CLIV. THE BIOLOGICAL FILTRATION OF DILUTE SUCROSE SOLUTIONS.

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1. INTRODUCTION.

IN a recent paper on this subject Jenkins [1931] has shown that in 0.1 % solution either pure sucrose, or mixtures of sucrose and lactic and acetic acids, are readily oxidised on a percolating filter containing $\frac{1}{8}$ to $\frac{1}{4}$ inch clinker to a depth of 54 inches when fed at a suitable rate of flow. The suitability of soluble carbohydrates for the nutrition of micro-organisms needs no emphasis and the more or less complete exhaustion of such solutions is only to be expected provided a sufficient number of the right type of organisms is present under suitable conditions for a sufficient period of time.

The fundamental factors in biological filtration are obviously

- (1) the microbial population;
- (2) the conditions under which this population exists as determined by
 - (a) the size of the particles constituting the medium,
 - (b) the rate of flow of the liquid;
- (3) the concentration of the nutrient solution.

The object of these investigations was the more precise determination of the above factors and their application to the treatment of some 250,000 gals. per day of liquor containing 0.2 % of sugar in solution.

The rate of flow used by Jenkins was 9 cc. per minute, equivalent to 86 gals. per cubic yard of filter bed per day. The treatment of 500,000 gals. of a 0.1 % sugar solution at this rate of flow would require a filter bed five-sixths of an acre in area with a depth of 54 inches. It is obviously of some importance to know if these dimensions are the minimum required for the effective solution of the problem.

The complete oxidation of sugar to CO_2 and water requires 1.12 times its weight of oxygen, so that a 0.2 % sugar solution, *i.e.* 200 parts of sugar per 100,000, will require 224 parts of oxygen per 100,000 of water. Since the solubility of oxygen in water is 1 part by weight per 100,000, the solution will require constant aeration during its passage through the filter, and the rate at which this occurs will be a very important controlling factor in the efficiency of the filter. The rate of aeration is determined by the size of particle and the rate of flow of the liquid.

The semi-commercial scale experiments conducted at Colwick, and referred to by Jenkins, showed that the use of fine clinker of $\frac{1}{8}$ to $\frac{1}{4}$ inch was liable to lead to the choking of the filter bed and insufficient aeration, resulting in a very much reduced purification.

Investigations of the inter-relation between concentration of nutrients, the size of particles and rate of flow and their bearing on the rate of oxidation are described in the present paper together with an account of the nitrogen and phosphate requirements of the biological film.

2. THE FIVE-DAY TEST.

This test measures the polluting value of an effluent by means of the biological oxidation of the organic matter. It therefore achieves the object of the filtration process. For this reason a study of the test will help towards a better understanding of biological filtration.

A sample of the water to be tested is diluted with water saturated with air at atmospheric pressure and 18° . By choosing a suitable degree of dilution the water will supply sufficient oxygen for the oxidation of the organic matter, provided of course that the right organisms are present. Determinations are made by Winkler's method of the dissolved oxygen in the water at the beginning and after 5 days' incubation at 18° , the difference being the amount of oxygen used in the oxidation of the organic matter.

With the object of testing the degree of correlation between sugar content and oxygen absorbed, 5-day tests were carried out on 0.2, 0.15, 0.10 and 0.05 % sugar solutions. The results are given in Table I, dilutions of 1 in 200 being used in each case.

Table I.

Concentration of sucrose solution %	Dissolved oxygen available in parts per 100,000	Dissolved oxygen required in parts per 100,000 (Calculated)	Dissolved oxygen absorbed in parts per 100,000 (Actual)	Sucrose accounted for %
0.20	180	220	131	60
0.15	180	165	102	62
0.10	180	110	62	56
0.05	180	55	35	63

In every case the amount of oxygen absorbed is little more than half the amount required for complete oxidation of the sugar. This discrepancy may be accounted for by (a) sugar undecomposed, (b) sugar metabolised but not oxidised. To what extent each of these two factors is responsible for the low oxygen absorption would be very difficult to determine. Assuming the unabsorbed oxygen to be due entirely to undecomposed sugar the maximum amount of sugar would be 0.0004 %. Actually most of it will exist as decomposition products of sugar and as cell tissue of the organisms effecting the decomposition. A more extended incubation period is obviously necessary for more complete oxidation. At the extreme dilutions of sugar used in this test the generation time of the organisms must be very considerable, probably 12 hours

or more (see p. 1432). Another series of tests was carried out in duplicate with the same dilution using a 0.15 % sugar solution. Ten bottles were incubated in each case and the temperature of incubation raised from 18° to 25° during the first 5 days. Determinations of oxygen absorbed were made at intervals during 20 days. The results are given in Table II.

Table II.

	Present at start	Dissolved oxygen in parts per 100,000 after								
		1	2	3	4	5	6	7	10	20 days
I	142	92	55	35	27	24	24	22	19	2
II	142	94	56	35	26	24	24	23	19	2

These results show that the absorption of oxygen is most rapid during the first 3 days and that 70 % of the sugar is oxidised after 4 days' incubation. This result is considerably higher than that obtained in the previous experiment.

Cook and Stephenson [1928] using pure cultures of *B. coli* measured the oxygen absorption in a Barcroft apparatus and found that for glucose only two-thirds of the theoretical amount was absorbed by the organism. They offer no explanation to account for the incomplete oxidation of the glucose and do not consider that bacterial growth may be responsible. In the 5-day test, however, there is an increase in bacterial numbers during the first 2 days and the writer is of opinion that bacterial growth and synthesis is responsible for the incomplete oxygen uptake. The differential rates of oxygen uptake as well as the different amounts recorded by Cook and Stephenson [1928] for various carbohydrates seem to point to a difference in value of these carbohydrates in metabolic synthesis (see p. 1431). This subject is being investigated in greater detail.

The determination of the possible variation due to the inoculation was made by preparing a series of tests using tap water only for dilutions, and another series with the addition before dilution of 1 % of a laboratory filter effluent to the sugar solution to be tested. The results are given in Table III.

Table III.

No. of sample		Oxygen absorbed per 100,000	Standard deviation
1)	Tap water only	91)	Mean = 92 ± 7.4
2)		87)	
3)		89)	
4)		108)	
5)		87)	
6)		89)	
7)	Tap water <i>plus</i> 1 % filter effluent	112)	Mean = 110 ± 3.1
8)		109)	
9)		115)	
10)		107)	
11)		113)	
12)		106)	

These results show that leaving the dilution water to a chance inoculation causes a decreased oxygen absorption and a greater variation in the amount than using a definite inoculation from a filter bed.

The standard deviation of the mean using a definite inoculation is 3.1 which means that a single determination may vary as much as 9.0 % from the mean value.

As an accurate method of determining the amount of organic matter present in a liquid this test is obviously not very reliable. A controlled inoculation is not practicable since there is no known method of producing cultures of bacteria in a uniform state of efficiency.

The possibilities of a chemical test answering the purpose are not encouraging. Recent work at Rothamsted has shown the 4-hour permanganate test to be unsuitable for estimating the oxygen requirement of organic acids [Water Pollution R.B. Report, 1929]. The only reliable method is that of combustion, and this could not be considered as a true measure of the polluting effect, since there are organic substances which are comparatively inert biologically. There appears therefore to be no alternative to this incubation test, and, provided too much reliance is not placed on single determinations, it may be considered to answer the purpose for which it was designed. In the succeeding experiments it has been used as a measure of the amount of purification effected by biological oxidation on percolating filters. In this case the calculations are made from determinations obtained by using identical inoculations.

3. THE BIOCHEMICAL OXIDATION OF SUCROSE.

A very extensive literature exists on this subject which for the purposes of this paper may be summarised as follows.

After hydrolysis of the sucrose the hexose sugars give rise to methylglyoxal ($\text{CH}_3 \cdot \text{CO} \cdot \text{CHO}$) which yields lactic acid ($\text{CH}_3 \cdot \text{CHOH} \cdot \text{COOH}$) and then by oxidation pyruvic acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{COOH}$). Pyruvic acid on hydrolysis yields acetic and formic acids. Formic acid is readily decarboxylated yielding CO_2 and H_2 . Alternatively pyruvic acid may be decarboxylated yielding acetaldehyde which on reduction yields alcohol and on oxidation acetic acid. Acetaldehyde may also form the starting-point of several organic syntheses by polymerisation and condensation forming higher fatty acids and alcohols.

In biological filtration any or all of these compounds may be produced according to the bacterial population and conditions of aeration of the filter bed. In any case the fundamental processes in all the different types of fermentation are the same, and the object of filtration, *i.e.* the complete oxidation of the carbohydrates to CO_2 and water, is best secured by reducing the synthetic reactions to a minimum. In accordance with Hopkins's dictum the production and accumulation of any particular compound in the filter bed, other than the final products of oxidation, is a measure of its uselessness to the organisms producing it [Stephenson, 1930]. From the point of view of

pollution the intermediate products, lactic and acetic acids, theoretically have the same oxygen absorption value as sugar, so that their production does not result in any reduction in the polluting effect of the liquid. Their decomposition requires the presence of hydrogen acceptors which may be provided either by free oxygen or oxygen combined as nitrate [Quastel, Stephenson and Whetham, 1925]. Since the solubility of oxygen in water is very slight, it may become the limiting factor determining the rate of oxidation. It has long been known that denitrification is part of the respiratory process of bacteria under conditions of restricted aeration. The presence of oxidised nitrogen either as nitrites or nitrates in a filter bed is generally considered as indicating its efficient working, but the possibility of their playing a part in the actual oxidation of carbohydrates does not appear to have been previously recognised.

4. EXPERIMENTS WITH SECTIONAL FILTERS.

With the object of obtaining more information as to the working of a biological filter, a special filter was designed by the writer and manufactured in earthenware by Messrs Doulton of Lambeth. The principle of this filter was adopted by Jenkins and described in a recent paper [1931]. The original apparatus consists of pipe sections each 1 foot in length and 4 inches in diameter fitted at one end with a perforated plate containing 5 holes $\frac{1}{2}$ inch in diameter. Each section was set on a separate earthenware saucer provided with a single drainage hole 1 inch in diameter. The object of the saucer was to collect the effluent from each section and deliver it to the centre of the next section below. In this way the possibility of the liquid passing down the inner surface of the pipes was obviated. A certain amount of spreading of the effluent over the surface of each section was effected by the presence of a ring of drip nipples round the lower edge of the drainage hole of each saucer.

The capacity of each section, assuming a 9 inch working depth, was such that when supplied with 1 litre of liquid per 24 hours it was equivalent to a filter bed receiving 100 gallons per cubic yard per day (g.y.d.). Five such sections placed in a column can be conveniently supplied with 5 litres of liquid per day from a 6-litre aspirator at this unit rate of feed. The possible criticism that such a filter would be subject to abnormal conditions on account of the supposed increased aeration due to the air gaps between each section is not valid. The contraction of the film in passing from one section to another would result in less aeration than if the filter medium were continuous. It should be remembered, however, that the choking of any one section not only affects that particular section, but in practice would also put out of action the whole column.

In filtration experiments the rate of flow of the liquid through the bed is obviously of great importance and precautions must be taken to ensure that the rate of flow is actually what was intended. In these experiments the aspirators were fitted with a Mariotte's constant head device which released

the liquid under a very low uniform hydrostatic pressure. The use of a "T" piece outlet obviated the possibility of gravitational pull by the dropping liquid and at the same time facilitated cleaning operations. The apparatus was kept free from both organic and mineral deposit by rinsing with hydrochloric acid each day. Before sampling, checks on the rate of flow were made by measuring the volume of effluent collected in 30 minutes.

A. The size and nature of the filter medium.

In this experiment two columns each of six sections were used. One column was filled with fine gravel of $\frac{1}{4}$ to $\frac{3}{8}$ inch and the other with clinker of $\frac{3}{8}$ to $\frac{5}{8}$ inch. Each column was inoculated with 100 cc. of a suspension of film obtained from a large scale experimental filter being operated at a Sugar Beet Factory. Both laboratory filters were fed with a solution of the following composition at the basic rate of 100 g.y.d.

			%
Sucrose	...	...	0.2
NaCl	...	...	0.03
K ₂ HPO ₄	...	...	0.03
KH ₂ PO ₄	...	...	0.02
MgSO ₄	...	...	0.01
(NH ₄) ₂ SO ₄	...	...	0.03
CaCO ₃	...	...	0.03 (supplied by the tap water).

The p_H was 6.8-7.0.

This experiment was run continuously for a period of 60 days. Samples of the effluents of each section were examined daily for p_H and mineral nitrogen and weekly for degree of purification by means of the 5-day test.

(a) p_H values. These were determined by means of the B.D.H. capillators, with bromocresol purple and bromothymol blue. The results were plotted as curves with the numbers of the sections as ordinates and the p_H values as abscissae. During the first week little or no change in the reaction of the liquid occurred in its passage down the filters so that the graph is almost a straight line. During the second week the p_H of the effluents from the second section became more acid (see Figs. 1 and 2).

By the end of the second week acidity developed in the effluent from the first section and continued to increase during the third and fourth weeks, but to a greater extent in the effluent from the gravel filter than in that from the clinker. This difference in acidity between the two filters continued throughout the remainder of the experiment. The p_H values of sections 1 and 2 of the gravel filter averaged 5.5 whilst in the corresponding clinker sections the average was 6.0. The effluents were always less acid from section 3 and generally reached neutrality in section 4. In sections 5 and 6 they became slightly more alkaline (p_H 7.0-7.1) than the original solution supplied from the aspirator. This development of alkalinity in the lower sections diminished after the 30th day with the increase in nitrification, more especially in the case of the

clinker filter in which the effluent gradually became more acid (p_H 6.5) after leaving section 4 (see Fig. 2).

Fig. 1. p_H of gravel filter.

Fig. 2. p_H of clinker filter.

The greater acidity developed in the gravel filter may be due to deficient aeration, unless some basic material in the clinker was exerting a neutralising effect.

An attempt was made to diminish the acidity of the gravel filter by placing glass dishes filled with broken chalk beneath the drainage holes of sections 1 and 2. Although the chalk was at first rapidly corroded by the acid liquor there was no significant change in the reaction of the effluents. After two days the chalk was covered with a gelatinous film which prevented any further corrosion. On testing the liquid in the dishes containing the chalk it was found that the p_H was even less than that of the liquid dropping on to it from the section above. The same lowering of the p_H also occurred on collecting the effluent in glass dishes without any chalk. In 2 hours the p_H dropped from 5.5 to 5.0 and in 20 hours 4.2 was recorded. The liquid at the surface gave a p_H value of 5.8. This higher p_H of the surface layer could only be accounted for by greater aeration.

In order to confirm the improbability of any buffering effect of the clinker medium the basicity of the media was determined by titration with both hydrochloric acid and acetic acid.

With both mineral and organic acids the gravel contains more basic material than the clinker. The difference between the two is greater in the case of the organic acid on account of the occurrence of sulphides in the clinker which are not decomposed by acetic acid.

Time of shaking (hours)	Gravel cc. of <i>N</i> /10 HCl acid neutralised	Clinker cc. of <i>N</i> /10 HCl acid neutralised
24	7.3	5.8
48	9.0	8.8
72	9.4	9.15
(Some H ₂ S was evolved by the clinker.)		
	cc. of <i>N</i> /5 acetic acid neutralised	
24	3.7	0.5
48	8.0	0.6
72	8.6	0.7

It must therefore be concluded that the difference in reaction between the two filters is due entirely to aeration and not to chemical composition. Jenkins [1931] states that "for acid to be produced the p_H of the nutrient solution must not be less than 6.7 to 6.8." It is difficult to reconcile this statement with the above observations or with any known theory of fermentation.

The cause of this difference in aeration must be attributed to the size of the particles of gravel. Although the total volume of interstitial space is independent of the size of particles, the permeability to water is proportional to the square of their diameter [Green and Ampt, 1912]. With small particles the interstitial space is also diminished to a greater extent by the film of liquid and bacterial slime. After running for 45 days the first section of the gravel filter was completely blocked with film and the liquid ran over the side of the section. This filter was therefore considered to be out of action since the lower portions only worked in virtue of their being sectional. The clinker filter continued working in a satisfactory manner for another month.

The gravel filter was therefore dismantled and the amount of film present in each section was determined in the following way.

(b) *Determination of weight of film in gravel filter.* The gravel from each section was shaken thoroughly with successive lots of 400 cc. of water and the total volume of suspended film made up to 2 litres. This was allowed to settle and 1 litre of clear water was decanted. The remaining litre of film suspension was then thoroughly agitated and 60 cc. of it were evaporated to dryness, dried at 100° and weighed again. It was then ignited and the ash determined.

The results are as follows.

Table IV.

Section	Total weight of film g.	Weight of ash g.	Weight of volatile matter g.	Ash %
1	14.6	4.8	9.8	33
2	14.0	6.2	7.8	44
3	11.4	6.2	5.2	54
4	9.4	5.2	4.2	55
5	12.6	7.2	5.4	57
6	5.2	3.4	1.8	65

These results show

(1) that the greatest amount of film occurs in the first section and that

the amount generally diminishes with depth; the irregularity of the fifth section is probably due to collection of film discharged from higher sections;

(2) that the ash generally increases in percentage amount with depth and constitutes the bulk of the film in the lower sections.

(c) *Reconstructed gravel filter.* This filter was then reconstructed with coarse gravel as follows:

Section 1	}	$\frac{1}{2}$ to $\frac{3}{4}$ inch	Coarse.
2			
3		$\frac{3}{8}$ to $\frac{1}{2}$ inch	Intermediate.
4	}	$\frac{1}{4}$ to $\frac{3}{8}$ inch	Fine as before.
5			
6			

It was then inoculated with the wash water from the previous medium.

As in the previous experiment acidity first developed in section 3 at the end of the first week, after which it occurred only in the first (p_H 5.6) and second sections (p_H 6.2). After the second week the p_H of the first section varied between 6.0 and 6.5 whilst that of the second section rarely fell below 6.5. The p_H curves obtained with this graded gravel were almost identical with those obtained with the clinker filter, thus confirming the conclusion that for the above experiment the size of the particles and not their chemical composition or texture determines the development and efficiency of the film.

Both filters continued to operate in a very satisfactory manner for 20 weeks when both were choked in the lower sections by copious discharge of film from the sections above.

(d) *Purification.* The purification effected by a filter is calculated from the oxygen absorption figures in the 5-day test of the original solution and of the effluent. The difference between these two determinations expressed as a percentage of the first is the percentage purification. The results obtained during the first period of the two filters are given in Table V.

Table V.

	Percentage purification								
Section	1st	2nd	3rd*	4th	5th	6th	7th	8th	week
	Gravel filter.								
1	42	43	16	21	22	21	3.3	15	
2	58	77	27	46	40	55	23	40	
3	55	90	43	78	66	68	41	32	
4	68	92	57	86	87	82	66	77	
5	68	90	69	96	95	84	85	87	
6	72	90	76	93	96.5	84.5	89	94	
	Clinker filter.								
1	27	52	42	60	22	38	34	36	
2	30	74	59	78	40	46	55	66	
3	52	76	68	90	54	65	80	93	
4	58	76	73	93	81	77	90	98	
5	72	86	81	96	90	85	90	100	
6	73	90	79	97	91	93	95	100	

* Nitrogen omitted.

These results lead to the following conclusions.

(1) There was no difference between the filters during the first 3 weeks. Both gravel and clinker media gave 90 % purification after 2 weeks.

(2) Omission of nitrogen and consequent retardation of the growth of film lowered the efficiency immediately.

(3) Most of the purification was effected in the first three sections and very little effect was produced by the sixth section (see Fig. 4).

(4) The fine gravel filter showed a fall in efficiency in the first section after the second week, but without any appreciable effect on the lower sections.

The changes in the purification of each section are shown in Figs. 3 and 4.

Fig. 3. Purification curves for fine gravel filter.

Fig. 4. Purification curves for clinker filter.

The purification results of the second period after reconstructing the gravel filter are as follows (Tables VI and VII).

These results show:

(1) that the coarse gravel ($\frac{1}{2}$ to $\frac{3}{4}$ inch) was much superior to fine gravel ($\frac{1}{4}$ to $\frac{3}{8}$ inch) and to clinker ($\frac{3}{8}$ to $\frac{5}{8}$ inch); the purifications in the first two

Table VI. *Coarse gravel (regraded).*

Section	Percentage purification							
	1st	2nd	4th	6th	9th	11th	13th	15th week
1	26	64	61	77	68	20*	46	69
2	45	88	74	86	85	73	69	90
3	60	90	84	91	86	85	82	92
4	75	94	93	95	89	95	92	95
5	83	94	95	95	96	95	95	95
6	85	95	94	95	96	95	97	97

* Drainage outlet closed by pebble.

Table VII. *Clinker.*

Section	Percentage purification					
	1st	3rd	5th	9th	12th	14th week
1	22	26	22	20	45	32
2	64	69	33	35	55	43*
3	80	85	59	40*	67	72
4	86	95	87	90	92	90
5	95	97	90	95	96	95
6	97	97	95	99	98	98

* Choked with larvae and debris, and cleared.

Fig. 5. Purification curves of regraded (coarse) gravel filter.

sections were much higher and showed less falling off in efficiency with age (Fig. 5);

(2) that starting the filter with the previous inoculation showed more rapid development of purification than with the original film from the large scale filter bed;

(3) that 3 feet of gravel ($\frac{1}{2}$ to $\frac{3}{4}$ inch) will give 90 % purification of 0.2 % sucrose solution after 2 weeks and maintain this for more than 16 weeks;

(4) that very little extra purification was obtained after the 4th section in either filter.

B. *Oxidation of lactic and acetic acids.*

The difference in acidity and purification between the coarse and fine filter media suggested a possible correlation between p_H and purification. An experiment was therefore carried out using 0.2 % solutions of lactic and acetic acids in place of sugar. The experiment ran for 14 days and was characterised by remarkably good purification figures and nitrification.

The results are given in Tables VIII and IX.

Table VIII. *Oxidation of lactic acid.*

Aspirator	p_H	$[H'] \times 10^7$	Purification %		
			2nd day	7th day	14th day
Section 1	3.7	2000	—	—	—
„ 2	4.8	158	16.6	43	58
„ 3	6.5	3.2	63.0	83	83
„ 4	6.9	1.1	85.0	91	99
„ 5	7.0	1.0	90.0	98	100
„ 6	7.1	0.8	93.0	99	100
„ 6	6.9	1.1	96.0	99	100

Table IX. *Oxidation of acetic acid.*

Aspirator	p_H	$[H'] \times 10^7$	Purification %				p_H
			2nd day	7th day	14th day	21st day	
			0.2 % acetic acid			0.2 % sucrose	
Section 1	4.0	1000	—	—	—	—	6.9
„ 2	4.6	250	40	36	38	44	6.6
„ 3	6.5	3.2	65	82	85	57	6.8
„ 4	6.8	1.6	87	94	97	78	6.9
„ 5	7.0	1.0	90	96	98	91	7.0
„ 6	7.1	0.8	93	96	98	98	6.9
„ 6	7.0	1.0	95	98	98	98	6.9

The reduction in $[H']$ is seen to be much greater in the first section than in any of the other subsequent sections, but the amount of purification effected is about equal in the first and second sections. Obviously the reduction in acidity is effected by other processes in addition to oxidation. The results show very conclusively that the development of acidity in a filter bed is not in itself detrimental to its efficiency. If anything, it appears that organic acids are more readily oxidised than sugar, a result which is not in agreement with conclusions reached by Jenkins [1931]. It follows therefore that the low purification of the fine gravel filter is not due to any possible toxic action of the organic acids, but solely to lack of oxygen. These results are also of great interest biologically since the protozoan population of sections 1 and 2 was almost entirely extinguished, so that the more efficient filtration process was carried on by bacteria and fungi supporting a population consisting of eel-worms and insect larvae.

The more rapid oxidation of organic acids compared with that of sugar may have a possible explanation in terms of energy transformation.

In the fermentation of sugar to lactic and acetic acids the organisms may derive energy for growth and metabolism without oxidising the material, but no growth is possible on lactic and acetic acids without oxidation. When sugar is supplied to a filter bed there is doubtless a development of organisms capable only of utilising intra-molecular energy, and these organisms must necessarily die out when the filter receives only organic acid, resulting in an increase in the oxidising organisms.

C. *The development of the film.*

The active portion of the filter medium consists of bacteria and fungi supporting a very variable population of protozoa, nematodes and insect larvae. It would be difficult to follow the possible fluctuations in the bacterial flora but they are doubtless reflected in the constantly changing fauna. In a filter bed the organisms develop in the form of colonies attached to the particles of the medium. On starting up a newly inoculated filter the liquid shows an increasing bacterial population as it flows through successive sections, until a certain maximum is reached about the third section, when clumping or colonisation begins, resulting in a much reduced dispersion of the bacteria in the lower sections. This process continues and gradually appears in the upper sections until all the effluents show a marked reduction in bacterial dispersion. The effect of this is shown in Table X by the results of direct chamber counts of bacteria in the effluents.

Table X.

Section	Bacterial numbers in millions per cc.	
	1st day	14th day
1	5.6	32-52
2	32	20-45
3	296	14-29
4	84	12-13
5	36	10-16
6	36	8-14

(These figures do not represent in any way the bacterial population of the film but only the free organisms in suspension. The numbers in the film vary from 3000 to 9000 millions per g.) As the fixed colonies proliferate they may become too heavy for the point of attachment and thus break away either as a whole or in portions. These small fragments of film are carried along with the liquid, provided the filter bed is coarse enough to allow them to pass through, and they may be seen in the effluent from all sections after the first or second week of operation. Under the microscope they appear as masses

of micrococci in the form of zoogloea with occasional yeast cells and funga hyphae. Frequently they carry with them large numbers of protozoa and eelworms. The movements of the larger eelworms and annelids may also play a part in the breaking away of the colony. The colour of the film is at first white especially in the upper sections, but after 3 weeks it changes gradually to a dark brown in the lower sections. This shedding of film is a constant process in the life of the filter and is quite distinct from the periodical sloughing which is associated with seasonal climatic changes.

The rate of growth of the film will be greatest in the first section where the concentration of food is greatest and will diminish with the depth of the section. This explains the greater purification effected in the upper sections of the filter bed. According to Penfold and Norris [1912] the time of generation of *B. typhosus* in peptone increases from 60 minutes at 0.2 % concentration to 90 minutes at 0.1 % and 180 minutes at 0.05 %. A similar result was obtained by Cutler and Crump [1923] for protozoa, the concentration of the nutrient in this case being expressed in terms of bacterial numbers. With a purification of 75 % in section 3 the concentration of nutrient in this section would correspond to 0.05 % sugar and, assuming the time of generation in mixed culture to be similar to that in pure cultures, it would be about three times that of the first section, so that the film would only grow at one-third the rate. Similarly the growth rate in the fourth, fifth and sixth sections would be reduced almost to nil. The figures given in Table IV however show much greater amount of film than would be expected from these growth rates. This difference from expectation must be attributed to the film continuously shed from the upper sections being retained by the fine gravel in the lower sections. The use of a coarse medium would allow an easier passage to this discharged film. The effect of this is seen in the reconstructed gravel filter. When the first three sections were filled with coarse gravel the discharge of film into the surface of section 4 (fine gravel) was so great that it became covered in a period of 12 weeks with a solid layer $\frac{1}{2}$ inch thick.

D. *Time of contact.*

The development of the film has an effect on the filter apart from its increase in population, *viz.* in increasing the "time of contact" or the length of time the liquid takes to pass through the filter. This factor is usually determined by Clifford's method [1907] which measures the mean time required to wash out a quantity of sodium chloride from the filter at a given rate of flow.

It is obvious that the time of contact will increase with the development of the film. The film consists of about 97 % by weight of water and the total volume in one of these experimental filters may be $1\frac{1}{2}$ litres. With a rate of flow of 5 litres per 24 hours the water of the film can be changed $5\frac{2}{3}$ times in 24 hours which gives a time of 7.2 hours for each change of water.

The volume of film in each section is measured by the difference between

the volume of the interstitial space of the clean gravel, and that after development of the film. This determination was carried out on one of the sectional filters filled with gravel of $\frac{1}{2}$ to $\frac{3}{4}$ inch after running 1 month. The results are given in Table XI. The time of contact is the ratio $\frac{\text{volume of film}}{\text{rate of flow}}$, since if X = volume of film and V = total volume of liquid per 24 hours, then V/X = number of times the film is filled per day, and $\frac{24}{V/X}$ = time of contact = $X \ 24/V$.

Table XI.

Section	Volume of film cc.	Rate of flow	Time of contact hrs.
1	450	5 litres per day	2.1
2	400		2.0
3	300		1.5
4	150		0.7
5	75		0.36
6	75		0.36

From the above table we find that out of a total of 7 hours of contact 5.6 hours are spent in the first three sections. The time of contact is greatest where nutrition and growth are greatest. By reducing the size of the gravel, thereby causing a retention of the film, the time of contact can be increased considerably but not without diminishing the aeration, and increased contact without aeration is of no value. Increase in the time of contact should be the result of growth rather than mechanical accumulation. A deficiency in time of contact only occurs when starting up a new filter, and an excess tends to occur with age. This initial deficiency can be best remedied by increasing the concentration of the nutrient solution thereby increasing the rate of growth of the film. If at the same time the rate of flow is diminished, the capacity of the filter will not be exceeded. As the film develops the concentration of the solution can be diminished and the rate of flow increased.

E. Rate of flow.

It has been shown that the development of film in a filter is greatest in the upper sections and that it is determined by the concentration of nutrients in the liquid. Increase in the rate of flow will diminish the time of contact. This will result in a lower purification gradient, which means a higher concentration of nutrient in the lower sections, and an increase in the development of film below the upper layer. Thus with a lower purification gradient a deeper filter becomes necessary.

A problem of long standing to the sewage engineer is the question whether it is better to filter a given amount of organic matter as a strong solution at a slow rate or a dilute solution at a quicker rate. Information on this point can be obtained from a consideration of the previous figures for the sectional purification. In Table VI by averaging the figures for the normal working periods, the 2nd, 4th, 6th, 9th, 13th and 15th week, we get the following percentage purification: section 1, 64 %; section 2, 82 %; section 3, 88 %.

Since the rate of flow through five sections is 100 g.y.d. the rate of flow through each section alone is five times as much or 500 g.y.d. A 0.2 % sucrose solution run at this rate undergoes a purification of 64 %, whilst the effluent of this section containing the equivalent of 0.072 % sucrose, run through the second section at the same rate, undergoes a purification of 18 % of $0.2 = 0.036$ or 50 % and the effluent of section 2 containing the equivalent of 0.036 % sucrose undergoes a purification of only 6 % of $0.2 = 0.012$ or 33 %.

Thus a reduction in the concentration of nutrient through a filter diminishes its efficiency, though the purity of the final effluent is increased. The effect of increasing the rate of flow with a reduction in concentration of sucrose was studied by means of two sectional filters filled with the same gravel, $\frac{1}{2}$ to $\frac{3}{4}$ inch. Both were inoculated with the same medium. One was run with a 0.2 % solution of sugar at 100 g.y.d. and the other with 0.1 % solution at 200 g.y.d. The experiment was run for 30 days after which the gravel was washed and the amount of film determined in each section.

The results of the purification are given in Table XII.

Table XII.

Section	Number of days							
	6		13		20		30*	
	0.2 %	0.1 %	0.2 %	0.1 %	0.2 %	0.1 %	0.2 %	0.1 %
	Purification %							
3	90	59	90	72	88	74	72	50
6	96	90	99	98	99	97	97	83

* Nitrogen omitted from both filters.

There was little difference between the final purification in both filters until nitrogen was omitted from the solution. But throughout the experiment the purification at section 3 was distinctly in favour of the more concentrated solution. The effect of dilution is not apparent with a filter 6 feet deep but with a shallow filter of only 3 feet the use of a more dilute liquid at increased rate of flow gives a lower purification.

The weights of film on the various sections are given in Table XIII.

Table XIII. *Distribution of film with different concentrations of sucrose.*

Section	0.2 % solution at 100 g.y.d.		0.1 % solution at 200 g.y.d.	
	Total film (g.)	% ash	Total film (g.)	% ash
1	16.5	26	15.8	26
2	16.0	32	14.9	33
3	10.4	37	12.5	38
4	6.0	42	7.8	44
5	4.3	44	5.6	57
6	4.1	46	4.5	59
Total	57.3		61.1	

It would seem that the amount of film is greater in the upper sections and diminishes more rapidly with the 0.2 % solution at 100 g.y.d. than with the

0.1 % solution at 200 g.y.d. With the more rapid rate of flow the amount of film is greater in the lower sections, part of this increase being due to a greater ash content.

F. *The influence of the C/N ratio on the film.*

The effect of omitting nitrogen from the sugar solution has been shown in the purification figures in Tables V and XII. Most of the previous work on biological filtration has been done with liquids of a high nitrogen content and having a C/N ratio less than 10. The pulp-press liquor from a beet sugar factory has a C/N ratio of about 20, but since most of the nitrogen exists as organic nitrogen it is not immediately available for the nutrition of the organisms of the filter. Such a liquor would thus resemble a liquid with a higher C/N ratio. The growth of the film in the filter bed has a certain definite nitrogen requirement without which its development will be retarded. An analysis of the film from the top and bottom sections of the gravel filter (see Table IV), is given in Table XIV.

Table XIV. *Nitrogen content of film.*

Section	Total nitrogen (Kjeldahl)	% organic matter	% nitrogen in organic matter
1	7.1	67.0	10.6 = 69 % protein
6	4.6	38.7	13.1 = 85 % protein

The percentage of nitrogen in the organic matter is high, especially in the film of section 6 which contains nitrifying organisms. It is equivalent to a protein content of 85 % so that the organisms of the lowest section have a very low non-protein content (15 %) similar to that of animals in a state of starvation. Probably they are living on their own tissues since there is practically no soluble organic matter in the liquid passing through this portion of the filter.

A C/N ratio of 20 is equivalent to a nitrogen content of 2.1 % of the weight of sugar. In order to increase this nitrogen content to that of the film in the upper section, *viz.* 10.6 %, 4/5 of the sugar must be oxidised to CO₂. The remaining 1/5 of the sugar is then just sufficient to combine with the 2.1 % of nitrogen to form the organic matter of the film. If more than 4/5 of the sugar were oxidised there would not be sufficient to combine with all the nitrogen, and the excess nitrogen would be excreted. Thus, the nitrogen requirements of the film can be measured by adding the nitrogen as ammonium sulphate and noting its rate of absorption in the various sections of the filter.

For this purpose four filters were run on 0.2 % sugar solutions containing different amounts of ammonium sulphate, so as to give C/N ratios of 5, 10, 15 and 20 respectively. The effluents from the various sections were tested for ammonia with Nessler's reagent. A large excess of ammonia occurred in all sections of the filters with ratios of 5 and 10. In the filter with a ratio of 15 the excess ammonia was very slight in sections 5 and 6, whereas with a ratio of 20 no ammonia was found in sections 4, 5 and 6. It appears therefore that the limit of the nitrogen requirements of the filter medium lies between the

ratios of 15 and 20, which is in agreement with the oxidation of 85 % of the sugar and the conversion of 15 % into organic matter of the film.

To test the possibility of nitrogen passing through the filter as amino-acid, formaldehyde titrations were carried out on the effluent from sections 2 and 3. The results are given in the following table.

Table XV. *Formaldehyde titration values of filter effluents.*

Sections	C/N ratio			
	5	10	15	20
	cc. of N/10 NaOH required per 100 cc. of effluent			
2	4.3	1.8	1.1	0.1
3	3.9	1.7	1.0	0.1

The titration value is negligible except in the case of a C/N ratio of 5. A titration value of 4 cc. is equivalent to 0.0056 % amino-nitrogen or 1/3 of the nitrogen supplied as ammonium sulphate. It must be concluded that amino-nitrogen is not produced in excess of the requirements of the organisms except in the case of a low C/N ratio. Similar determinations on the effluents of the fine gravel filter showed an increase in the formaldehyde titration value from 2.0 to 3.7 when ponding occurred, which suggests that the formation of amino-acid in the filters was due to some disturbing factors: in the one case possibly excess of NH_4 ions (ammonium sulphate), and in the other lack of aeration. The absence of ammonia from the effluents of the filter with a C/N ratio of 20 can therefore only be due to nitrogen shortage.

The percentage purification results obtained with the various C/N ratios are given in Table XVI.

Table XVI. *Percentage purification.*

Days	Section	C/N ratios			
		5	10	15	20
2	6	83	86	79	78
7	6	93	91	92	83
14	1	41	36	30	32
	2	68	60	71	58
	3	91	73	88	76
	4	94	91	89	84
	5	95	92	90	90
	6	95	92	92	92
21	1	58	59	56	53
	2	74	76	75	72
	3	95	82	85	86
	4	98	97	94	92
	5	99	98	97	96
	6	99	98	98	98
28	1	18	28	45	43
	2	42	57	51	61
	3	60	67	83	73
	4	81	71	87	94
	5	91	90	90	96
	6	97	96	96	97

It will be seen that in the early stage of the development of the film a low C/N ratio gives a better purification, but this difference does not persist beyond 14 days. On the 21st day there is practically no difference between the various filters as regards purification in any of the sections. On the 28th day the filters with the low C/N ratio show a distinct falling off in purification in the first three sections, but there is no significant difference between the final effluents.

The filters were dismantled after 30 days and the amounts of film on each section determined. The results are given in Table XVII.

Table XVII. *Effect of C/N ratio on dry matter of film.*

Section	C/N ratio							
	5		10		15		20	
	Film g.	Ash %	Film g.	Ash %	Film g.	Ash %	Film g.	Ash %
1	18.7	21	18.6	20	16.8	25	17.1	22
2	17.9	25	17.7	27	16.4	31	15.3	27
3	12.8	33	11.8	34	10.4	37	9.7	37
4	9.7	40	9.7	40	6.4	42	7.0	43
5	4.8	56	5.0	62	4.3	44	6.6	62
6	4.1	59	4.2	63	4.1	51	4.0	63
Total	72.3		71.0		58.4		63.3	

Total weight of sugar consumed per filter = 300 g.

Deducting the ash content of the film we obtain a total weight of film produced of 50.0 g. from the C/N 5 filter and 39.2 g. from the C/N 15 filter, representing 17 % and 13 % respectively of the weight of sugar consumed. This leaves 83 % and 87 % to be accounted for by the sugar oxidised and that remaining as impurity in the final effluent. These results are rather better than those obtained in the 5-day test (p. 1421), but inferior to the 90 % obtained by Cutler and Crump [1923] in the pure cultures with continuous aeration.

From these results it would appear that the better purification obtained in the early stages with a low C/N ratio (5–10) is due to a more rapid growth of film, which may later become excessive and diminish aeration with consequent reduction of efficiency.

Some control over the growth of film would appear to be desirable for the maintenance of efficiency. Of the various limiting factors to bacterial growth, two only appear capable of application in practice, *viz.* (1) dilution, (2) reduction of nitrogen (only applicable where nitrogen has been added). Direct biological control of the population has not yet been attempted, but experiments in this direction would doubtless supply useful information.

A reduction in growth rate of the organisms can be effected by diminution in the nitrogen supply to a C/N ratio of 100. This occurred when ammonium sulphate was omitted from the solution, but in such cases there was an immediate drop in the purification. It must therefore be concluded that a certain growth rate is essential for efficient filtration.

The maintenance of mature organisms with a high CO_2 output does not appear a possible solution. The most hopeful line of biological control of the film would appear to be in the use of a metazoan population to effect a more rapid consumption and discharge of film (*cf.* acetic acid experiment, p. 1430).

G. Nitrogen fixation.

Microscopic examination of the effluents of the various sections revealed the presence of organisms resembling the capsular and sarcina forms of *Azotobacter*, the nitrogen-fixing organism. Various workers on filtration have suggested the possibility of nitrogen fixation taking part in the processes of the filter bed. A sectional filter was therefore run for 14 days on 0.2 % sugar solution *plus* mineral salts but without added nitrogen. The tap water used for dilution contained 8 parts of nitrate-nitrogen per million, equivalent to a C/N ratio of 100. The gravel was washed before commencing the trial and a small inoculation of the previous film was used. To assist the establishment of a film 0.006 % nitrogen as ammonium sulphate was added to the first day's charge in the aspirator.

The purification figures and weights of film obtained are given in Tables XVIII and XIX.

Table XVIII. *Purification of nitrogen-free solution.*

Section	Percentage purification	
	After 4 days	After 14 days
3	52	58
6	65.5	76

Table XIX. *Weights of film from nitrogen-free solution.*

Section	Weight in g.		Total nitrogen %
	Total	% ash	
1	17.6	8.1	2.02
2	10.1	11.0	
3	12.1	14.5	
4	7.7	21.0	
5	6.9	34.0	1.46
6	5.9	35.0	

Total nitrogen of the film (Table XIX):

$$\left. \begin{array}{ll} \text{From sections 1, 2 and 3} & 39.8 \times \frac{2.02}{100} = 0.8039 \\ \text{From sections 4, 5 and 6} & 20.5 \times \frac{1.46}{100} = 0.2993 \end{array} \right\} = 1.1032.$$

Nitrogen supplied in solution:

$$\left. \begin{array}{ll} 5 \text{ litres} \times 0.00006 \text{ g. N per cc.} & = 0.3 \text{ g.} \\ 13 \times 5 \text{ litres} \times 0.000008 \text{ g.} & = 0.52 \text{ g.} \end{array} \right\} = 0.82$$

Gain in nitrogen = 0.2832 g.

Sugar passed through filter

$$14 \times 5 \text{ litres} \times 0.002 = 140 \text{ g.}$$

Assuming 70 % consumption of sugar by the organisms

Sugar consumed = 98 g.

Ratio of sugar consumed/nitrogen fixed = $\frac{98}{0.2832} = 345$.

These results show a small but definite gain in nitrogen similar in amount to that obtained by various workers on nitrogen fixation. The effluent contained no mineral nitrogen but probably contained a small amount of organic nitrogen in the form of living organisms, so that the total fixation of nitrogen was no doubt greater than the above figures show.

From the point of view of biological filtration nitrogen fixation cannot be said to be of any real importance. With a C/N ratio of 100 the amount of nitrogen fixed is only adequate to affect a purification of 76 % after 2 weeks' running with a 6 ft. filter. Assuming the same increase in purification with depth as in previous experiments it would require a filter at least 12 ft. deep to effect a purification of 98 % using a solution with a C/N ratio of 100.

H. *Nitrification.*

The production of nitrates in a filter effluent has always been a criterion of purification. In the experiments with sectional filters the effluents from each section were tested daily for nitrite by the Griess-Ilosvay reagent. In starting up a new filter with 0.2 % sugar a period of 20 days may elapse before any nitrite appears in the effluent. The effluents from the solutions of C/N ratio 5 and 10 showed the presence of nitrite several days before the effluent from a C/N ratio of 15 or 20, and the amount of nitrite was always greater from the solution of low C/N ratio.

The amount of nitrite produced diminishes from section 6 upwards to section 4, but none is found in the upper sections irrespective of the C/N ratio. The presence of nitrifying organisms in these upper sections has been shown by applying the Griess-Ilosvay test to the dilution water after incubation in the 5-day test. These sections therefore provide an example of the suppression of nitrification even with a C/N ratio of less than 5. It would appear therefore that the degree of purification has a greater influence on nitrite formation than has the C/N ratio [*cf.* Jensen, 1929]. When this figure reaches 85 to 90 %, *i.e.* equivalent to not more than 0.03 % sugar, nitrification may appear. It has been shown that bacterial growth is much more rapid in the upper sections, being 6 times more rapid in section 1 than in section 4. This growth would have a large oxygen requirement which would be met by the reduction of any available reducible substance. In these circumstances nitrous oxide could not persist. In sections 4, 5 and 6 aeration is generally more than sufficient to maintain the oxygen requirements of the organisms. For these reasons nitrite formation only persists during the final stages of purification.

The presence of oxidisable carbohydrates does not in itself necessarily inhibit nitrification. Nelson [1931] has recently shown that *Nitrosomonas* in

pure culture may continue to oxidise ammonia even in the presence of 10 % glucose.

In the case of the filters run on lactic and acetic acids the rate of purification was more rapid and strong nitrification appeared in section 3.

Certain pure strains of bacteria isolated from the filter bed at Colwick appeared to be capable of oxidising ammonium lactate to CO_2 and nitrous acid simultaneously, but in the presence of restricted aeration the nitrite did not persist [Rothamsted, 1929].

In the case of the clinker filter supplied with a solution of C/N ratio 15, the amount of nitrite in the final effluent reached 3 parts per million on the 30th day, and increased to a maximum of 35 parts on the 50th day. With the increase in nitrification all trace of ammonia disappears from these sections. The maximum amount of nitrite-nitrogen obtained in this experiment is slightly more than half the total amount supplied to the filter as ammonia. This means that nearly half the nitrogen added with the sugar is retained in the filter as organic matter and is only slowly released by decomposition.

Considerable fluctuations in the amount of nitrite were recorded from time to time, but they were generally traceable to irregularities in the rate of flow or mechanical stoppages resulting in deficient aeration. In the fine gravel filter nitrification steadily declined after the 30th day as a result of the diminishing purification of this filter. After the regrading of the upper three sections nitrification reappeared in the lower sections and continued vigorously.

The production of nitrates was detected 10 days after the appearance of nitrites, but it developed more slowly, apparently requiring the stimulus of larger amounts of nitrite. Increase in the amount of nitrate occurred at the expense of the nitrite, until all trace of nitrite disappeared from sections 5 and 6 and only a small amount remained in section 4. The amounts of nitrate in the final effluent varied considerably from time to time from 1 part N to as much as 10 parts N per 100,000, which is more than the rate of supply of nitrogen to the filter. To some extent these variations were due to differences in aeration but not entirely. The fact that they were of a much higher order than occurred in the previous period of nitrite formation suggests that the variation is of a biological nature. Fluctuations in the C/N ratio of the original solution had no direct effect on the amounts of nitrate in the effluent.

To obtain further information on this point, section 6 of the filter was removed and treated as a separate filter. Solutions containing nitrogen in different forms and amounts were run through this section at the rate of 2 litres per day (Table XX). The solution of each substance was run through the filter for not less than 24 hours. Estimations of the three forms of mineral nitrogen were made by the colorimetric methods as described in the Ministry of Health Bulletin [1929], ammonia by Nessler's reagent, nitrite by Griess-Ilosvay reagent, and nitrate by the pyrogallol test.

Table XX.

All quantities expressed in parts per million of solution.

Source of nitrogen	Total N	Amount of N in effluent as			Gain or loss	
		Ammonia	Nitrite	Nitrate		
Ammonium lactate	200	40	4	120	—	-36
Ammonium lactate	150	100	8	44	+ 2	—
Nil. Water only	—	—	0.5	40	+ 40	—
Ammonium lactate	60	40	1.0	24	+ 25	—
Urea	60	30	0.2	4	—	-15
Urea	30	1	0.2	32	+ 2	—
Nil. Water only	60	10	0.2	16	—	-13
Ammonium sulphate	—	—	—	13	—	-37
Ammonium sulphate	140	40	1	12	+ 12	—
Sodium nitrite	60	50	—	100	+ 1	—
Sodium nitrite	40	—	15	16	+ 6	—
Nil. Water only	20	—	0.2	40	+ 15	—
Sodium nitrite	60	—	18	40	+ 20	—
Sodium nitrite	—	—	—	120	+ 78	—
Sodium nitrite	60	—	8	20	+ 20	—
Sodium nitrite	100	—	15	80	+ 28	—
Sodium nitrite	100	—	0.2	120	+ 35	—
Sodium nitrite	100	—	10	200	+ 100	—
Sodium nitrite	100	—	0.2	160	+ 70	—
Sodium nitrite	180	—	48	160	+ 60	—
Nil. Water only	—	—	—	320	+ 188	—
Nil. Water only	—	—	—	32	+ 32	—
Sodium nitrite	—	—	—	24	+ 24	—
Sodium nitrite	100	—	16	160	+ 76	—

From these results it appears that the only nitrogenous substance capable of stimulating nitrate formation is sodium nitrite, which also gives rise to more nitrate-nitrogen than is supplied as nitrite. The increased nitrogen excreted in presence of nitrite is more than can be accounted for by the normal oxidation of the nitrogen in the film, since water alone extracts much smaller amounts of nitrate-nitrogen. It is suggested that the toxicity of the nitrite exerts some selective action on the mixed bacterial flora of the filter, resulting in increased breakdown and oxidation of the film. The symbiosis between these ammonifying and nitrifying bacteria is the subject of detailed bacteriological investigation.

J. *Phosphate metabolism of the film.*

In the preceding experiments the mineral salts added to the sugar solution included 0.05 % potassium phosphate (p. 1424). The amounts of available phosphoric acid in each effluent of the sections was estimated by the colorimetric method of Fiske and Subbarow [1925].

The results are plotted in curve A, Fig. 6, the amounts of phosphoric acid found in the various effluents being expressed as percentages of that supplied in the sugar solution.

As would be expected the absorption of phosphate is confined to the first three sections or the region of growth of the filters. In the 6th section there is a slight increase in the amount of soluble phosphate in the effluent probably due to the oxidation of organic phosphorus and the nitrification process.

These changes in phosphate content of the effluents may be correlated with changes in the p_H of the sectional effluents. In Figs. 1 and 2 it has been shown that the p_H of sections 4 and 5 tends to increase above that of the original solution, whilst in section 6 it tends to become less. The absorption of phosphoric acid by the growing film in sections 1 to 3 would result in the formation of free potash which would combine with any organic acid produced by the decomposition of the sugar. Oxidation of these salts would then result in the formation of potassium carbonate and a consequent rise in p_H . In section 6 the oxidation of organic phosphorus and nitrification would result in a lower p_H in this section.

Fig. 6. Absorption curves for phosphoric acid in sectional filter.

Attempts to measure the phosphate requirements of the film by the method employed in the case of nitrogen were not successful. Reducing the amount of phosphate in the original solution even to 1/6 the amount did not result in complete absorption of the phosphate by the film (see Curves B, C and D, Fig. 6). Each reduction in phosphate supply merely diminished the phosphate gradient down the various sections. This phenomenon is probably due to the division of the phosphate between the demand for nutrition or tissue formation and the temporary requirement of the enzyme reactions involving the formation of hexosephosphate. The hydrolysis of the hexosephosphate by the mineral acid used in the colorimetric determination would account for the apparent presence of uncombined phosphate during phosphate starvation.

The effect of phosphate deficiency on the working of the filter was determined as follows.

The five sectional filters were washed and re-inoculated with equal amounts of wash water. They were then run for 3 weeks on a basic solution of 0.2 % sugar and 0.03 % ammonium sulphate to which was added potassium phosphate in different amounts as shown in Table XXI.

The highest purification figures were given by filter C receiving 0.008 % P_2O_5 . Increasing the amount of phosphoric acid did not improve the filtration

Table XXI. *Percentage purification at section 3 with different amounts of phosphates in the crude liquor.*

Filter ...	A	B	C	D	E
P ₂ O ₅ % ...	Nil*	0.004	0.008	0.016	0.024
Days of run					
2	55	57	68	64	64
8	60	65	75	73	75
15	54	74	95	95	90
21	51	75	74	72	70

* The tap water used in this experiment contained 0.08 part P₂O₅ per million.

though Fig. 6 shows that it results in an increased retention of phosphoric acid by the film. This probably indicates a greater growth of film and since it is not accompanied by a corresponding increase in purification it must be assumed that aeration has become the limiting factor. This would explain the falling off in efficiency after the 15th day.

A smaller amount of phosphoric acid than 0.008 % appears to delay the development of the film, and in the almost complete absence of phosphate the film is permanently reduced in oxidising power.

CONCLUSIONS.

Biological filtration consists of two processes, *viz.* (1) deposition of organic matter by growth of film; and (2) oxidation through respiration. Both processes must be provided for, and since they are dependent upon one another the dominating factor in a filter bed is aeration, which is determined initially by the interstitial space and subsequently by the growth of the film. In this connection it should be realised that the minimum size of the particles is the determining factor and not the average size.

The growth of film is an essential part of the process in the filter, and any condition which delays or prevents it has an adverse effect on the purification. The most important factors influencing this growth are the concentration of food material or organic matter in the solution and its composition. Both these factors are more prominent in the initial stages of the working of the filter and their effect diminishes with age. Similarly their effect is greatest in the upper layers of the bed and diminishes with depth. An adequate supply of nitrogen and phosphorus is essential to the growth of the film and when the C/N ratio is more than 15 and the C/P₂O₅ ratio more than 10 the development and efficiency of the film is reduced. The lower portion of the filter bed then becomes dependent on nitrogen fixation and the discharge of organic nitrogen and phosphorus from the upper layer. In the early stages both these sources of nitrogen are inadequate and consequently delayed purification results. This effect of a high C/N ratio is probably one of the chief difficulties in the treatment of sugar beet factory effluents.

Physiologically a satisfactory filter bed is characterised by the absence of putrefaction (*i.e.* the production and accumulation of readily decomposed

organic compounds). (See Table XV.) The energy of decomposition is consumed in growth so that the occurrence of dead organisms is only a momentary phase in the metabolism of the film. Considering the enormous protozoan population, the occurrence of dead organisms is very rare. Under anaerobic conditions however decomposition products may accumulate and result in a change in the p_H of the medium and a higher formaldehyde titration value.

The clumping and colonisation of the organisms in the bed of the filter is also a very characteristic feature. It results in a much lower bacterial population in dispersion through the solutions than would normally occur in a liquid culture. This clumping or agglutinating process appears to be connected with the degree of aeration. The effluents from the fine gravel filter were generally opalescent and passed through a filter paper with difficulty, whilst those from the coarse filters were clear and filtered through paper readily. The degree of opalescence diminishes with the depth of the filters.

Nitrification is an essential feature of an efficient filter bed since oxidised nitrogen provides the most effective hydrogen acceptor in biological oxidation.

Nitrite formation occurs when the concentration of sucrose and its derivatives in solution falls below 0.03 %. The absence of nitrification in the presence of a higher concentration of soluble organic matter does not imply the absence of the nitrifying organisms but rather that the potential rate of decomposition of the nitrite is more rapid than its production. The production of nitrite ultimately gives rise to the production of nitrate, and this process increases until nitrite formation is hardly detectable.

SUMMARY.

1. There are two limitations to the 5-day oxygen absorption test, *viz.* (a) it is not possible to estimate carbohydrate in terms of oxygen absorption by a biological process owing to organic synthesis being an essential part of the process; (b) the nature of the inoculum affects the relative amounts of oxidation and synthesis.

Recognising the above limitations, the 5-day test is the most satisfactory measure of pollution available.

2. The production of organic acids is a stage in the decomposition of sugar, but their rapid destruction is a property of an efficient filter bed.

3. The organic acids lactic and acetic are more rapidly oxidised than sucrose.

4. The formation of oxidised nitrogen in the presence of organic matter promotes the dehydrogenation (oxidation) of lactic acid and probably of other compounds.

5. The growth of film is an essential part of the purification process and the film itself accounts for 15 % of the sucrose supplied.

6. Aeration is the dominant factor in biological filtration and is determined by the minimum size of the material which provides the interstitial

space for the growth of the film and also for its discharge. A fine medium should not be used to secure time of contact.

7. If grading of the medium is desired, the smaller particles should be placed at the bottom where the smallest amount of film will develop, but to ensure free passage of discharged film nothing passing through $\frac{1}{2}$ inch mesh should be used.

8. The rate of flow is the best means of controlling the time of contact and purification. Within limits a slow rate of flow with high concentration will increase the time of contact more rapidly than the reverse conditions. As the time of contact increases, the rate of flow may be increased without reduction in purification.

9. The growth and efficiency of film is dependent upon definite nitrogen and phosphorus requirements, *viz.* a C/N ratio of 15 and C/P₂O₅ ratio of 10.

10. Nitrogen fixation occurs in the filter bed but results in the growth of film of low N content and low purification.

11. A low C/N ratio and a high concentration of organic matter promote the rapid development of the film and attainment of high purification. They also lead to excessive growth of the film and where possible should be used only in the first month of the working of the filter.

12. With a C/N ratio of 15 or less a filter bed 5 ft. deep will give complete purification of 0.2 % sugar solution at a rate of flow of 100 g.y.d. using coarse gravel or clinker. With a C/N ratio of 50 to 100 a much deeper filter would be required, and better results could more easily be secured by the addition of nitrogen than by deepening the filter.

13. Both stages of nitrification occur in the filter bed and are subject to considerable fluctuations not correlated with the supply of nitrogen in solution.

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