found that organic acids may be produced in the upper part of a percolating filter if 0.2 % solutions of sugar are filtered: a reduction in p_H is observed in that part of the filter but the acid is oxidised as the solutions pass downwards, with corresponding increases in the p_H values of the effluents.

SUMMARY.

1. Experiments have been carried out on the biological oxidation in percolating filters of (a) 0.1 % sucrose *plus* different amounts of phosphate as K_2HPO_4 and with NH_4Cl or NH_4HCO_3 as the source of nitrogen, using broken glass as the filtering medium; (b) 0.1 % sucrose *plus* NH_4HCO_3 and different amounts of phosphate, with glass tubes as the filtering medium; (c) diluted press water from a beet-sugar factory; in this case the effect of organic compounds of phosphorus on the oxidation of organic matter was studied in the presence of sources of nitrogen readily available to micro-organisms, and in the presence of compounds of nitrogen available only with difficulty.

2. The amount of phosphate required to ensure rapid oxidation of sugar in a 0.1 % solution is influenced to a large extent by the nature of the filtering medium. To ensure freedom from choking, the medium should be of a large size and of a suitable shape. Under these conditions a 0.1 % solution of sugar requires at first a maximum of about 1.5 parts P_2O_5 and 2.2 parts N per 100,000 for complete oxidation of the sugar; once the film becomes mature 0.3 of P_2O_5 and 1 of N.

3. Excess of phosphate supplied to the filters passes out apparently unchanged. No organic compounds of phosphorus were ever found in the effluents from any of the sections of the different filters.

4. The organic compounds of phosphorus in the press water of a beet-sugar factory are only slowly available to the micro-organisms on a percolating filter as compared with potassium hydrogen phosphate, and this may limit the rate of oxidation of organic matter by a percolating filter. In the absence of a form of nitrogen which is readily assimilated by micro-organisms the full effect of a supply of available phosphorus is not shown.

5. When percolating filters filled with glass medium were supplied with sugar, ammonium chloride and various amounts of K_2HPO_4 the p_H values of the solutions before and after filtration were:

- (a) 5.6 and 5.0 respectively, without P_2O_5 ;
- (b) 5.4 and 3.2 respectively, with 0.3 part P_2O_5 per 100,000;
- (c) 5.0 and 2.8 respectively, with 3.0 parts P_2O_5 per 100,000.

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The cause of acid production was found to be the removal of NH_3 from NH_4Cl and the liberation of free HCl . Quantitative tests and measurements of the dissolved organic matter present in the effluents showed that this acidity was not due to organic acids.

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