XX. THE BIOLOGICAL OXIDATION OF CARBOHYDRATE SOLUTIONS.

PART I. THE OXIDATION OF SUCROSE AND AMMONIA IN SECTIONAL PERCOLATING FILTERS.

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THE manufacture of sugar from the sugar beet gives rise to large volumes of waste water of a polluting nature. About five-sixths of the beet sugar factory effluent originates from the water which is used to wash and convey the beet from open storage silos or from trucks into the factory. Under usual conditions the conveying or fluming water requires only simple treatment so that it may be used again in the factory. The remaining one-sixth of the total effluent is obtained from the beet pulp, which after extraction of sugar by diffusion, is dried and used as cattle food. Before the spent pulp is dried, as much water as possible is squeezed out of it. A "press water" is thus obtained which is highly polluting by reason of dissolved organic substances. Numerous attempts have been made on the Continent to devise a process which would destroy the dissolved substances but the methods tried have not been attended with great success.

Press water is a liquid of varying composition, but to illustrate its nature the following analysis of a sample taken from a factory is given; sucrose 230, protein 23, pectin 4.3, pentosan 13.4 and saponin 0.2 parts per 100,000. In view of the pollution already experienced and the increase that would probably follow as a result of the extension of the beet sugar industry, investigations were begun in 1927 at the Rothamsted Experimental Station and at the Colwick factory of the Anglo-Scottish Beet Sugar Corporation, Nottingham, for the Department of Scientific and Industrial Research with the object of ascertaining whether liquids such as press water could be satisfactorily purified by biological oxidation on percolating filters. The results of the semi-commercial scale experiments at Colwick have already been briefly described in the first three annual reports of the Water Pollution Research Board of the Department of Scientific and Industrial Research [1928, 1929, 1930]. At Rothamsted preliminary experiments showed that effective oxidation of solutions of the approximate composition of press water could be achieved, after dilution, by filtration through biological or percolating filters. The filters consisted of earthenware drainpipes 24 inches deep and 6 inches diameter filled with 1/8 to 1/4 inch clinker. In order to obtain artificial press water, sliced mangolds

or sugar beet were first boiled with water, the sugar extract strained off, and the residual pulp was then squeezed in a press. The runnings were diluted to the desired strength. Such artificial press liquors were most effectively oxidised when diluted so as to contain 0.1 % of sucrose, and at this strength they were allowed to trickle continuously on to the filters at a rate of 4.6 cc. per minute, equivalent to 100 gallons per cubic yard of filter per day. In this manner 90–95 % of the sugar was oxidised.

It was observed that the mangold or beet extracts rapidly became acid, owing to the fermentation of the sugar. Thus the p_H value of the freshly made solutions fell from 7.5–8.0 to 3.0–5.0 in less than 24 hours. The effluent obtained from the filtration of such an acid solution, however, was invariably neutral or slightly alkaline. When the acid fermented solutions were made alkaline with milk of lime and then filtered, the activity of the micro-flora seemed to be impaired in some way and an effluent of inferior quality resulted. In later experiments it was found that when a solution made from beet press water was fermented for 8 hours at 35°, the products were approximately 45 % unchanged sugar, 40 % hydroxy-acids and 15 % aliphatic acids. The behaviour on filtration of solutions composed of sugar and such typical products of fermentation as acetic and lactic acid was therefore studied in some detail. The results of these experiments proved conclusively that the course taken by natural fermentation provides a suitable liquid for oxidation on percolating filters; a mixture of sugar, acetic and lactic acids derived from a fermented solution of sugar is more easily oxidised than the original sugar solution. Only a partial conversion of sugar to acid is desirable. It seems that the acids alone do not provide the filter organisms with the most suitable food material to encourage the growth of a highly efficient biological film; the acids are then only partially oxidised. Moreover, filters fed with either acetic or lactic acid require added nitrogen, whereas those provided with a mixed diet of sugar and acid appear able to utilise very small amounts of nitrogen or possibly to fix atmospheric nitrogen. An interesting observation made by Dr Sandon, of the Rothamsted Experimental Station, was that all the filters, whether fed with sugar, sugar plus acetic acid, sugar plus lactic acid, lactic acid, acetic acid or any similar combination with different forms of added nitrogen, seemed to support the same protozoan and insect population.

One of the most significant observations made during the 1928 experiments was the presence of nitrates in incompletely oxidised effluents. These experiments were carried out with the following solutions: (a) 0.1 % sugar with 0.003 % of nitrogen added as ammonium chloride, and made up in tap-water containing 0.0005 % of nitrogen as nitrate; (b) as (a) but with 0.003 % of nitrogen added as albumin. The effluents regularly contained 0.0015 % of nitrogen as nitrate when as much as 0.01 % of sugar was present at the same time. There was thus some evidence that nitrification may occur in presence of organic matter. This conflicts with the generally accepted view in sewage purification that oxidised nitrogen does not appear in an effluent until the

organic matter has been decomposed. Oxidation of carbon precedes that of nitrogen when solutions of organic nitrogenous compounds are stored in bottles and supplied with an ample quantity of oxygen. With asparagine, for instance, Adeney [1908] found that hydrolysis first takes place, giving aspartic acid and ammonia. This is followed by complete oxidation of the aspartic acid before the ammonia is nitrified. Although the results of previous investigations on the oxidation of dissolved substances have been confirmed, the generally accepted view that easily oxidisable carbonaceous matter inhibits nitrification on percolating filters was not supported by the results obtained with drainpipe filters. The frequent presence of nitrate and sugar in the laboratory filters might conceivably be explained by assuming that some of the sugar in the fresh liquor always side-tracked down the filter or ran straight through the medium and that such solution would form one of the numerous integral parts of the whole effluent. This possibility is dealt with in the present paper.

In some earlier experiments on biological filtration in 1927, mangold extracts containing 0.1 % of sucrose were run through drainpipe filters 2 feet deep. The disappearance of organic matter was measured at depths of 6, 12, 18 and 24 inches by means of the oxygen taken up by the sample from acid permanganate in 4 hours, with the result that the upper half of the filter removed the greater part of the organic matter just as in sewage filters. It is apparent that where the food supply is richest and the air supply is plentiful, micro-organisms will become more abundant. They are then able to utilise the strong solutions which are run on to them. Moreover, with liquids such as sewage, the bacteria and zoogaea can coagulate colloidal or suspended organic matter and then live on the coagulum. The lower part of a biological filter is thus apparently inefficient and contains less vital film only because its food supply is limited by the activity of the upper part.

The work described in the present paper was undertaken with the following objects:

- (1) to account for the advantage of oxidising a solution of sugar partly fermented to acids over that of an unchanged solution of sugar;
- (2) to follow the course of sugar decomposition and nitrification of ammonia through different levels of a percolating filter;
- (3) to ascertain the function of different sections of a percolating filter.

EXPERIMENTAL.

Apparatus and methods of analysis.

Apparatus (Fig. 1). The sectional filter used in these experiments consisted of 6 glass cylinders each 6 inches in diameter and 9 inches deep. The cylinders rested in perforated zinc trays supported on the ledges of a vertical frame. Liquid passing through the perforations was caught on a funnel and then dropped on to the next section. In order to obtain a constant flow of sugar

solution through a tube wide enough to be cleaned easily of bacterial growths, the apparatus shown in Fig. 1 was used. This device controls the hydrostatic head of solution in an aspirator by means of a length of fine capillary tubing. The sections were filled with 1/8 to 1/4 inch clinker which had been well washed after use in similar filtration experiments.

Methods of analysis. Sucrose. (a) This was determined by the Hagedorn-Jensen method as modified by Hanes [1929]: 2.5 cc. of the solution were inverted with 1 cc. NH_2SO_4 by heating on a boiling water-bath for 20 minutes and then neutralised with 1 cc. of $NNaOH$. The sucrose was returned as glucose. The limits of the Hanes modification are from 1.74 to 4340 mg. glucose per 100 cc.

(b) An alternative modification has been used for the range of 0.347 mg. to 3470 mg. glucose per 100 cc. A solution is made up containing 3.3 g. potassium ferricyanide and 10.6 g. sodium carbonate per litre. 10 cc. of this solution and from 0.1 to 10 cc. of the unknown solution are heated according to the usual procedure and the residual ferricyanide is finally determined by reducing it with KI and titrating with $N/200 Na_2S_2O_3$.

Invert sugar. The Hagedorn-Jensen solutions were used but 10 cc. of the ferricyanide solution were taken instead of 2 cc. and the volume of invert sugar made up to 10 cc. The range of the method is thus greatly increased, the difference between the blanks on successive days is also reduced and more reliable results are obtained than in the original Hagedorn-Jensen method. While the limits of the latter method are from 0.868 mg. to 347 mg. glucose per 100 cc. those of the modified method are from 0.174 mg. to 1735 mg. per 100 cc.

Nitrogen as NH_3 . (a) Whenever the volume of solution permitted 250 cc. were distilled with ammonia- and nitrogen-free $NaOH$ made according to the Ministry of Health Methods of Analysis [1929, p. 20]. The distillate was titrated with $N/25 H_2SO_4$ using cochineal as indicator.

(b) When the above method was impracticable 50 cc. of the solution were evaporated to dryness in a water-bath and the residue was taken up in 5 cc. $NNaOH$ and 50 cc. 95 % alcohol. This solution was then steam-distilled in Foreman's apparatus [1928] until 100 cc. of distillate were collected and the ammonia was titrated with $N/25 H_2SO_4$. It was found that this method gave a complete recovery of ammonia from dilute solutions of ammonium salts.

Nitrogen as NO_2 . This was determined colorimetrically as described in the Ministry of Health Methods [1929, p. 8].

Nitrogen as NO_3 . The residue in the steam distillation flask left after the determination of ammonia by Foreman's method was neutralised with normal acid and made up to 200 cc. One-half of this solution was reduced by incu-

Fig. 1. Sectional percolating filter.

bation overnight at 35° with a zinc-copper couple; the ammonia was then distilled off and estimated by nesslerisation. The remaining half of the solution was incubated without the addition of a reducing agent, distilled and the distillate nesslerised. This represents the blank on the method. Details of the method are given in the Ministry of Health Methods [1929, p. 11].

Oxygen absorbed from permanganate in 4 hours. 10 cc. of $N/8$ $KMnO_4$ and 10 cc. of 25 % H_2SO_4 were incubated for 4 hours at 35° with sufficient solution of sugar or effluent to reduce not more than one-half of the permanganate. The unchanged permanganate was estimated with $N/20$ sodium thiosulphate. The amount of permanganate reduced is practically proportional to the sugar in the solution, provided the quantity of permanganate decomposed remains constant.

Biological oxygen demand, i.e. the oxygen absorbed from solution in 5 days or 20 days. In order to carry out this determination the dissolved oxygen present in a mixture of tap-water and the solution under examination is found. The mixture is then incubated for either 5 or 20 days at 20° and the oxygen absorbed from solution in the oxidation of the organic matter is obtained from a second oxygen determination. Under rigidly controlled conditions the oxygen taken up is approximately proportional to the organic matter present in the diluted solution.

p_H value. This was determined with the Hellige apparatus. The figures obtained gave good agreement when checked by the quinhydrone electrode method.

Total nitrogen. Before the solution was analysed as in the Kjeldahl method the nitrates and nitrites present were first reduced in the way described under *Nitrogen as NO₃*.

EXPERIMENTS.

Exp. 1. Filtration of a mixture of sucrose, and acetic and lactic acids. A solution composed of 0.033 % sucrose, 0.033 % acetic acid and 0.033 % lactic acid was made up in Harpenden tap-water and run on to the sectional filter at a rate of 9.0–9.5 cc. per minute. For the first 42 days the removal of organic matter from solution was measured only by the oxygen absorbed from permanganate and the biological oxygen demand tests. Adequate information on the rate of maturation of these filters had already been obtained and this information will shortly be published; hence no detailed analyses were made in the early stages of the experiment. The experiment proved that the acids were rapidly oxidised in the first and second sections. Thus the *p_H* of the original mixture which was 4.65 increased to 6.0 in the first section and 6.5 in the second; it then increased uniformly until the liquid was discharged from the sixth section at a *p_H* of 7.5. It should be noted that the nitric nitrogen in the final effluent during this period never exceeded 0.2 part per 100,000, although in the tap-water used to make up the nutrient solution there was 0.5 part of nitric nitrogen present.

Exp. 2. Filtration of a solution of sucrose and ammonium chloride. The further filtration of sucrose, and acetic and lactic acids was postponed on the 43rd day until suitable methods for the exact analysis of the substances in dilute solutions were available. From that day until the 88th day the following solution was fed to the filter at a rate of 9.5 cc. per minute.

Sucrose	...	...	...	1.0 g.
NH ₄ Cl	...	...	...	0.1528 g. (increased on 120th day
K ₂ HPO ₄	...	...	...	0.002 g. to 0.191 g.)
MgSO ₄ ·7H ₂ O	...	...	...	0.0006 g.
NaCl	...	...	...	0.0002 g.
CaCl ₂ ·6H ₂ O	...	...	...	0.1375 g.
Fe ₂ Cl ₆	...	...	...	Trace
Distilled water	...	...	...	1 litre

C/N ratio until 120th day = 10/1; thereafter 8/1.

Analyses were made every few days over a period of 45 days and the results obtained are given in Table I.

Table I. *Percentage of sugar oxidised.*

Section	...	1	2	3	4	5	6
By 4 hours' test		22.5	39.0	46.0	53.2	58.4	67.7
By 5 days' test		25.2	42.6	44.4	50.2	56.5	65.5

The nutrient solution was made up daily and adjusted to a p_H value of 7.0.

As the filter began to mature a drop in p_H of the effluent from the first section indicated that acid was being produced in this part of the filter. For example, on the 58th day of the experiment the p_H figures were:

	Section					
Fresh liquid	1	2	3	4	5	6
7.0	6.6	6.9	7.3	7.3	7.4	7.4

Similar observations have previously been made in the Microbiology Department at Rothamsted.

The p_H figures given were obtained during about the first half hour, at the commencement of each day's run. Thereafter the p_H of the fresh liquid fell progressively during its 24 hours' storage. Even by careful sterilisation of the aspirator it was difficult to prevent the p_H of the nutrient solution from falling below 5.5 to 6.0 at the end of the day. As a rule, however, the aspirator was cleaned out daily with distilled water and every few days with hydrochloric acid. Under these conditions a liquid of p_H 6.5 or less was filtered for the greater part of the day and there was no measurable production of acid. Typical examples are given in Table II.

Table II. p_H of nutrient solution and sectional effluent.

	Section					
Sucrose solution	1	2	3	4	5	6
6.5	6.8	7.3	7.4	7.4	7.4	7.4
5.5	6.6	6.9	7.0	7.1	7.2	7.2
4.6	6.4	6.8	7.0	7.1	7.2	7.2

Nitrogen assimilation. The nitrogen supplied as ammonium chloride was assimilated by the micro-organisms present in all the sections, but by those in No. 1 more than any other. It was evident from inspection that No. 1 section contained the bulkiest film. Compared with the other sections it oxidised the largest proportion of sugar. This is clearly shown in Table III.

Table III. *Nitrogen present in nutrient solution and effluents.*

Day of experiment	Nutrient solution	Section					
		1	2	3	4	5	6
54th	4.000	3.752	2.960	2.738	—	—	1.949
73rd	4.000	2.744	2.632	2.464	2.464	2.072	1.624
86th	5.000	4.704	3.472	—	3.248	—	2.688
112th	4.000	3.892	3.612	3.416	3.258	2.940	2.310
122nd	5.000	4.021	3.789	3.428	3.092	2.890	2.576
128th	5.000	4.372	4.230	—	—	—	—

It is apparent that nitrogen is continuously removed from the solution. This does not always signify an increase in vital film and therefore a correspondingly greater percentage oxidation of sugar; nitrogen may be lost by the decay of the organic film and its discharge as humus or by the removal of living micro-organisms in the final effluent or by the breeding and growth of flies on the filter, as rapidly as it is assimilated as ammonia.

Nitrification. In proportion to the amount of nitrogen locked up by the filter the quantity of nitrogen oxidised to nitrous and nitric acid is quite considerable. There appears to be a fine balance between the amount of nitrogen assimilated and carbohydrate oxidised on the one hand, and the amount of nitrogen oxidised. Where the film is thick and air passage somewhat restricted as in the upper sections, carbohydrate combustion is vigorous and nitrogen assimilation is appreciable. But in the lower sections, where organic matter is present in low concentrations (from 0.01 to 0.05 % sucrose) the filter is specialised for nitrification. It will be seen from Table IV, however, that

Table IV. *Nitrification in presence of ammonia and sucrose.*

Section	86th day Rate 9 cc. per min.				99th day Rate 5 cc. per min.				112th day Rate 5.85 cc. per min.			
	% sugar oxidised	N present as			% sugar oxidised	N present as			% sugar oxidised	N present as		
		NH ₃	NO ₂	NO ₃		NH ₃	NO ₂	NO ₃		NH ₃	NO ₂	NO ₃
1	9.4	4.66	0.01	0.03	10.8	5.0	0.01	0.0	37.6	3.89	0.05	0.17
2	9.7	3.43	0.01	0.03	24.0	4.3	0.01	0.12	4.4	3.61	0.05	0.20
3	6.7	—	0.01	0.03	5.3	4.2	0.02	0.24	6.3	3.42	0.05	0.29
4	8.7	3.17	0.01	0.07	28.6	3.0	—	—	9.9	3.26	0.06	0.32
5	10.7	2.08	0.01	0.15		2.5	0.03	0.25	8.3	2.94	0.07	0.37
6	22.0	2.57	0.02	0.10	6.6	—	—	0.52	11.6	2.31	0.05	0.61
Total	66.2				75.3				78.1			

complete absence of carbohydrate is not essential before nitrification can occur. On the 112th day, for instance, the effluent from Section No. 4 contained 44 parts of sucrose per 100,000, 0.06 part of nitrogen as nitrite and

0.32 part of nitrogen as nitrate. The solution passing through the filter receives a thorough mixing and it is therefore highly improbable that a small volume of nutrient solution passes through four sections and escapes oxidation while the rest of the solution undergoes a more complete oxidation. In other words, bacteria capable of oxidising ammonia to nitrite and nitrite to nitrate are actively present in a solution which contains free ammonia and carbohydrate.

Exp. 3. Interchange of sections Nos. 1 and 2. The effect of interchanging section No. 1 with No. 2 was studied from the 129th day of the experiment to the 157th. While section No. 2 showed a rapid increase in efficiency, due to the fact that it received a stronger nutrient solution, No. 1 fell off slowly. The net effect of the change, therefore, is to increase the capacity of the two upper sections to oxidise sugar. This is shown in Table V.

Table V. *Percentage of sugar oxidised.*

Percentage of sugar oxidised.							
Section	Day before change	1st day of change	3rd day of change	6th day of change	14th day of change	20th day of change	29th day of change
No. 1	32.1	10.7	24.2	22.9	26.9	32.2	40.3
No. 2	8.0	34.4	35.9	24.4	21.4	17.0	26.8
No. 1 + No. 2	40.1	45.1	60.1	47.3	48.3	49.2	67.1
All sections	78	—	—	—	89.1	—	100.0
1 to 6	(about)						

It was shown in Exp. 2 that the production of acid in Section No. 1 depends on the p_H of the nutrient solution. If this is less than 6.7 or 6.8 then increase in acidity cannot be detected. The interchange of Sections Nos. 1 and 2 gave additional evidence in support of the view that the p_H of the nutrient solution controls acid formation. Thus in Table VI it is seen that for 2 days after Section No. 1 had been placed in No. 2 position, it received a liquid of p_H 6.8. This section still retained its powers of acid production, for the p_H was lowered to 6.6. A few days after the change, however, it was unable to produce acid from a liquid of p_H 6.8.

Table VI also indicates the rapid oxidation of acid, even by an immature section such as No. 2.

Table VI. *Effect of interchange on p_H values.*

	Day before change	1st day of change	2nd day of change	3rd day of change	6th day of change	14th day of change	29th day of change
Nutrient solution	6.0	6.3	6.8	6.0	4.4	6.0	6.6
Section No. 1	6.4	6.8	6.8	6.6	6.0	6.8	6.6
Section No. 2	6.4	6.6	6.6	6.8	6.4	6.9	6.8

Estimations of nitrate in the effluents of the sections interchanged were made at frequent intervals. These analyses are given in Table VII. It may be stated that the average amount of nitrate present in the effluent from the first section did not exceed 0.05 part N per 100,000, while in the second section this increased to 0.1 part. When the change of section was made nitrate was

definitely absent from the first effluent after the 6th day. The effluent from the next section (No. 1 temporarily in the place of No. 2) contained appreciable amounts of nitrate, though the results were somewhat erratic. However, sufficient nitrate was present to prove that nitrification had actually occurred in the presence of sucrose and invert sugar, equivalent in concentration to 0.06 to 0.07 % of glucose.

Table VII. *Nitrogen as nitrate.*

		Parts per 100,000.				
Day before change	Section	1st day of change	3rd day of change	6th day of change	14th day of change	29th day of change
Section No. 1 0.105	No. 2	0.095	0.056	0.024	0.0	0.0
Section No. 2 0.114	No. 1	0.125	0.111	0.048	0.378	0.200

Exp. 4. Interchange of Sections Nos. 1 and 6. In order to confirm the results obtained in Exp. 3, Section No. 6 was made the first section and No. 1 the last. This experiment brings out the effect of a 0.1 % sucrose solution on the biological activity of nitrifying organisms. It also demonstrates the reaction of a mixed biological flora to solutions of ammonia and nitrite in which little carbohydrate is present.

Turning to the consideration of the course of the sugar oxidation, it is seen that the oxidising power of Section No. 6 rapidly increased in its new position. Prior to the interchange the progressive removal of sucrose as the solution percolated through the filter was of the following order: Section No. 1, 40.3 %; No. 2, 26.8 %; No. 3, 6.8 %; No. 4, 11.8 %; No. 5, 12.0 %; No. 6, 2.3 %; total 100 %. When these figures are compared with those given in Table VIII, the development of active film in Section No. 6 is shown by the greater proportion of sugar removed by this section. Within one month it was as active as either Sections 1 or 2, when these received the fresh solution first.

Table VIII. *Percentage of sucrose oxidised.*

Day before change	Section	1st day of change	4th day of change	7th day of change	11th day of change	27th day of change
Section No. 1 40.3	No. 6 (now No. 1)	14.9	18.4	21.5	14.5	42.5
Section No. 6 2.3	No. 1 (now No. 6)	—	—	—	15.8	12.5

It is a remarkable fact that the filter, considered as a whole, did not suffer by the sudden change of conditions in this experiment. This is due partly to the fairly slow reduction in rate of oxidation shown by Section No. 1, partly to the efficiency of this latter section in the last position even after one month and partly to the increasing activity of Section No. 6 in the first position. That the overall efficiency of the filter was not impaired is seen from the following example of the percentage removal of sugar effected by the sections in the order they were present in the filter: Section No. 6, 42.5 %; No. 2, 13.9 %; No. 3, 14.9 %; No. 4, 9.0 %; No. 5, 1.7 %; No. 1, 12.5 %; total 94.5 %.

For the first few hours of the change of Sections Nos. 6 and 1, nitrite and nitrate were found to be present to the extent of 0.018 and 0.190 part N per 100,000 respectively in No. 6 effluent (temporarily No. 1); in No. 1 effluent (temporarily No. 6), the amounts were 0.036 and 0.230 respectively. Within one week nitrifying activity had almost ceased in No. 6, only 0.008 part of nitrogen as nitrite and 0.04 part as nitrate per 100,000 being obtained after 6 days. Section No. 1, however, became more active in this respect: the effluent contained 0.04 part of nitrogen as nitrite and 0.44 part as nitrate. There seemed to be a tendency for nitrate to appear suddenly in Section No. 6 and then disappear. Insufficient evidence exists on which to base any satisfactory explanation of this apparent anomaly.

Inversion of sucrose during filtration. When sucrose solutions were run on to non-sectional percolating filters only small amounts of invert sugar were found in the effluents. Analysis of samples taken at various levels of the sectional filter used in the previous experiments proved that a considerable fraction of the total sucrose was invariably changed into invert sugar. Typical results are given in Table IX.

Table IX. *Sucrose and invert sugar.*

Sample	Calculated as mg. glucose per 100 cc.							
	102nd day of experiment		122nd day of experiment		157th day of experiment		185th day of experiment	
	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar
Fresh solution	108.02	1.10	112.34	0.86	130.9	—	111.50	—
Eff. No. 1	81.57	8.67	73.39	2.25	67.35	10.85	53.54	10.54
Eff. No. 2	68.04	9.88	56.88	4.83	33.28	9.72	37.90	10.75
Eff. No. 3	55.23	11.97	48.23	6.97	28.82	5.38	22.31	9.68
Eff. No. 4	37.66	15.26	36.15	11.15	10.44	8.26	15.26	6.73
Eff. No. 5	33.02	12.85	34.63	11.37	0.00	3.05	14.47	5.66
Eff. No. 6	35.25	11.75	32.11	11.69	0.00	—	4.07	2.34

It is seen from Table IX that appreciable amounts of invert sugar are present in certain sections of the filter. The quantity of invert sugar produced by any particular section does not remain even approximately constant during the development of the biological film. It is not improbable that the limiting factor in the oxidation of sucrose is the rate of oxidation of the invert sugar. Hence, when the filter was still immature (up to about the 130th day) invert sugar would tend to accumulate in the lower sections, owing to the inability of the organisms in these sections to decompose as much invert sugar as they produced. After the 130th day the filter reached maturity. The lower sections were then able to oxidise invert sugar *in situ* as rapidly as it was formed, in addition to part of that presented by the upper sections.

DISCUSSION.

The experiments described in this paper prove that a biological film may be built up on a filter made up of sterile clinker by allowing a 0.1 % solution of sucrose and an adequate supply of available nitrogen to percolate slowly

through the filter. If the rate of flow of this nutrient solution is kept low and aerobic conditions are maintained, any desired degree of oxidation can be effected. Before the microbial population has become established the clinker removes a considerable proportion of the sugar by adsorption but once the organisms begin to develop loss of sugar due to purely physical forces becomes insignificant. The sugar is then oxidised entirely by the micro-organisms. When the part played by each section of the filter used for these experiments is examined, it should be remembered that the first section is given an advantage in the initial stages which becomes more pronounced as the filter matures. This advantage lies in the concentration of sugar in the nutrient solution which is run on to No. 1 section. Table I exhibits the beginning of the superiority of Section No. 1 over all other sections. As this section oxidised 25 % of the sugar, the second section can only decompose 18 % of the total sugar, assuming an efficiency equal to that of the first section. Each development of film in the top section therefore increases the difference between the amount of sugar oxidised by Section No. 1 and by lower sections. Further advantages which the top section gains are due to the fact that the water-holding capacity of the film and its absorptive power increase with the growth of film. The sugar therefore remains in contact with micro-organisms for a longer period in this part of the filter than in any other. A quantitative expression of the average time of contact of liquids with filter medium has already been worked out by an elaboration of Clifford's method [1907] and it has been shown that the time of contact might increase to two or three times the contact of the clean, sterile medium, as a result of film development. The sectional filter was not regarded as mature until it was able to oxidise 90 to 100 % of the sugar it received. The percentage of sugar oxidised by the different sections was then approximately: No. 1, 40 %; No. 2, 25 %; No. 3, 12 %; No. 4, 10 %; No. 5, 7 %; No. 6, 6 %.

Under suitable conditions each part of a filter is able to perform the work of any other section merely by interchanging the sections. An example of the operation of this principle is shown in Exp. 4. In this case the sixth section, which contributed very little towards the total oxidising effect of the whole filter, but was highly specialised for nitrification, was made the first section for one month. During this period microbial counts showed that there was a rapid development of yeasts, bacteria and protozoa in this section which corresponds with a parallel increase in the amount of sugar oxidised. At the end of the month as much sugar was completely oxidised to CO_2 and water by the sixth section (temporarily the first) as by the section previously in the first position. Even after one month, however, the latter section was able to decompose more sugar than the original sixth section and at the same time effect a considerable nitrification. It thus appears that a filter gains in efficiency when its various parts are matured by receiving in turn the strong nutrient solution.

The sequence of changes which the sugar undergoes in its ultimate

breakdown to CO_2 and H_2O is so far mainly a matter for speculation. A mixed flora exists on the filter and contains organisms which may bring about almost every known type of fermentation with the appropriate end products. In a mature aerobic filter the population co-operates in oxidising the sugar and only such organisms will exist in any quantity as can effectively make use of the sugar and of the waste products of other organisms. Intermediary products will thus tend to be oxidised as soon as they are formed. There is an alternative possibility that the usual products of fermentation are not formed to any extent under aerobic conditions of a percolating filter, or if formed are capable of rapid oxidation *in situ* by the organism which produces them. This idea gains in probability when it is remembered that typical products of bacterial fermentation are relatively stable in anaerobic cultures but break down to CO_2 and water when air is admitted to the cultures. Lactic acid, for example, accumulates in anaerobic yeast fermentations but disappears when the yeast is aerated [Fürth and Lieben 1922], or when the fermented liquid is treated on a percolating filter (unpublished work). There is little doubt, however, that a considerable proportion of the sucrose is first inverted as an intermediate stage in the oxidation. There is some evidence that an acid product is also formed, but the nature of this substance is as yet unknown. It has already been stated that the drop in p_{H} —evidence on which this claim is based—is recorded as the nutrient solution passes out of the first section. The latter is thick with film, which might restrict the passage of air and so produce partially anaerobic conditions and acid fermentation in those parts of the film not exposed to air.

Turning to a consideration of the role of nitrogen in the sectional filter, it is apparent that a constant supply of available nitrogen is necessary for the development and maintenance of the biological film; the greater the amount of active film the greater the demand for nitrogen. It has been shown in large-scale filters (unpublished work) that for reasons already stated the quantity of film steadily decreases from the top of the filter to the bottom. The results of the work described in this paper also show that there is a gradient of nitrogen assimilation, or protein synthesis, corresponding to the amount of film present in different levels of the filter. When an excess of nitrogen over and above this amount is added, nitrites and nitrates are produced. These compounds appear in effluents from the middle and lower sections of the filter even in the presence of appreciable quantities of sugar. For example, pronounced nitrification has occurred over long periods in solutions containing up to 60 parts of sucrose per 100,000. Similar observations were made two years ago when, as previously stated, the filter was not divided into sections. Since the work of Winogradsky [1891], it has been held that small concentrations of organic substances inhibit or prevent nitrification. This belief has repeatedly been attacked, as, for instance, by Beijerinck [1914]. There is, in fact, much conflicting evidence in support of either view. Until recently no satisfactory explanation of these contradictory facts could be

given. Cutler [1930], however, has reported that a number of organisms capable of producing nitrite from ammonia have been isolated from Rothamsted soil and percolating filters similar to the one described in this paper. Runov [1926, 1928] and Mischustin [1926, 1928], described nitrifying bacteria which multiply in media containing organic matter. The discovery of other organisms which transform nitrites into nitrates in the presence of organic matter therefore appears to await the solution of a suitable technique for their growth and isolation. The experiments described in this paper suggest the existence of such organisms.

SUMMARY.

1. The experiments described have been carried out with a percolating filter. This consisted of six independent sections, each 6 inches diameter and 9 inches deep, and filled with 1/8 inch to 1/4 inch clinker.

2. When a mixture of 0.033 % sucrose, 0.033 % acetic acid and 0.033 % lactic acid is fed to the filter described in (1) at a suitable rate of flow, it is readily oxidised by a mixed microbial population. A biological film is built up and the organic matter is oxidised even if the C : N ratio of the nutrient solution is 80 to 1.

3. If a 0.1 % solution of sucrose is allowed to trickle through a mature portion of the sectional percolating filter, some formation of acid and invert sugar takes place. For acid to be produced the p_H of the nutrient solution must not be less than 6.7 to 6.8. The maximum p_H drop recorded has been from an initial p_H of 7.0 to one of 6.6. The nature of the acid product is as yet unknown. It is suggested that the acid arises either as a normal intermediate product of sucrose oxidation which is destroyed almost instantaneously, or that its presence is due to the growth of a bulky film within which an acid, anaerobic fermentation occurs.

4. When the sectional filter was working well within its capacity, *i.e.* more than able to oxidise the organic matter with which it was supplied, it removed sucrose from a 0.1 % solution in approximately the following manner: Section No. 1, 40 %; No. 2, 25 %; No. 3, 12 %; No. 4, 10 %; No. 5, 7 %; No. 6, 6 %; total 100 %.

5. Each section of the percolating filter may be replaced by any other without the filter losing in efficiency. In fact the total working capacity of the filter could probably be increased by making each section the first in turn for a few weeks.

6. Oxidation of ammonia proceeds best in the lower sections where the concentration of sugar is least. It is not inhibited by concentrations of sugar up to 0.05 to 0.06 %. Therefore nitrification and carbohydrate oxidation take place simultaneously in several of the sections. Ample evidence exists in support of the view that bacteria which nitrify ammonia may be active in presence of organic matter.

7. Both nitrites and nitrates are produced by micro-organisms in the presence of 3 to 4 parts per 100,000 of nitrogen as ammonia.

8. Each section, irrespective of its previous function, rapidly shows nitrifying activity when placed in a suitable part of the filter.

The unpublished results referred to in the Introduction were obtained by Messrs E. H. Richards, R. B. Dawson, S. W. Johnson, J. T. Martin and the author. Mr E. H. Richards, Head of the Fermentation Department, has been personally responsible for the direction of the work described in this paper and his keen interest and stimulating criticisms have been highly valued.

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