

## **Effect of treatment in percolating filters on the numbers of bacteria in sewage in relation to the composition and size of filtering medium**

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### INTRODUCTION

It has been shown in a number of investigations that a progressive reduction in bacterial numbers takes place during the different processes of treatment of sewage at a sewage works. These include the activated-sludge process, single and alternating double filtration, sand filtration, and grass-plot irrigation (Allen, Tomlinson & Norton, 1944; Allen, Brooks & Williams, 1949; Keller, 1959; Kabler, 1959). In percolating filters, in general, the proportion of bacteria removed decreases as the rate of application of sewage to the filters increases. However, owing to wide variations in the composition of the sewage, construction of filters, and methods of operation, it is difficult to evaluate the effect of any one factor on the removal of bacteria.

The present investigation was instituted at the suggestion of the Ministry of Agriculture, Fisheries and Food in relation to prevention of pollution by sewage effluents of estuarine waters in which shell fisheries are situated. The interdependence of the work of sewage purification and that of water supply has also been emphasized by water engineers responsible for supplies drawn from rivers. It is obviously of prime importance to have information on the most economical means of reducing to a minimum pollution due to bacteria of human origin.

The object of the present work was to investigate the effect of different types of filtering media on the reduction in numbers of bacteria in an entirely domestic sewage treated at a constant rate in eight pilot-scale percolating filters containing different types of filtering medium at the Water Pollution Research Laboratory, Stevenage.

### DESCRIPTION OF PERCOLATING FILTERS

The filters, each 8 ft. 7 in. long, 8 ft. 4 in. wide and 6 ft. deep, were arranged in two rows of four. Sewage was supplied from a central trough extending the whole length of the two rows. It was distributed over the surface of the filters through nozzles spaced 6 in. apart in a pair of pipes on carriages travelling up and down the row of filters on either side of the trough and supplied from the trough by means of siphons. Each pipe discharged when travelling in one direction only so that each filter received a dose of sewage at 6 min. intervals at an average daily rate of 100 gallons per cubic yard of filtering medium. The sewage was entirely domestic in character with a biochemical oxygen demand (B.O.D.) of about 300 p.p.m. This

gave a B.O.D. loading of about 0.3 lb. oxygen per cubic yard per day, a rather higher value than that usually employed in designing single filters in Great Britain.

The filters contained four types of medium—clinker, blast-furnace slag, rounded gravel, and rock—in nominal sizes of about 1 in. and 2½ in. The specific surface, defined as the area in square feet of 1 cu.ft. bulk volume of medium, of the eight samples of medium was determined by Schroeffer's (1951) method. The surface area was obtained by comparing the weight of paint retained after dipping a known bulk volume of medium with that retained by materials of known surface area such as bricks and wooden blocks. To eliminate the effect of differences in the absorptive capacities of the various surfaces the samples were dipped twice, allowing the paint to dry after each dipping, before a third dipping, the results of which were used in calculating surface areas. Results for the different media given in Table 1 range from 19.7 sq.ft./cu.ft. for the 2½ in. rounded gravel to 61.5 sq.ft./cu.ft. for the 1 in. clinker. A full description of the filters and their performance is given by Truesdale, Wilkinson, & Jones (1961).

Table 1. *Area in square feet of 1 cu.ft. bulk volume of different types and sizes of filtering medium*

(From Truesdale, Wilkinson & Jones, 1961.)

Medium	1 in. nominal	2½ in. nominal
Slag	59.8	33.0
Clinker	61.5	37.4
Rock	43.3	27.6
Gravel	44.5	19.7

## METHODS

### *Sampling*

Samples of sewage and effluents were collected in sterile 12 oz. glass-stoppered bottles at about 9 a.m. once a week. As far as possible sampling took place on different days of the week from Monday to Thursday in order to take into account variations in the character of the sewage. The sewage had been pumped out of a sewer during the previous morning and had therefore been retained for about 20 hr. in the holding tank before sampling.

Samples of effluent were taken from the outlet of the effluent collecting trough at the base of each filter just before sewage was applied. Two samples of sewage were taken from the trough before and after taking the effluent samples by dipping a clean beaker just below the surface. Samples for analysis were decanted into sterile 5 oz. bottles after 30 min. quiescent settlement.

### *Bacteriological techniques*

The membrane filter technique of Taylor, Burman & Oliver (1955) as modified by Burman (1960 and private communication) was adopted for the total colony and *coli-aerogenes* counts. Suitable series of tenfold dilutions of the sample in ¼-strength Ringer's solution were filtered through 5 cm. Oxoid membranes which were then

placed on no. 17 Whatman filter pads soaked in the appropriate medium in 3 in. glass Petri dishes. These were placed in the incubator in an aluminium tray with a lid to prevent excessive evaporation.

The colony count was made with a medium containing 4% Bacto-peptone and 0.6% Yeastrel in distilled water, pH 7.2, after incubating the membranes for 24 hr. at 30° C.

The *coli-aerogenes* count was made with a medium containing 1% Bacto-peptone, 3% lactose, 5% (v/v) Teepol L (Jameson & Emberley, 1956), 0.5% sodium chloride, 1.2% (v/v) of a 1% alcoholic solution of brom-cresol purple in distilled water, pH 7.4. The membranes were incubated for 4 hr. at 30° C (resuscitation) followed by 20 hr. at 37° C. Yellow colonies only were counted.

Haemocytometer counts were made in a Thoma chamber 0.02 mm. deep with a 4 mm. objective and  $\times 10$  eyepiece.

## RESULTS

All counts have been expressed as number/ml. Owing to the wide variability of the individual counts results were averaged over successive periods of 5 weeks and these moving averages plotted against the date of the first count of each 5-week period; biochemical oxygen demand and turbidity were averaged in the same way.

### *Sewage*

Average total and *coli-aerogenes* counts of the settled sewage, the biochemical oxygen demand, and mean monthly temperature of the sewage, are shown in Fig. 1.

On average the total colony count was about five times that of coliform bacteria. Three peaks occurred in March–April, June–July, and October–November. The *coli-aerogenes* count increased from 100,000 in January 1960 to a maximum of 3 million in May. The numbers decreased during summer and autumn falling to a minimum of 400,000 in December. The increase in numbers of bacteria in settled sewage during the warmer months has been noted on previous occasions and Allen *et al.* (1949) suggested that growth may take place in the sedimentation tank during the warmer months of the year. Table 2 gives the results of storing fresh sewage in completely filled bottles for different periods at 6 and 20° C. Temperature had no significant effect on the total count after storage for 24 hr. With regard to the *coli-aerogenes* count, however, numbers were significantly greater after storage at 20° C. compared with storage at 6° C.

The numbers of bacteria in the sewage do not appear to be correlated to the strength as given by the biochemical oxygen demand or to the appearance as indicated by turbidity (Fig. 5).

### *Effluents*

Table 3 shows that the annual mean reduction in total count of sewage after treatment ranged from 99.2% in the filters containing 1 in. slag and clinker medium to just over 95% in those containing 2½ in. rock and gravel.

Reductions in the *coli-aerogenes* count were even higher. They ranged from 99.7% in filters containing the smaller size of slag and clinker to 97-98% in those containing the larger size of rock and gravel.

Fig. 2 shows, in general, that the settled effluent had a lower total count in

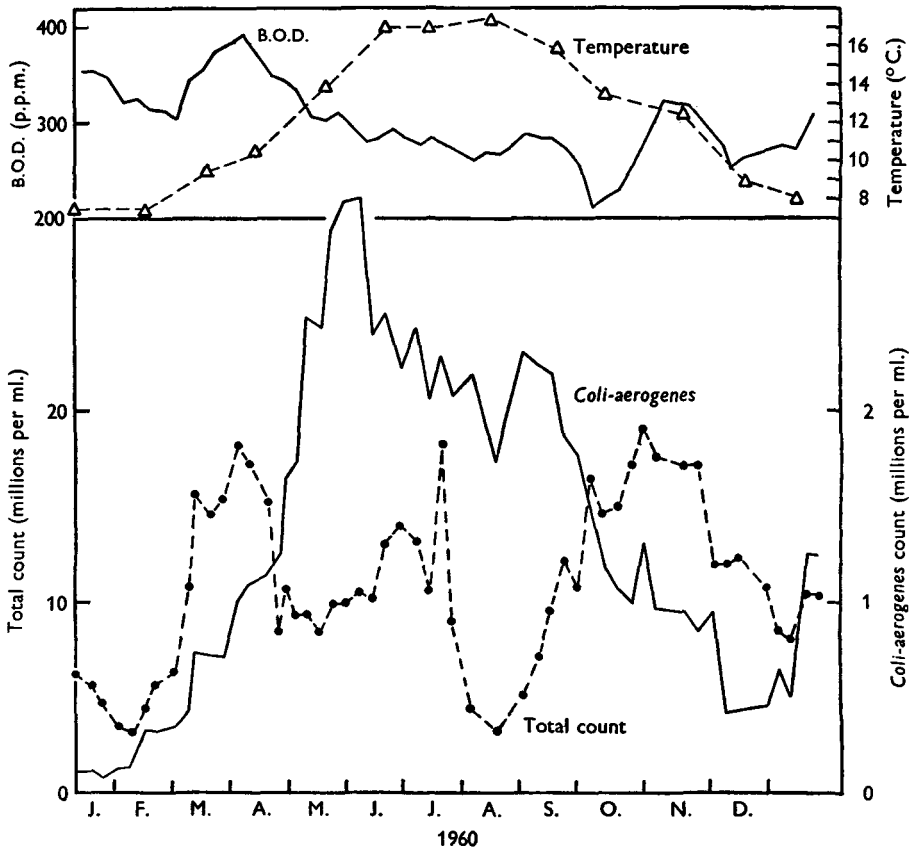


Fig. 1. Variations in numbers of bacteria, biochemical oxygen demand, and temperature of settled sewage.

Table 2. Changes in numbers of bacteria in sewage after storage for different periods at 6°C and 20°C

Period of storage (hr.)	Samples at 6° C.						Samples at 20° C.					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Total count (% of initial count)												
4	106	65	70	43	56	68	100	81	132	74	124	102
7	136	38	64	30	20	58	100	39	134	90	68	86
24	160	27	265	72	125	130	56	16	217	123	135	110
<i>Coli-aerogenes</i> (% of initial count)												
4	76	46	93	31	59	61	93	31	400	47	220	158
7	34	31	130	17	132	69	22	210	900	106	470	342
24	72	51	210	48	700	216	320	210	600	153	820	420

summer than in winter and consequently that the percentage removal was greater during the warmer months. A striking feature is the rise in numbers towards the end of 1960, the increase beginning at different times in the different filters. This rise did not coincide with the rise in biochemical oxygen demand of the effluent which occurred at about the beginning of November in all eight filters. In the filters containing 1 in. medium the increase in total count began in October in the filter containing rock, in December in those containing slag and gravel, and in January 1961 in the filter containing clinker. In the filters containing 2½ in. medium the rise in numbers occurred earlier but the order in which the rise took

Table 3. Annual average numbers of bacteria in, and biochemical oxygen demand of, sewage and effluent from eight percolating filters

Filtering medium	Total count		<i>Coli-aerogenes</i> count		Biochemical oxygen demand	
	Numbers per ml.	% reduction	Numbers per ml.	% reduction	p.p.m.	% reduction
1 in. clinker	93,500	99.2	5,300	99.7	11.3	96.3
slag	94,200	99.2	5,100	99.7	9.7	96.8
rock	254,000	97.8	27,700	98.3	18.1	94.1
gravel	217,000	98.1	8,550	99.5	21.8	92.9
2½ in. clinker	390,000	96.7	25,000	98.5	21.0	93.1
slag	337,000	97.1	27,700	98.3	23.3	92.4
rock	561,000	95.2	36,100	97.8	28.2	90.8
gravel	509,000	95.7	40,600	97.5	30.5	90.0
Sewage	11,700,000	—	1,600,000	—	305	—

Table 4. Recovery of *Serratia indica* added to sewage treated in percolating filters

Date (1960)	...	...	19 January	16 February
Medium	...	...	1 in. gravel	1 in. slag
Number added in the sewage			$2.32 \times 10^{12}$	$1.45 \times 10^{13}$
Number recovered in effluent (%)				
After 2 hr.			0.145	9.6
5 hr.			0.305	10.6
24 hr.			0.995	13.7
144 hr.			1.425	14.0
Number recovered in effluent (%)				
Total count			7.1	9.2
<i>Coli-aerogenes</i> count			13.7	11.5

place was the same as that of the filters containing 1 in. medium. In those containing rock and slag the numbers began to increase in August and in those containing clinker and gravel in October and November respectively.

The *coli-aerogenes* counts of the settled effluent do not show the wide variation characteristic of the sewage since the percentage removal was greater during the warmer months. Most of the filters reacted to the high coliform density in the sewage in May and June by discharging more bacteria in the effluent. Two filters containing 2½ in. slag and rock had peak discharges in August–September cor-

responding to a secondary peak in the *coli-aerogenes* count of the sewage. The rate of discharge of *coli-aerogenes* bacteria from all the filters increased in December 1960 and January 1961.

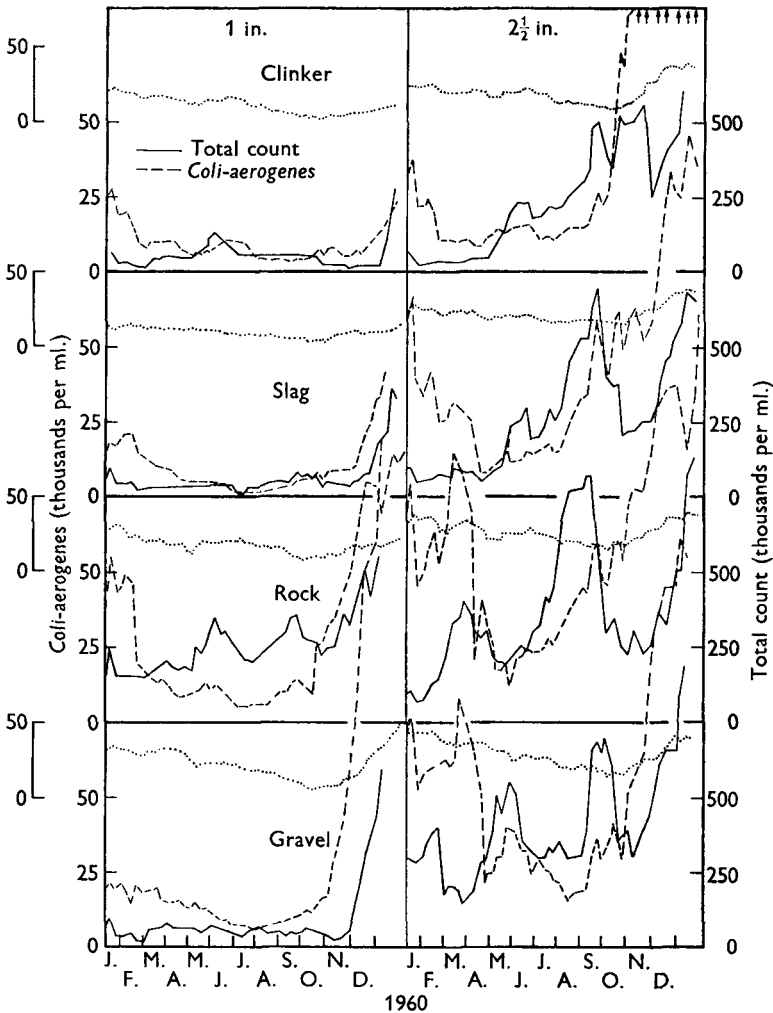


Fig. 2. Variations in numbers of bacteria in, and biochemical oxygen demand of, settled effluent from percolating filters containing different media.

#### *Experiments with an indicator organism*

Although it is convenient to think of the wide difference between the numbers of bacteria in the effluent and sewage in terms of removal, this may not be strictly accurate since active growth of bacteria takes place in a filter and some of the bacteria in the effluent will undoubtedly have originated there.

Some information about the history of bacteria in sewage and in a filter was obtained by adding an indicator organism, *Serratia indica*, to the sewage and following the course of its discharge in the effluent. This organism, a culture of which was obtained from Mrs June Robson of the Atomic Energy Research

Establishment, Harwell, produces magenta colonies and is easily distinguished from other bacteria in the effluent.

In the first experiment, carried out in January 1960, 250 ml. of a broth culture of *S. indica* containing  $2.32 \times 10^{12}$  bacteria was added to the sewage, as a single dose, applied to the filter containing 1 in. gravel medium. Over a period of 6 days about 1.4% of the organisms were recovered in the effluent and about half of these were recovered within 12 hr. (Fig. 3).

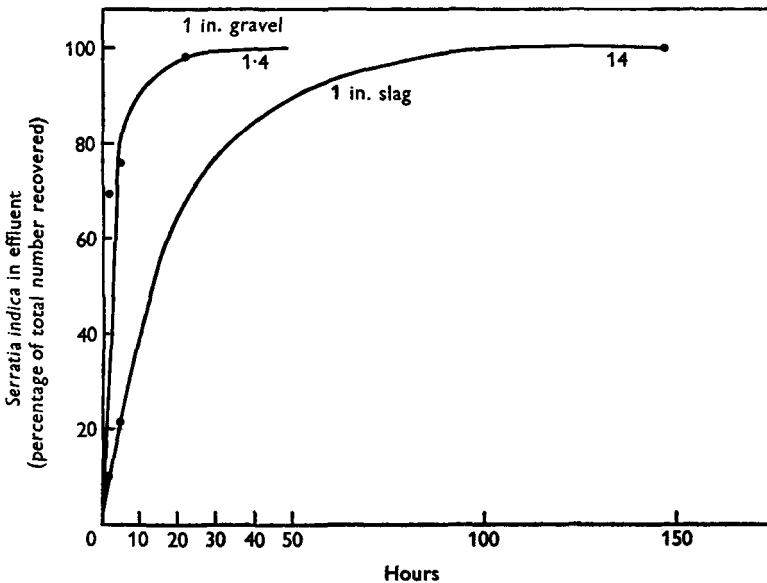


Fig. 3. Recovery of *Serratia indica* in effluent from filters treating sewage to which *S. indica* was added. Total recovery (as % of number added) printed against curves.

Table 5. Types of coliform bacteria isolated from sewage and filter effluent on two occasions

Type	Group 1				Group 2		
	Sewage		Effluent		Sewage	Effluent	
	(%)		(%)		(%)	(%)	
<i>Escherichia coli</i>	Type I	28	66.5	37	80.5	59	63
	Type II	3	7	0	0	1	1
Intermediate	Type I	1	2	1	2	0	0
	Type II	0	0	0	0	3	2
<i>Aerobacter aerogenes</i>	Type I	0	0	2	4.5	1	1
	Type II	2	5	3	6.5	0	1
<i>Aerobacter cloacae</i>		0	0	0	0	0	1
Irregular	Type I	2	5	2	4.5	2	7
	Type II	0	0	0	0	0	0
	Type VI	0	0	0	0	2	2
	Other types	6	14	1	2	32	22
<b>Total</b>		<b>42</b>	<b>100</b>	<b>46</b>	<b>100</b>	<b>100</b>	<b>100</b>

Table 6. *Average haemocytometer counts of particles suspended in sewage and filter effluents and total bacterial counts for the period March–May 1960*

Filter medium	Haemocytometer count	Total count of bacteria	Bacterial count
			Haemocytometer count (%)
1 in. clinker slag rock gravel	$2.0 \times 10^7$	$8.35 \times 10^4$	0.42
	$2.4 \times 10^7$	$10.0 \times 10^4$	0.42
	$1.9 \times 10^7$	$12.9 \times 10^4$	0.68
	$2.3 \times 10^7$	$13.0 \times 10^4$	0.57
$2\frac{1}{2}$ in. clinker slag rock gravel	$1.9 \times 10^7$	$10.0 \times 10^4$	0.54
	$2.6 \times 10^7$	$22.6 \times 10^4$	0.86
	$6.1 \times 10^7$	$61.7 \times 10^4$	1.0
	$7.9 \times 10^7$	$59.7 \times 10^4$	0.76
Sewage	$48.5 \times 10^7$	$133.9 \times 10^4$	2.76

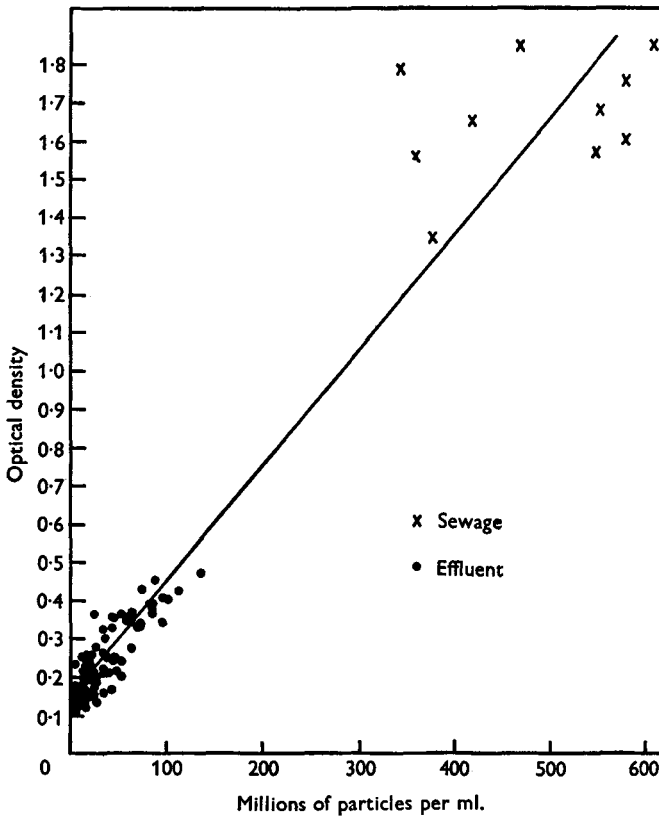


Fig. 4. Relation between concentration of microscopic particles and optical density of sewage and effluents.

In the second experiment in February the filter containing 1 in. slag medium was dosed with  $1.45 \times 10^{13}$  *S. indica*; 14% of the organisms were recovered, 70% of these in the first 2 hr. Table 4 shows that, in the first experiment, the efficiency of removal of *S. indica* was very much<sup>1</sup> higher than the reduction in total or



Table 7. Reduction in turbidity, total count of bacteria and coli-aerogenes count of sewage after treatment in percolating filters (%)

Filter medium	Optical density	Total count	Coli-aerogenes count
1 in. clinker	95.1	99.2	99.7
slag	95.2	99.2	99.7
rock	93.25	97.8	98.3
gravel	91.35	98.1	99.5
2½ in. clinker	92.9	96.7	98.5
slag	92.9	97.1	98.3
rock	88.0	95.2	97.8
gravel	86.1	95.7	97.5

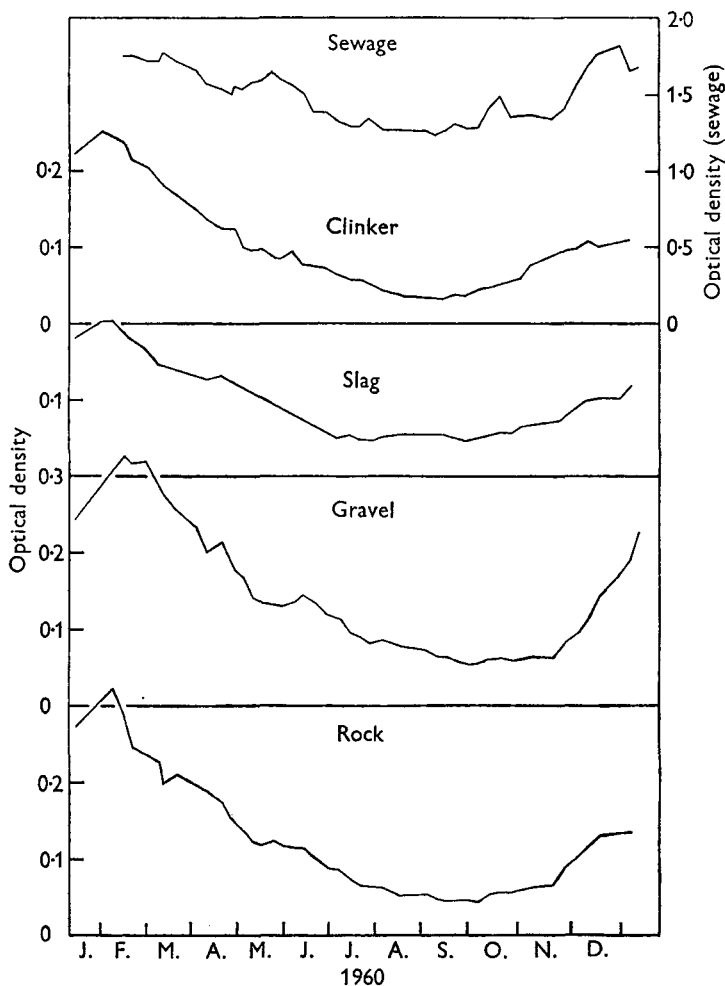


Fig. 5. Variation in turbidity of settled sewage and settled effluents from filters containing 1 in. medium.

*coli-aerogenes* counts, whereas in the second experiment recovery of the three types was of the same order.

The proportion of *S. indica* recovered in the effluent from 1 to 6 days from the start of the experiment was of the order of 0.4%; very few were found after 6 days.

It would appear that of the bacteria removed from sewage in a percolating filter, comparatively few are stored in the filter for longer than a day and subsequently discharged as isolated viable cells. Presumably the bulk of those retained in the filter are incorporated in the film and eventually discharged as 'humus'.

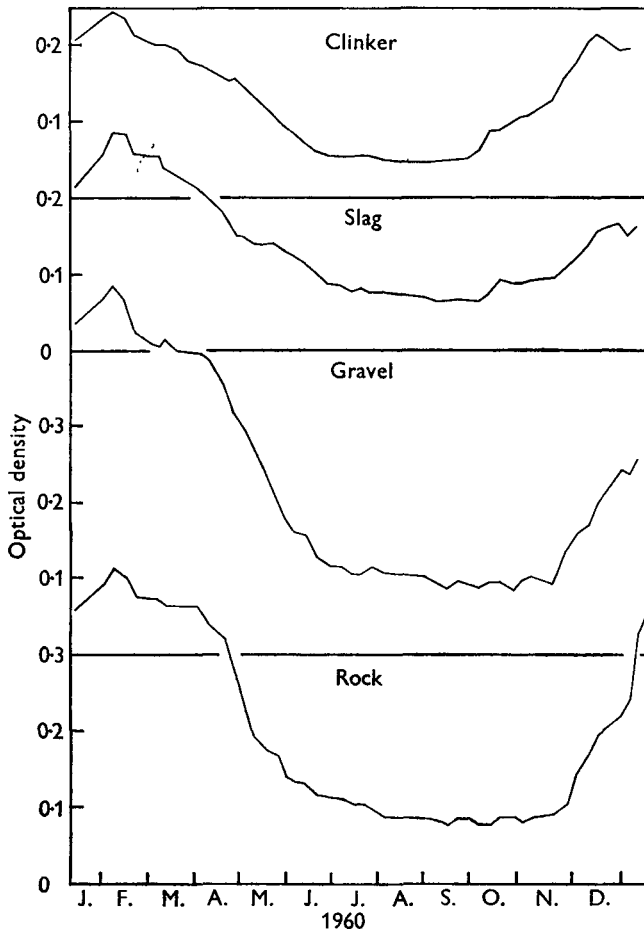


Fig. 6. Variation in turbidity of settled effluents from filters containing  $2\frac{1}{2}$  in. medium.

*Proportion of different types of coliform bacteria in sewage and effluent*

On two occasions colonies of coliform bacteria were picked off and typed according to the classification in the Ministry of Health *Report* no. 71 (1956). Table 5 shows that on the first occasion when forty-two cultures were obtained from sewage and forty-six from effluent, 66.5% of the sewage and 80.5% of the effluent isolates were faecal *Escherichia coli* Type 1. On the second occasion when

100 cultures were obtained from sewage and 100 from effluent, 59 % of those from sewage and 63 % of those from effluent were *E. coli* Type I. These proportions are similar to those found in sewage and filter effluent by Allen *et al.* (1949) and bear out their conclusion that the filter does not have a significant selective action on the different types of coliform organisms.

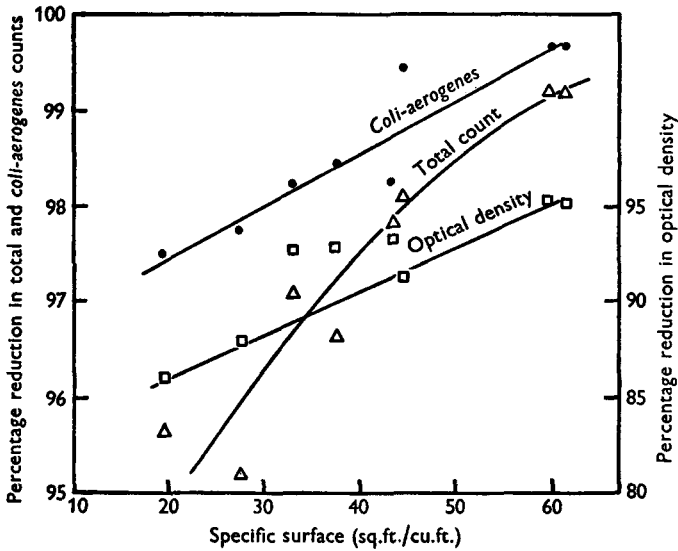


Fig. 7. Removal of bacteria and other particulate matter in relation to specific surface of filter medium.

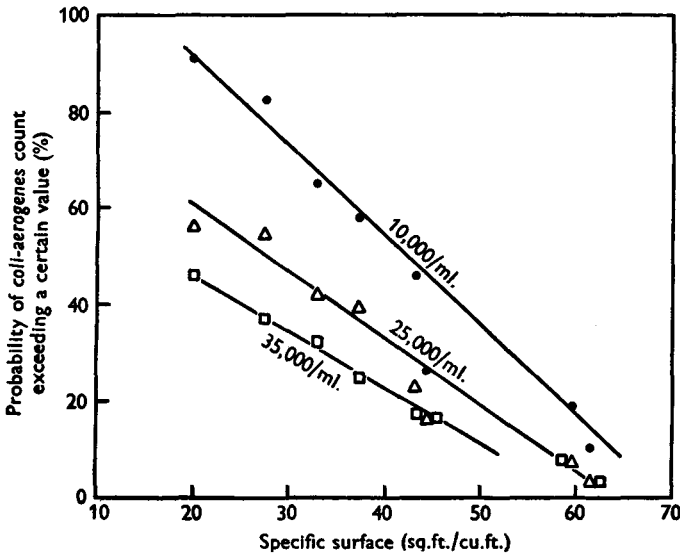


Fig. 8. Probability of obtaining *coli-aerogenes* counts equal to or greater than the number indicated.

*Removal of particulate matter from sewage*

The total viable count of bacteria in sewage and filter effluent represents only a small proportion of the particulate matter in suspension. Average results in Table 6 of haemocytometer counts made from March to May 1960 show that the sewage contained  $4.85 \times 10^8$  particles per ml., whereas the concentration in the effluents ranged from about 2 to  $8 \times 10^7$  per ml. The total viable count of the sewage accounted for 2.76% of the haemocytometer count, whereas those of the effluents ranged from about 0.4 to 1%.

Fig. 4 shows that the optical density of sewage and effluent is correlated to the haemocytometer count, and it may be assumed that the plots of optical density in Figs. 5 and 6 reflect changes in concentration of particles of microscopic size. Taking averages for the whole year, Table 7 shows that the apparent percentage 'removal' of all particles is lower than that of all viable bacteria or coliforms, but with one exception the filters fall in the same order of efficiency. Filters containing 1 in. clinker and slag are the most efficient and those containing  $2\frac{1}{2}$  in. gravel and rock are least efficient. But while the filter containing 1 in. gravel comes third in respect of removal of viable bacteria its efficiency in removing all particulate matter places it sixth.

Fig. 7 shows that, in general, the efficiency of removal of bacteria and other suspended matter increases as the specific surface of the filter medium increases. A linear relation also exists between the efficiency based on chemical criteria and specific surface (Truesdale *et al.* 1961).

Another way of expressing the results is shown in Fig. 8. It may be important to know the probability of a filter discharging more than a certain concentration of coliform bacteria. The individual counts have been used in these calculations and not the moving averages shown in Figs. 1 and 2. It will be seen that the probability of the concentration of coliform bacteria in the effluent exceeding a certain value varies inversely as the total surface area of filtering medium. Within a certain range of concentrations it follows that the probability of the concentration of coliform bacteria exceeding a certain value will decrease as the efficiency of a filter increases.

## SUMMARY

For a period of a year the total count, *coli-aerogenes* count and turbidity were determined on samples of sewage and effluent taken every week from pilot-scale percolating filters treating domestic sewage. The eight filters contained four types of filtering medium in two sizes.

Numbers of coliform bacteria in the settled sewage were much higher during the warmer months but the total count showed no regular seasonal variation. Treatment in all the filters reduced the numbers of bacteria in both groups by over 95%. In general the reductions in numbers of bacteria and in numbers of all particles in suspension were proportional to the total area of filtering medium with which the sewage came into contact. The probability of discharging more than 10,000 *coli-aerogenes* per ml. ranged from 10% in the most efficient to 90% in the least efficient filter.

Experiments in which *Serratia indica* was added to the influent sewage showed that only 0.4% were recovered in the settled effluent after 24 hr. Treatment in the filters had little selective action on the different types of coliform bacteria.

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