EFFECT OF TREATMENT AT THE SEWAGE WORKS ON THE NUMBERS AND TYPES OF BACTERIA IN SEWAGE

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(With 9 Figures in the Text)

It is well known that successive stages of treatment at a sewage works reduce the quantity of suspended solids, the content of organic matter, and the biochemical oxygen demand of sewage, so that the polluting strength of the final effluent is a small fraction of that of the sewage received at the works. Much less is known, however, about the effect of treatment on the bacterial flora of sewage. An investigation has therefore been made at two sewage works at each of which treatment in percolating filters and humus tanks is followed by treatment in a sand filter or an anthracite filter. Samples taken at various stages of treatment from both works over a period of several months were submitted to bacteriological examination to find the plate count on nutrient agar and the counts of *coli-aerogenes* bacteria, of faecal *Bacterium coli* and of *Streptococcus faecalis*. A large number of pure cultures of coliform bacteria and of streptococci were isolated and their characters were determined. Chemical analyses were made on the same samples as were used for bacteriological examination.

The data as a whole gave a general picture of the extent to which treatment at the works reduced the numbers of bacteria in different groups, of the relative parts played by percolating filters and by sand filters in this process, and of the numbers of bacteria discharged to a river with a final treated effluent.

PREVIOUS WORK

One of the earliest processes of sewage treatment consisted in applying settled sewage intermittently to slow sand filters. Early work at the Lawrence Experiment Station (Massachusetts State Board of Health, 1887–1915) established the fact that if the interval between doses was sufficiently long to allow the bed to drain and dry on each occasion, intermittent filtration through fine sand yielded an effluent in which the bacterial count was a small fraction of that of the settled sewage applied to the bed. Thus a filter containing coarse clean mortar sand (effective size 0·48 mm.), 5 ft. deep, which consistently received sewage at a rate of 74,000 gallons*/acre/day removed on an average 94 % of the organic matter and 98 % of the bacteria. A similar filter with finer sand (effective size 0·06 mm.) treated 43,000 gallons/acre/day and removed 97·5 % of the organic matter and 99·99 % of the bacteria from sewage.

As with slow sand filters used in the treatment of water, the efficacy of these filters depends largely on the presence of a biological film which reduces the volume of the interstices between the grains of sand and at the same time removes organic matter in the water by using it as a source of food for growth and energy for the micro-organisms in the film.

Because of the high content of organic matter and the turbid character of sewage filtration is slow, extensive areas of sand are necessary, and the upper layers of the bed frequently become clogged. For these reasons other methods of treatment were tried and for some time the contact bed was in common use. This consists of a shallow bed, often lined with concrete, and filled with coarse gravel, stone, or similar material. After use for some time the surface of the medium becomes coated with a biological film consisting of a mixed population of bacteria, fungi, and protozoa. Sewage is filled into the bed so as not to disturb this film and a period of contact of about 2 hr. is allowed. During this period a proportion of the constituents of the sewage is adsorbed by the biological film, and a further proportion is simply deposited. The effluent is then drawn off and the bed is allowed to stand empty for a period to allow microbial oxidation of the adsorbed and deposited organic matter.

Difficulties caused by clogging and the comparatively low rates of dosing led to attempts to improve on the design of the contact bed. These resulted in the evolution of the percolating filter, in which settled sewage trickles continuously through a bed filled with a suitable medium such as coke or stone, the pieces of which are commonly 1–2 in. in diameter. This process allows a comparatively rapid rate of treatment and effects a considerable reduction in polluting strength.

* Throughout the paper 'gallons' refers to Imperial gallons.

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Experiments at the Lawrence Experiment Station (loc. cit.) showed that treatment in percolating filters removed from 68 to 96 % of the total numbers of bacteria (plate counts on nutrient agar at 20° C.) and from 76 to 96 % of *coli-aerogenes* bacteria from settled sewage, depending on the nature of the medium and the rate of dosing. The results also suggested that at slow rates of filtration most of the bacteria were removed in the first 2 ft. of filter, but that with increasing rates of application a larger proportion of the total numbers tended to pass through the filter.

Results recorded by Houston (1910) for the Royal Commission on Sewage Disposal showed that, although treatment at various sewage works reduced the total count and the count of '*B. coli*' appreciably, there were still high counts in the final effluents. Boyce, MacConkey, Grunbaum & Hill (1902) investigated the effect of further filtration through 4 ft. of sand or earth on the counts of bacteria in effluents from contact beds. An initial count of 9800 '*B. coli*' per ml. was reduced to a count varying from 49 to 590 with different rates of filtration. At a rate of 157,000 gallons/acre/day the count was frequently less than 10/ml. It was concluded that the chance of *Bact. typhosum* appearing in an effluent under these conditions was very small.

Winslow (1905) found average total microscopic counts (millions/ml.) of 29 for crude sewage, and 30, 24, 17, and 0·65 for the respective effluents from the septic tank, contact filter, trickling filter, and sand filter of an experimental plant in Massachusetts.

Gaub (1924) made a bacteriological study of the sewage-disposal plant at Plainfield, U.S.A., in which screened sewage was passed through Imhoff tanks and the effluent from these was treated in percolating filters. Bacterial counts showed a progressive decrease throughout the process of treatment, the total count being reduced by 93 %, and the *coli-aerogenes* count by 98·9 %; the effluent showed an average total count of 93,000, and a count of *coli-aerogenes* bacteria of 9000/ml. Heukelekian (1927), in tests with the same plant, found that the percentage of *coli-aerogenes* bacteria removed by the percolating filters in the period between August and March ranged from 53 to 100, with an average of 89.

Rudolfs, Heukelekian, Lacy & Henderson (1930) found that the average count of *coli-aerogenes* bacteria in the effluent from the Tenafly (New Jersey) activated-sludge plant was reduced from 3090 to 134/ml. by further treatment in a sand filter. Rudolfs, Chamberlain & Heukelekian (1932) found that the effect of the higher temperature during summer was to increase the speed of chemical changes taking place in a percolating filter and to increase the percentage of bacteria removed.

Allen, Tomlinson & Norton (1944) investigated the effect of treatment in percolating filters, in an experimental plant at Minworth, on the bacterial counts of settled sewage. The percentage reduction in the plate count in a single percolating filter treating sewage at a rate of 60 gallons/cu.yd. of filter medium per day was consistently high—above 90 %—in the warmer months of the year, but it fluctuated considerably and was sometimes quite low in the winter months. In an alternating double filtration plant treating similar sewage at an average rate of approximately 240 gallons/cu.yd./day the bacterial count of the primary effluent was usually high and the count of the secondary effluent was on the average considerably higher than that from the single filter. The chemical quality of the effluent was found to be no criterion of its bacterial quality. The count of *coli-aerogenes* bacteria was reduced in the single filter by an average of 95 % in the colder months and of 99·5 % in the warmer months of the year.

In present-day practice although sand filters are rarely used for treatment of crude or settled sewage they are not infrequently used to improve the quality of effluents from percolating filters, or from activated sludge plants, in places where a final effluent of high quality is required (Streander, 1935, 1940). The filters used are usually shallow gravity filters, or sometimes pressure filters; the medium may be silica sand, fine coal, crushed iron ore (magnetite), or paper pulp.

DESCRIPTION OF SEWAGE WORKS

The two sewage works studied in the present investigation differed in several particulars, but chiefly in size and in the character of the sewage treated, one works serving a small rural district and the other a large industrial town.

Sewage Works No. 1

This Works, which serves a population of approximately 1700, receives only domestic sewage. The sewage is first treated in a sedimentation tank and the tank effluent runs by gravity to two dosing chambers, from which it is discharged by siphon to two percolating filters in parallel. Each filter is 42 ft. in diameter and is filled with clinker to a depth of 6 ft. Effluent from the filters drains into a common channel which serves two humus tanks in parallel. Only one humus tank is in operation at any time, duplicate tanks being provided to facilitate cleaning. Effluent from these tanks flows over a weir into a channel which feeds two sand beds in parallel. Each sand bed measures 71 ft. × 20 ft. and consists of a 9 in. layer of gravel surmounted by 9 in. of sand. The underdrains are 4 in. glazed stoneware pipes perforated underneath. A mechanical analysis of the sand carried out at the Department's Building Research Station gave the following results:

B.S. sieve size (in.)	Percentage by weight passing B.S. sieve
$\frac{3}{8}$	100
$\frac{3}{16}$	96
$\frac{3}{32}$	77
$\frac{3}{64}$	49
$\frac{3}{128}$	32
$\frac{3}{256}$	14
$\frac{3}{512}$	4

Effluent from the humus tanks is discharged through a bell-mouth pipe on to the surface of the sand at one end of the bed. No attempt is made to ensure even distribution and when the bed has been freshly cleaned the liquid wets only a comparatively small area of the sand near the inlet. As the surface becomes clogged with humus from the effluent being treated, more and more of the area of the bed becomes wetted until the operator considers that it is time to put that bed out of commission and re-condition it. This is done by turning the sand over with a spade. The bed is then left until the alternative bed is in its turn put out of commission. Effluent from the sand beds is discharged to a brook.

The flow of sewage to the works is not recorded, but measurements were made by calibrating the dosing chambers and making observations of the frequency of discharge. Results showed that the rate of flow of sewage in dry weather was about 28,000 gallons a day, corresponding to a rate of application to each percolating filter of 70–80 gallons/cu.yd. of filtering medium a day. Under these conditions each sand filter when in use treated 900–1100 gallons of sewage effluent an hour. Rates of treatment on the occasions of sampling ranged from 43·1 to 133·0, with an average of 65·1, gallons/day/cu.yd. of filter medium in the percolating filters, and from 410,000 to 1,265,000, with an average of 666,000, gallons/acre/day in the sand filters.

Sewage Works No. 2

The dry-weather flow of sewage to this Works per day is about 6½ million gallons, of which approximately one-third is trade wastes; an appreciable proportion of the total flow consists of condenser cooling water which has the effect of maintaining the temperature of the sewage, particularly in winter, at a comparatively high level. The sewage first passes through screens, detritus tanks, and primary and secondary sedimentation tanks, and is then treated by the bio-flocculation process in aeration tanks supplied with diffused air. Effluent from this process is further treated in rectangular percolating filters and after passing through humus tanks is discharged to a river.

As part of the experiments undertaken by the Water Pollution Research Laboratory to investigate possible methods of improving the chemical quality of the effluent, two semi-scale pressure filters were installed, each consisting of a vertical cast iron cylinder 3 ft. deep with internal diameter of 2 ft. One filter contained sand 24 in. deep, graded -10 $+20$ mesh, and the other contained a lower layer of about 10 in. of -4 $+8$ mesh anthracite, supporting an upper layer of 14 in. of -8 $+18$ mesh anthracite. Effluent was drawn off at a steady rate through rate controllers, a centrifugal feed pump increasing the pressure of delivery as resistance increased in the filter. The length of run of each filter before the medium was back-washed varied during the course of the experiments, but was usually between 24 and 48 hr.

Rates of treatment in the percolating filters were estimated from the recorded rates of flow of sewage to the works after making allowance for the time taken for the sewage to pass through the sedimentation tanks and the bio-aeration plant. On the occasions of sampling, rates of treatment ranged from 110 to 178, with an average of 139, gallons/day/cu.yd. of filter medium.

In the pressure sand filter rates of treatment ranged from 62 to 125, with an average of 101, gallons/sq.ft./hr., and in the pressure anthracite filter rates ranged from 74 to 186, with an average of 116, gallons/sq.ft./hr.

EXPERIMENTAL

Sampling

Samples of sewage at various stages of treatment were taken periodically from both works, all samples being collected in sterile glass-stoppered bottles. From Sewage Works No. 1 the following samples were collected: (1) settled sewage, taken from the distributors to the percolating filters; (2) unsettled effluent from percolating filters—this was taken from the effluent channel when the liquid was sufficiently deep and on other occasions from the influent end of the humus tank; (3) settled effluent from the percolating filters, taken from the bell-mouth pipe delivering effluent to the sand bed; (4) final effluent from the sand bed, taken from the channel discharging effluent to the brook.

The following samples were collected from Sewage Works No. 2: (1) settled sewage, taken from the influent pipe to the bio-aeration plant; (2) settled effluent from the bio-aeration plant, taken from the weir of the separating tank; (3) unsettled filter effluent, taken from the influent end of the humus tank; (4) settled filter effluent, taken from the sampling cock on the pipe leading from the humus tank to the sand filter; (5) final effluent from the sand filter; (6) final effluent from the anthracite filter.

Samples of effluent from the percolating filters were allowed to settle for 1 hr. in the laboratory and from the supernatant liquid a portion, amounting to about one-third of the whole volume, was decanted into a second sterile bottle. The remaining samples were examined without further settlement. Before preparing serial dilutions in Ringer solution each sample was shaken twenty-five times in a sterile stoppered bottle which was not more than three-quarters full.

Bacteriological methods

Plate counts on nutrient agar at 20° C. and presumptive counts of *coli-aerogenes* bacteria in MacConkey broth at 37° C. were determined by methods laid down by the Ministry of Health (1939), except that triplicate, instead of duplicate, plates were poured. Presumptive counts of faecal *Bact. coli* were determined by sub-culturing all positive tubes in the 37° C. test to fresh tubes of MacConkey broth which were incubated in an accurately controlled water-bath at 44° C. From the number of positive tubes after incubation for 2 days the most probable number of faecal *Bact. coli* was computed from statistical tables.

Faecal streptococci were enumerated by the method described by Hannay & Norton (1947) using a medium of the following composition: 10 g. peptone, 5 g. NaCl, 5 g. glucose, 5 g. K_2HPO_4, 2 g. KH_2PO_4, 3 g. yeastrel, 0·25 g. sodium azide, 2 ml. alcoholic solution of brom-cresol purple (1·6 %), distilled water 1000 ml. (pH 6·6–6·8).

After preparing suitable dilutions of a sample of sewage or effluent, 1 ml. quantities of each dilution were inoculated into five parallel tubes of the medium, which were then incubated at 45° C. in an accurately controlled water-bath, results being recorded after 48 hr. Preliminary trials showed that for sewage and sewage effluents this medium gave higher counts and a more decided change of colour than the medium devised by Hajna & Perry (1943). Results of comparative tests are shown in Table 1.

Chemical methods

Chemical purification effected by treatment at the sewage works was assessed by determining on each sample the biochemical oxygen demand during incubation for 5 days at 18·3° C., and the value for oxygen absorbed from N/80 acid permanganate in 4 hr. at 26·7° C.

RESULTS

In the course of the investigation, between 400 and 500 samples were submitted to both chemical and bacteriological examination. Results are therefore too numerous to be presented in full, but the main trends may be seen from the results* of averages and from curves.

Ranges in temperature and in numbers of bacteria during the course of the investigation, illustrated graphically in Fig. 1, show that although wide fluctuations occurred at frequent intervals there was a general tendency for bacterial counts in settled sewage to be higher in the warmer months of the year. This conclusion is supported by the figures in Table 2 which gives average results of bacteriological and chemical examination of all samples throughout the investigation, figures for the warmer and for the colder months of the year being shown separately. The temperatures recorded at Sewage Works No. 1 (average 7·8° C. between November and April and 14·2° C. between April and October) were lower than at Works No. 2, where the sewage was warmed by the condenser cooling water it contained. The lower count in winter does not appear to be due to greater dilution, judging from the values for biochemical oxygen demand and for oxygen absorbed from permanganate, which were approximately the same in the two periods. This suggests that in the warmer months of the year some growth of bacteria may occur in the sewage before its discharge from the sedimentation tank.

Table 1. *Comparative counts of* Streptococcus faecalis *in two different media*

Sample	Counts (per ml.) of *Strep. faecalis* in following media: Hajna & Perry (1943)	Hannay & Norton (1947)
Settled sewage	700	800
Effluent from:		
Percolating filter	60	110
Humus tank	17	90
Sand filter	13	90
Settled sewage	900	1700
Effluent from:		
Percolating filter	17	110
Humus tank	13	35
Sand filter	11	35

Average total counts are much higher in the sewage containing trade waste than in the domestic sewage for the same period, but numbers of bacteria of faecal origin are appreciably smaller. This may be because trade wastes encourage the growth of

* A small proportion of the results of bacteriological examination (2·7 % of the total) and one result of chemical examination were well outside the range of figures obtained for the remaining samples in that group. These results were considered to be abnormal owing possibly to the difficulty of obtaining from a sewage works a small sample which is representative of the whole of the liquid being treated, and were therefore not included when computing averages.

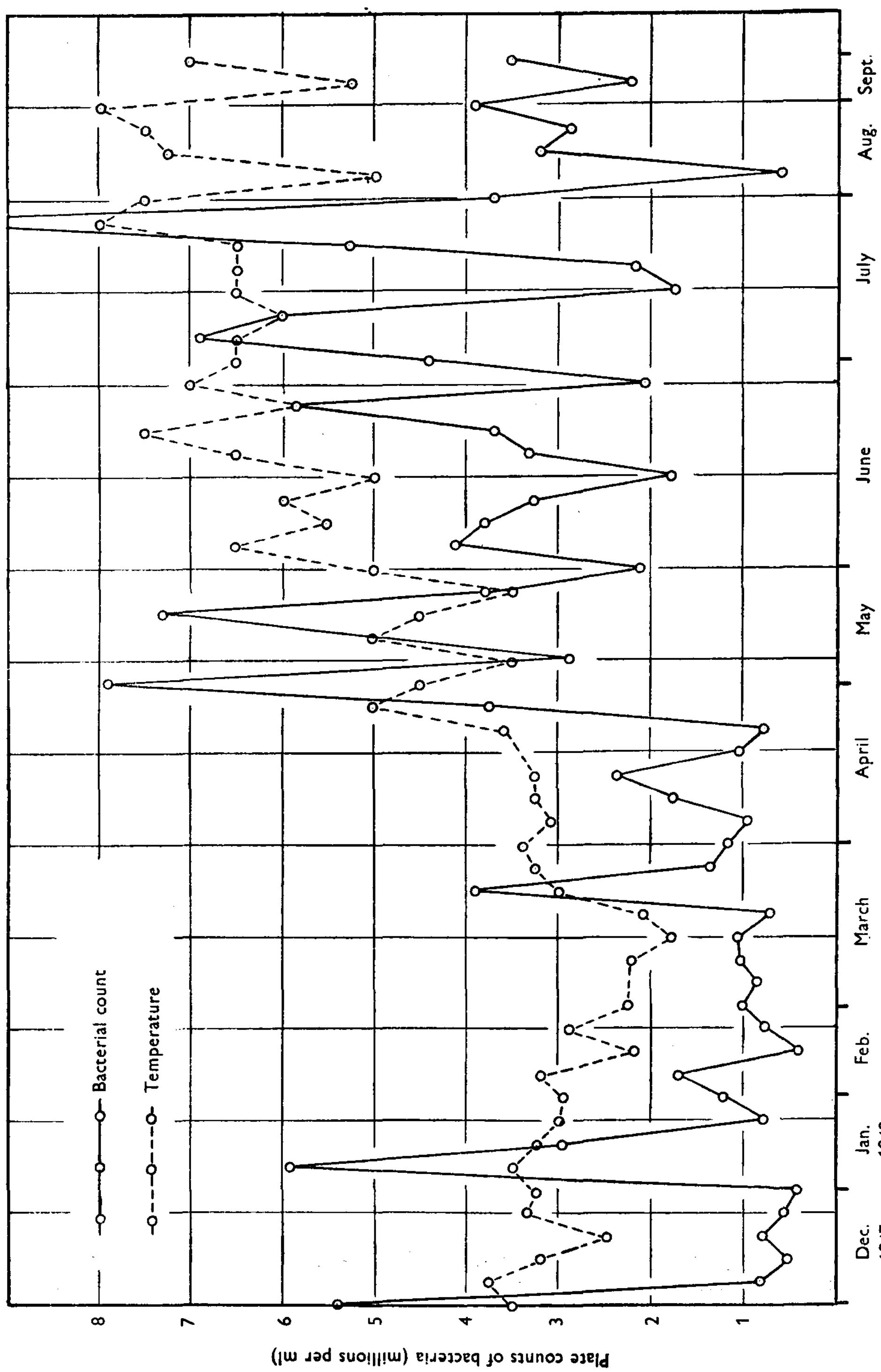

Fig. 1. Fluctuations during the period of the investigation in the temperature and in the bacterial count of settled sewage at Sewage Works No. 1.

extraneous organisms or because they contain substances inimical to *Bact. coli* and to streptococci.

The plate counts, on nutrient agar at 20° C., of samples taken at different stages of treatment throughout the investigation are shown graphically in Figs. 2 and 3, the counts being plotted on a logarithmic scale. Counts of samples from any one of the stages of treatment covered a wide range owing to the large fluctuations which occurred, but it is clear that the numbers of bacteria are greatly reduced by the treatment received at the works. Nevertheless, the final effluents still contain a very large number of bacteria. Thus the effluent from Sewage Works No. 1 conveys to the stream into which it is discharged an average of about 160,000 cells of faecal *Bact. coli* and about 20,000 faecal streptococci with every 100 ml. of liquid. Average figures for Sewage Works No. 2 are even higher. Both these works, it should be noted, are designed according to the best modern practice and give full biological treatment; the effluent from Works No. 1 is of exceptionally high chemical quality.

Proportion of bacteria removed by different treatments

In Table 3 are shown the proportions of the different groups of bacteria removed by the various filters, each considered separately. Results for the percolating filter were calculated from the counts for settled sewage and for the effluent from the filter after settlement in the laboratory for 1 hr. At Sewage Works No. 1 the percolating filter removed on the average more than 90 % of the bacteria in settled sewage and the treatment appeared to exert no selective effect on faecal or non-faecal types. Subsequent treatment in the sand filter removed a much smaller proportion of the bacteria remaining, the average percentage reduction in plate count being 57·4. The range of fluctuation encountered was also much higher. On fifty occasions, when samples from both stages of treatment were examined, treatment in percolating filters, followed by settlement in humus tanks, removed an average of 91·1 %, and the subsequent treatment in sand filters removed an average of 6·1 %, of the total number of bacteria (plate counts) initially present in the settled sewage.

Treatment in percolating filters at Sewage Works No. 2 removed a much smaller proportion of bacteria than at Works No. 1, the average percentage reduction being little more than 80. Conditions at Works No. 2 differed in three respects from those at Works No. 1. At Works No. 2 incoming sewage contained a high proportion of trade waste, the liquid passed to the percolating filters was the effluent from a bio-aeration unit, and the rate of filtration was high, averaging 136 gallons/cu.yd./day. At Works No. 1 the filters treated only settled domestic sewage, and at a much slower rate, averaging about 72 gallons/cu. yd./day. It was found by Allen *et al.* (1944) that in a single percolating filter treating sewage at ordinary rates appreciable removal of bacteria did not occur until the lower levels of the filter were reached, and that the effluent from a primary filter treating sewage at a high rate frequently showed a bacterial count of the same order as that of sewage applied to the filter. This suggests that the main reason for the smaller percentage removal of bacteria at Works No. 2 was probably the higher rate of treatment.

The anthracite pressure filter at this Works removed about the same proportion of bacteria from the liquid applied to it as the gravity sand filter at Works No. 1 (53 and 57 %, respectively). For the smaller number of occasions when the sand pressure filter was tested the average proportion removed was appreciably higher—about 72 % of the numbers in the liquid supplied to the filter—but on these same occasions the average removal accomplished by the anthracite pressure filter was about 65 %. Table 4 shows the plate counts of the influent (that is, the settled effluent from the percolating filters) and of the effluent from each of the pressure filters at Sewage Works No. 2 at different intervals after the beginning of a filter run. The sand filter consistently removed a higher proportion of bacteria than the anthracite filter, the effect being more marked during the first 4 hr. than subsequently; for both filters the proportion removed at the beginning of the run was appreciably lower than at later stages. Results in Table 5 show that the small proportion removed in the early stages was a fairly consistent feature.

Treatment in the bio-aeration plant did not markedly reduce the number of bacteria in settled sewage. Thus, on the twenty-five occasions when samples were taken at both stages of treatment, plate counts of settled sewage ranged from 740,000 to 38,400,000, with an average of 13,400,000, and plate counts of the effluent from the bio-aeration plant ranged from 750,000 to 28,200,000, with an average of 9,700,000, per ml. On twelve occasions the count of the effluent was lower, and on thirteen occasions it was higher, than the count of settled sewage sampled at the same time. On fifteen occasions when samples were taken from the pressure sand filter, treatment in the bio-aeration plant, percolating filters, and humus tanks removed an average of 79·3 % and the sand filter removed a further 10·5 % of the bacteria initially present in the settled sewage.

On the nineteen occasions when samples were taken from the anthracite pressure filter an average of 8·0 % of the bacteria were removed by this filter and 82·7 % by the previous treatment.

The total percentage reduction in the counts of different groups of bacteria resulting from treatment of settled sewage in percolating filters and humus

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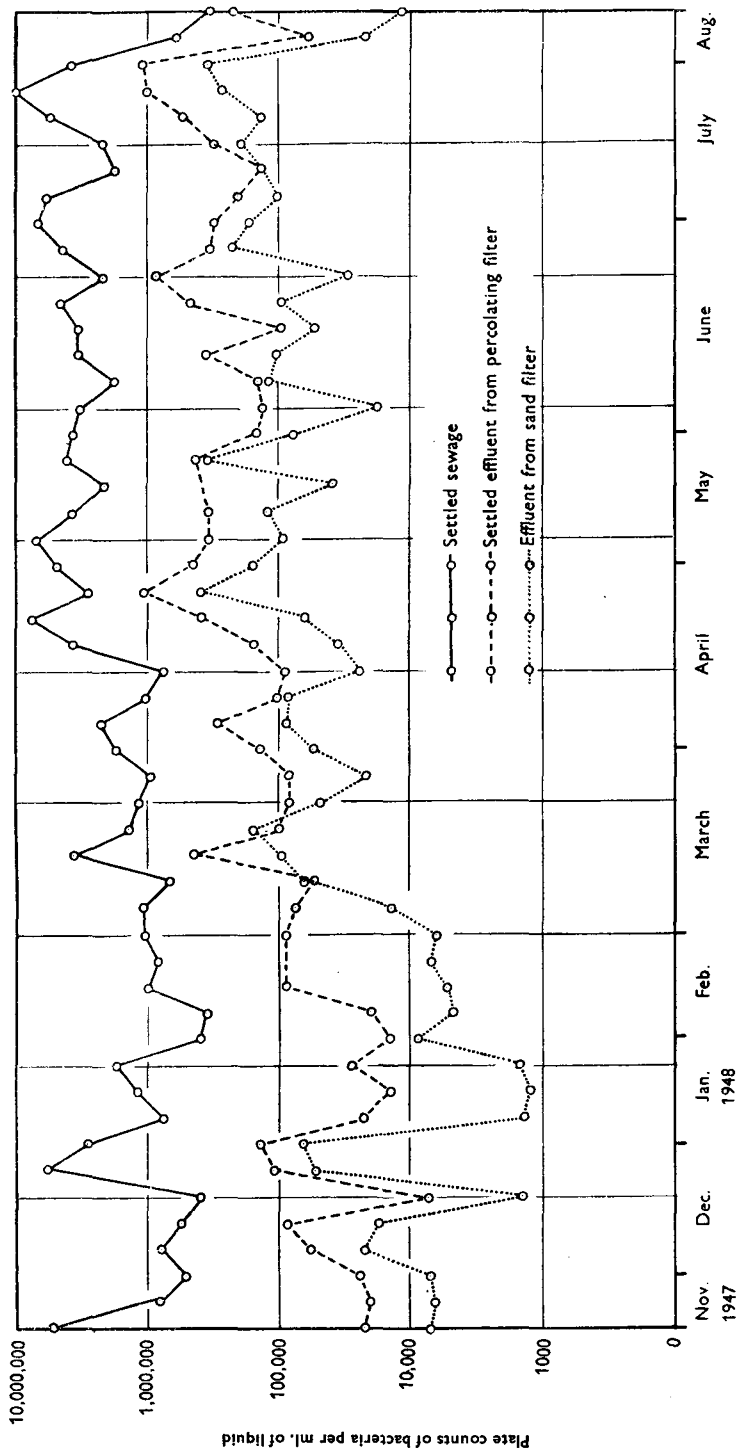

Fig. 2. Plate counts of bacteria at different stages of treatment of sewage at Works No. 1.

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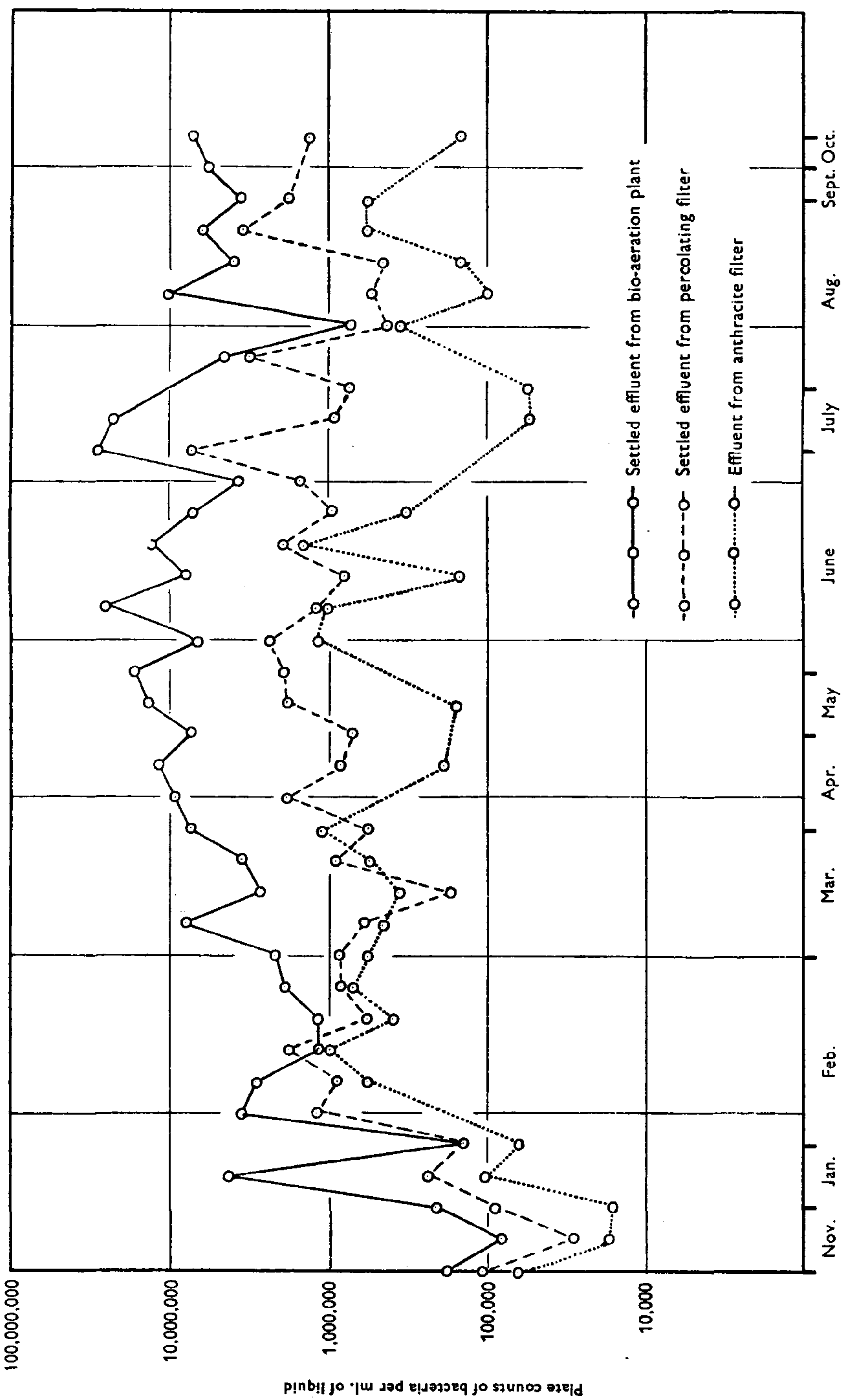

Fig. 3. Plate counts of bacteria at different stages of treatment of sewage at Works No. 2.

Table 2. *Average results of bacteriological and chemical examination of samples taken at different stages of treatment at two sewage works*

	Sewage Works No. 1			Sewage Works No. 2				
	Settled sewage	Settled effluent from percolating filter	Effluent from gravity sand filter	Settled sewage	Effluent from bio-aeration plant	Settled effluent from percolating filter	Effluent from pressure sand filter	Effluent from pressure anthracite filter
Period 11 November 1947 to 8 April 1948. Average temperature of settled sewage = 7·8° C. Average temperature of effluent from bio-aeration unit* = 14·9								
Bacterial counts (thousands per ml.):								
Plate counts at 20° C.	1540	89	36·1	—	3090	930	—	488
Coli-aerogenes at 37° C.	81	2·94	1·65	—	45·4	5·63	—	3·12
Faecal *Bact. coli* at 44° C.	74	2·83	1·55	—	26·1	3·16	—	1·91
Strep. faecalis	9·6	0·26	0·10	—	0·81	0·17	—	0·22
Chemical results (parts per million):								
Biochemical oxygen demand in 5 days at 18·3° C.	143	6·0	2·4	—	95	33	—	20
Oxygen absorbed from acid permanganate in 4 hr. at 26·7° C.	43	9·4	7·1	—	56	25	—	20
Period 9 April to 11 October 1948. Average temperature of settled sewage = 14·2° C. Average temperature of effluent from bio-aeration unit* = 18·8° C.								
Bacterial counts (thousands per ml.):								
Plate counts at 20° C.	4070	390	120	15,800	10,000	1730	475	444
Coli-aerogenes at 37° C.	169	4·66	2·24	68	67·8	6·0	4·05	4·31
Faecal *Bact. coli* at 44° C.	102	3·35	1·76	29·7	35·6	4·9	3·66	3·31
Strep. faecalis	17·6	0·90	0·23	4·1	3·4	0·38	0·19	0·19
Chemical results (parts per million):								
Biochemical oxygen demand in 5 days at 18·3° C.	141	6·0	2·7	97	65	27	8·4	7·7
Oxygen absorbed from acid permanganate in 4 hr. at 26·7° C.	43	10·5	6·8	52	46	20	14	15

* On the occasions when samples both of settled sewage and of this effluent were taken the temperatures were approximately the same.

Table 3. *Efficiency of percolating filter, of sand filter, and of anthracite filter in removing bacteria.*

(Reduction in bacterial count expressed as percentage of count of liquid applied to each filter. Figures in brackets indicate number of samples tested.)

	Sewage Works No. 1					Sewage Works No. 2					
	Percolating filter				Sand filter	Percolating filter				Sand filter	Anthracite filter
	Plate count (35)	Coliforms at 37° C. (56)	Coliforms at 44° C. (54)	*Strep. faecalis* (44)	Plate count (50)	Plate count (34)	Coliforms at 37° C. (39)	Coliforms at 44° C. (38)	*Strep. faecalis* (36)	Plate count (17)	Plate count (28)
Minimum	72·7	54·3	48·0	72·2	13·0	35·7	36·0	25·0	50·0	31·1	7·5
Maximum	99·5	99·8	99·8	99·9	97·0	98·6	99·0	99·0	98·9	95·5	94·0
Mean	92·9	95·0	91·6	94·0	57·4	80·4	82·4	82·7	84·1	72·0	52·8

tanks, and from further treatment in a sand or anthracite filter, is shown in Table 6. It is clear that additional treatment yields an effluent of appreciably higher bacterial quality. This may be seen by applying the figures to a hypothetical case. If settled sewage with a count of 100,000 faecal *Bact. coli* were treated at Works No. 1 in percolating filters and humus tanks only, the final effluent discharged would, on the average, show a count of 5500 per ml. If, however, the effluent were passed through the sand filter before discharge the count would, on the average, be reduced to 2700. Similarly, an initial count of 10,000 faecal streptococci would be reduced to 530 by treatment in percolating filters and humus tanks and to 190 by further treatment in sand filters.

General effect of different treatments over a long period on the chemical and bacterial quality of sewage

The general picture of the effect on sewage of treatment in percolating filters, in gravity sand filters, and in pressure filters is shown graphically in Figs. 4–9. The following conclusions may be drawn:

(1) Figs. 4 and 7 suggest that a percolating filter removes a larger and more constant proportion of bacteria from the liquid supplied to it than does a sand filter.

(2) Figs. 5 and 8 show the effect of additional treatment in a gravity sand filter or in a pressure filter in smoothing out fluctuations in the performance of a percolating filter and so tending to produce an effluent of consistently higher bacterial quality.

Table 4. *Proportion of bacteria (plate counts at 20° C.) removed by pressure filters at Sewage Works No. 2 at different intervals after the beginning of filter run*

Period after beginning of run (hr.)	Plate count of: Influent	Effluent from: Sand filter	Effluent from: Anthracite filter	Reduction in count due to treatment (%): Sand filter	Reduction in count due to treatment (%): Anthracite filter
¼	1,320,000	720,000	1,400,000	45·5	6% increase
1	860,000	330,000	520,000	61·6	39·5
2	1,380,000	224,000	500,000	83·7	63·9
3	910,000	183,000	264,000	79·8	70·9
4	720,000	189,000	289,000	73·7	59·8
5	1,250,000	239,000	274,000	80·8	78·1
6	1,890,000	212,000	251,000	88·7	86·7
23	2,270,000	420,000	510,000	81·4	77·5

Table 5. *Percentage of bacteria (plate counts at 20° C.) removed by sand or anthracite pressure filters near beginning of filter run*

Date of sampling	Period after beginning of filter run when sample was taken (hr.)	Bacteria removed (%): Sand filter	Bacteria removed (%): Anthracite filter
5. ii. 48	1	—	7·5
12. ii. 48	1½	—	41·4
17. ii. 48	¼	—	14·2
4. iii. 48	1	—	22·6
8. iii. 48	¼	—	40·0
3. vi. 48	¾	36·6	14·6
5. viii. 48	¼	9·1	13·6
30. viii. 48	1	76·0	67·5

For similar sewage treated at Works No. 2 the counts of faecal coliform bacteria in the settled effluent from percolating filters would be reduced by further treatment in pressure filters from 17,400 to 6200, and the counts of faecal streptococci from 1060 to 510, per ml.

The gravity filter at Works No. 1 appeared to be more effective in this respect than the anthracite pressure filter at Works No. 2. Figs. 6 and 9 show that, in general, chemical quality was improved by additional treatment in much the same way. On the other hand, there is certainly no direct correlation between chemical and bacterial quality.

(3) The fluctuations in the counts of the different groups of bacteria in the final effluents discharged at both sewage works show that sand filters of the type investigated in this work cannot be relied upon to produce an effluent of good bacterial quality. This could only be attained by methods similar to those used in water-works practice—for example by means of a slow sand filter or by using a pressure filter preceded by chemical coagulation.

Types of coliform bacteria in sewage and in treated effluent

It is generally agreed that with samples of water positive results in MacConkey broth incubated at 44° C. are, with few exceptions, due to faecal strains of *Bact. coli*. To test the validity of this assumption

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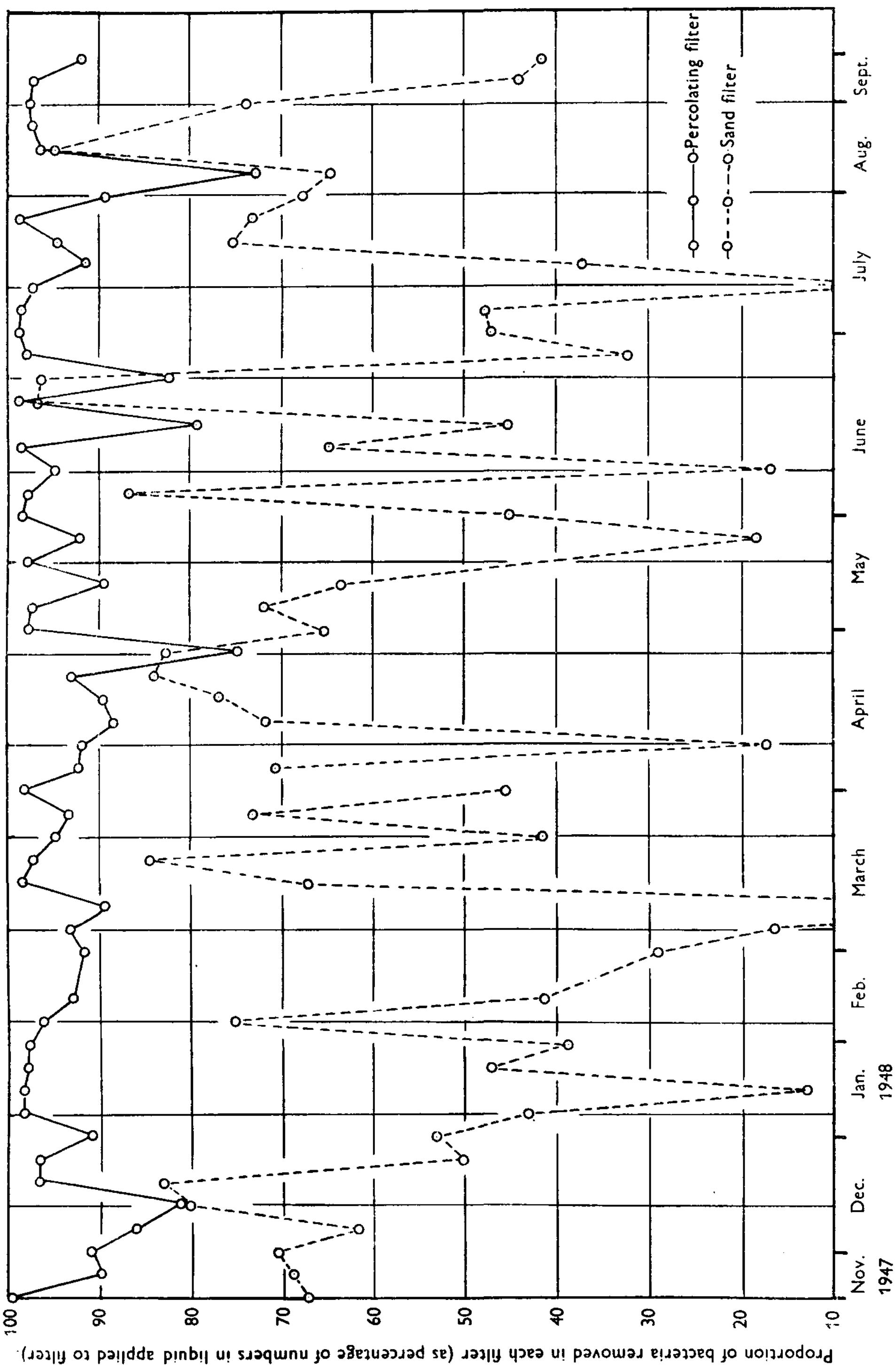

Fig. 4. Percentage of bacteria (plate counts at 20° C.) removed at Sewage Works No. 1: (*a*) from settled sewage by treatment in percolating filters; and (*b*) from percolating filter effluent by treatment in gravity sand filters.

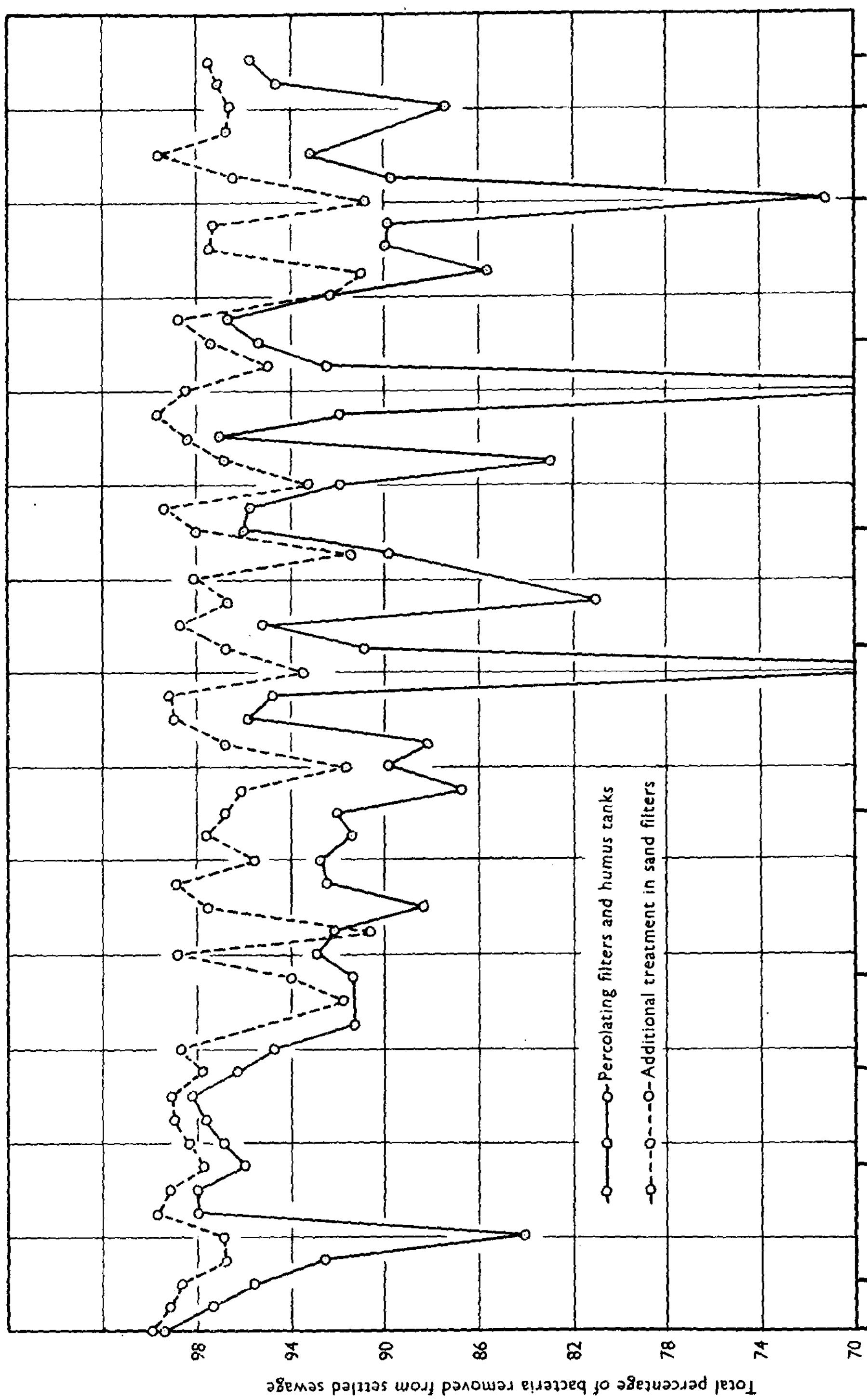

Fig. 5. Total percentage of bacteria (plate counts at 20° C.) removed from settled sewage at Sewage Works No. 1: (*a*) by treatment in percolating filters followed by settlement in humus tanks; and (*b*) by further treatment in gravity sand filters.

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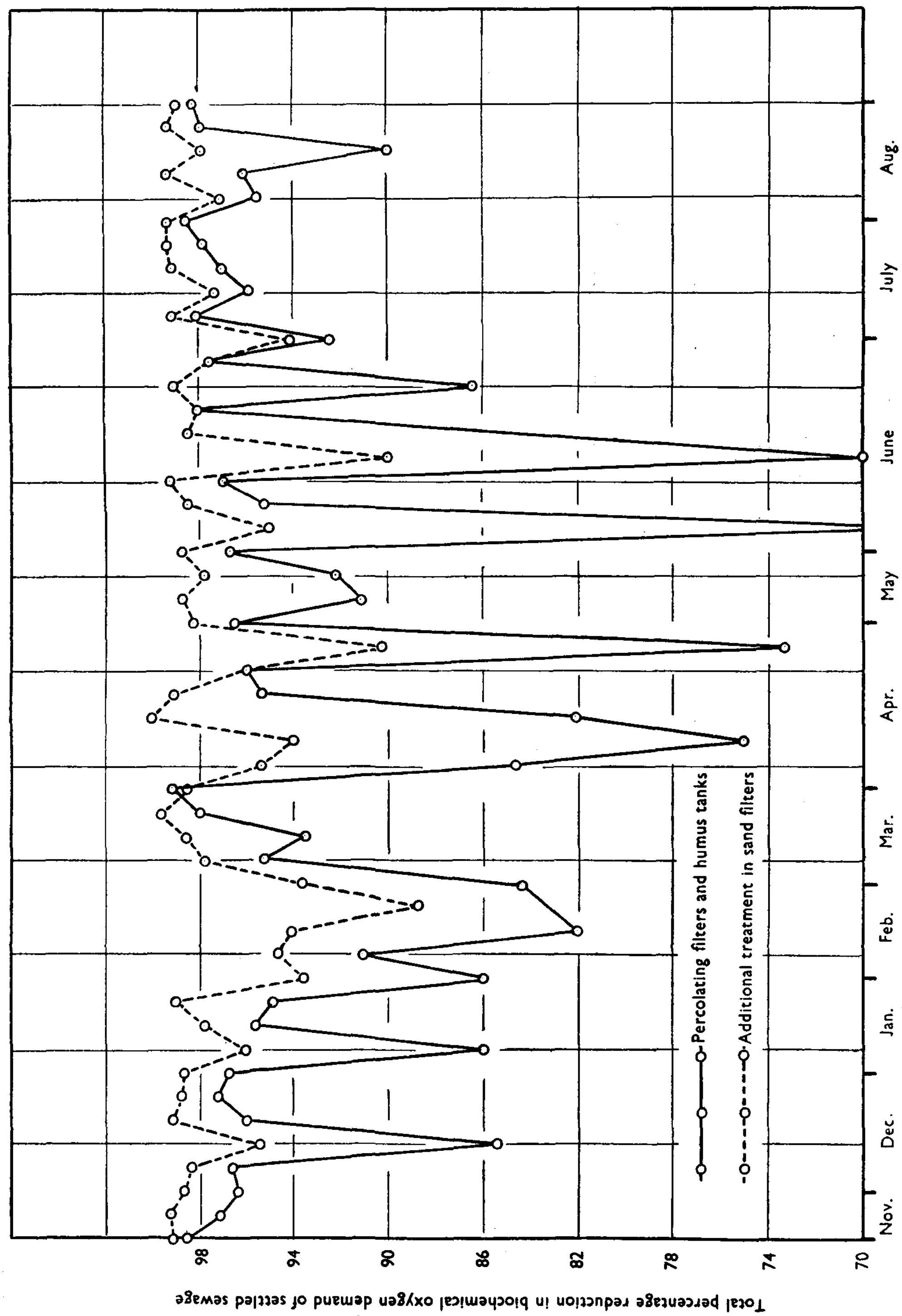

Fig. 6. Total percentage reduction in biochemical oxygen demand of settled sewage at Works No. 1: (*a*) by treatment in percolating filters followed by settlement in humus tanks; and (*b*) by further treatment in gravity sand filters.

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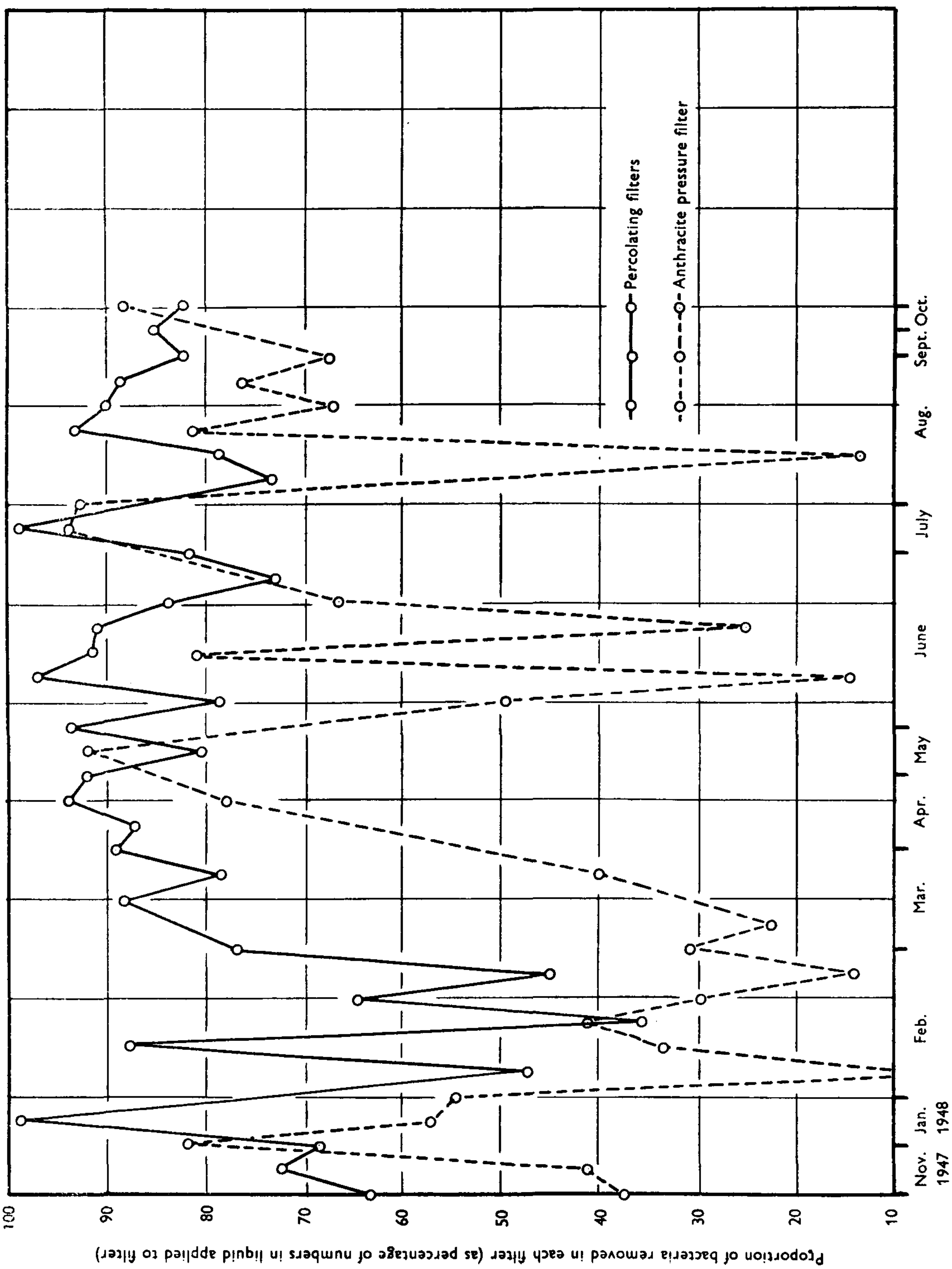

Fig. 7. Percentage of bacteria (plate counts at 20° C.) removed at Sewage Works No. 2: (*a*) from effluent from bio-aeration plant by treatment in percolating filters; and (*b*) from percolating filter effluent by treatment in anthracite pressure filter.

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in the tests made with samples of sewage and sewage effluent, confirmatory tests were run on a number of samples throughout the investigation. Pure cultures were isolated from tubes of MacConkey broth giving positive results in the 44° C. test by streaking on MacConkey agar, incubating at 37°C., and picking off single colonies into peptone water. These cultures were subjected to the indol, methyl red, Voges-Proskauer, and citrate tests. Of 167 pure cultures (97 from Sewage Works No. 1, and 70 from Sewage Works No. 2) 159 proved to be *Bact. coli* type I and five were Irregular type II. The remaining three strains gave anomalous reactions, two strains being negative in both the V.P. and the M.R. tests, and one strain giving the reactions for a 44° C. positive strain of Intermediate type II. It is clear from these results that in this investigation the 44° C. test was almost specific for faecal *Bact. coli* as suggested by the Ministry of Health (1939) for water.

In many of the samples of sewage or of the effluents from the various filters, faecal types were predominant in the coliform flora, so that counts in the 37° and the 44° C. tests were the same. In other samples faecal types of *Bact. coli* formed only a fraction of the total numbers. On the average, for all samples taken at Sewage Works No. 1, faecal strains formed 75 % of the total coliform flora in settled sewage, 77 % in the effluent from a percolating filter, and 76 % in the effluent from the sand filter. For Sewage Works No. 2 the corresponding figures were 65, 63, and 68 % respectively. Evidently the process of treatment at the works did not exert any significant selective action as between faecal and non-faecal types of coliform organisms.

Isolation and typing of strains of Streptococcus faecalis

The specificity of the method used for enumerating *Strep. faecalis* was tested by isolating pure strains from a number of samples throughout the investigation and subjecting these strains to the four 'tolerance tests' devised by Sherman & Stark (1934) and modified by Shattock & Mattick (1943), and Shattock & Hirsch (1947). These tests were (1) growth at 45° C. (tests were conducted in yeastrel dextrose broth), (2) growth in yeastrel dextrose broth containing 6·5 % sodium chloride, (3) ability to survive heating for 30 min. at 60° C., (4) growth in dextrose broth buffered to pH 9·6.

Pure cultures of the streptococci were isolated by streaking positive tubes of the glucose-azide broth used for enumerating faecal streptococci on to

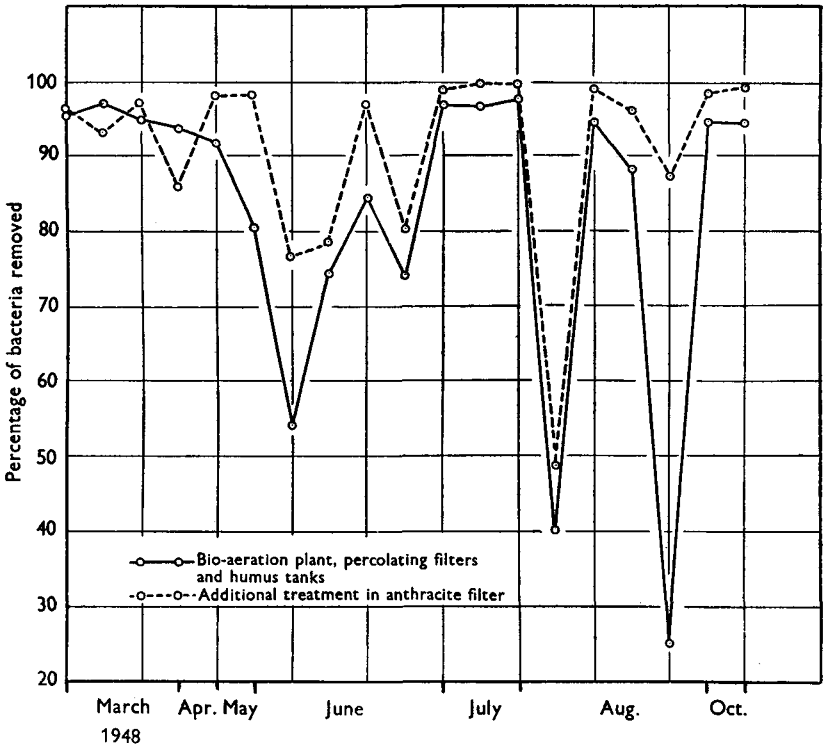

Fig. 8. Total percentage of bacteria (plate counts at 20° C.) removed from settled sewage at Works No. 2: (*a*) by treatment in bio-aeration plant, percolating filters, and humus tanks; and (*b*) by further treatment in anthracite filter.

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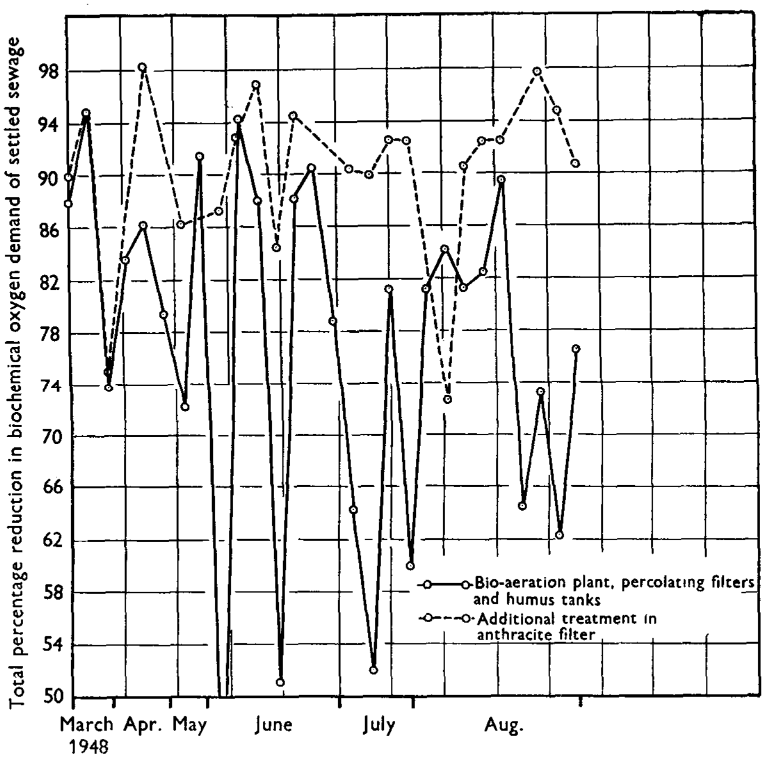

Fig. 9. Total percentage reduction in biochemical oxygen demand of settled sewage at Works No. 2: (*a*) by treatment in bio-aeration plant, percolating filters and humus tanks; and (*b*) by additional treatment in anthracite pressure filter.

Table 6. *Total precentage reduction in bacterial count of settled sewage produced by treatment in percolating filters and humus tanks, and by further treatment in a sand filter or an anthracite filter*

(At Sewage Works No. 2 the settled sewage was given preliminary treatment in a bio-aeration plant. Figures in brackets indicate numbers of samples tested.)

	Sewage Works	Treatment in percolating filters and humus tanks: Plate count	Coli-forms at 37° C.	Coli-forms at 44° C.	*Strep. faecalis*	Additional treatment in sand filter: Plate count	Coli-forms at 37° C.	Coli-forms at 44° C.	*Strep. faecalis*	Additional treatment in anthracite filter: Plate count	Coli-forms at 37° C.	Coli-forms at 44° C.	*Strep. faecalis*
		(52)	(53)	(51)	(43)	(55)	(57)	(55)	(46)				
Minimum	No. 1	57·6	70·0	48·0	82·2	90·6	73·4	73·3	88·0				
Maximum		99·6	99·7	99·7	99·6	99·9	99·9	99·9	99·9				
Mean		91·1	95·6	94·5	94·7	96·8	97·5	97·3	98·1				
		(19)	(29)	(26)	(25)	(15)	(18)	(17)	(16)	(19)	(22)	(21)	(18)
Minimum	No. 2	21·0	43·5	22·2	68·6	45·9	64·3	64·3	77·1	48·6	54·3	64·3	82·9
Maximum		97·8	98·7	98·8	99·9	99·8	99·8	99·9	99·99	99·8	99·8	99·9	99·99
Mean		82·7	81·9	82·6	89·4	89·9	92·4	93·8	94·9	90·7	91·4	92·6	96·5

glucose-azide agar, incubating at 37° C., and picking off the colonies into yeastrel-dextrose broth which was incubated at 45° C. This process was repeated to ensure purity. All strains so isolated proved to be Gram-positive cocci, occurring (in nutrient broth) as diplococci and short chains. None of the strains liquefied gelatin. Of 57 strains tested (36 from Works No. 1 and 21 from Works No. 2), 55 were positive in all four 'tolerance tests'; the remaining two strains were positive in three of the tests, but failed to grow at pH 9·6. Each of the 57 strains was also inoculated into litmus milk. Only two failed to clot the milk and, with the exception of two strains isolated from the same sample which clotted the milk in less than 24 hr., the remaining strains were slow clotters, the period of incubation at 37° C. required to curdle the milk varying from 3 to 30 days with different strains.

SUMMARY AND CONCLUSIONS

Over a period of about 10 months samples were taken at frequent intervals from various stages of treatment at two sewage works; one of these was a small rural works treating only domestic sewage, and the other treated the sewage of an industrial town. These samples were submitted to bacteriological and to chemical examination.

The total plate counts, the counts of *coli-aerogenes* bacteria, and the counts of *Streptococcus faecalis* in settled sewage were higher in the warmer than in the colder months of the year.

Though the average total numbers of bacteria in the sewage containing trade wastes were appreciably higher than that of the domestic sewage the counts of faecal organisms were somewhat lower.

Treatment at the works considerably reduced not only the polluting strength of the sewage but also the numbers of bacteria. At the works receiving sewage from an industrial town an average of about 80 % of bacteria in settled sewage were removed by treatment in the bio-aeration plant, percolating filters, and humus tanks, and a further 8–10 % were removed by subsequent treatment in pressure sand or anthracite filters. At the small rural works, where the rate of treatment was much lower, rather more than 90 % of the bacteria were, on the average, removed by treatment in percolating filters and humus tanks, and a further 6 % by subsequent passage through shallow gravity sand filters.

The final effluents discharged from the works still contained large numbers of bacteria. Their effect on the bacterial content of the streams to which they are discharged may be gauged from the fact that, in the effluent from the small rural sewage works, which was of consistently good quality when judged by chemical criteria, there were on the average about 160,000 cells of faecal *Bacterium coli* and about 20,000 faecal streptococci per 100 ml. Numbers in the effluent from the works in the industrial town were even higher. Sand filters of the type investigated here could therefore not be relied upon to produce an effluent of good bacterial quality.

The 44° C. test in MacConkey broth, prescribed by the Ministry of Health (1939) for examination of water, was found, when applied to samples of sewage or sewage effluent, to be almost specific for *Bacterium coli*, type I. Growth with formation of acid in sodium azide broth incubated at 45° C. was found to be an almost specific test for *Streptococcus faecalis*.

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