

**Report to the corporation of Belfast on a bacterial investigation of the experimental contact beds for the treatment of sewage / [J. Lorrain Smith].**

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# REPORT TO THE CORPORATION OF BELFAST ON A BACTERIOLOGICAL INVESTIGATION OF THE EXPERIMENTAL CONTACT BEDS FOR THE TREATMENT OF SEWAGE.

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## THE NATURE OF THE SAMPLES.

The material of which the beds\* are composed consists in fragments of brick or coke, measuring from  $\frac{1}{2}$  to 3 inches in diameter. In the first series of nine observations, the samples were of sewage which had been in contact with bricks only. In the second series, the upper of the two beds was composed of coke and the lower of brick.

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\*The beds are fully described in Mr. Bretland's Report, May, 1900.

The sewage, after it had been screened and sedimented, was passed into the upper bed, and allowed to remain there for a period of three hours. It was then passed into the lower bed, and left for a similar period. We have therefore to deal with it at four different stages. (1) Sewage as it passes from the main sewer into the tank where sedimentation takes place. The samples taken at this stage naturally vary in their composition, and in many ways are not comparable with each other, or with the samples which are taken at the subsequent stages. (2) Sewage after it has been in the sedimenting tank for a period of 6-12 hours. The sample of this was taken as it passed over into the upper bed, and could therefore be compared strictly with (3), the sewage as it passed into the lower bed after it had been in contact with the bricks of the upper bed for three hours. Finally, (4) a sample was taken of the sewage as it was leaving the lower bed to be poured into the Lough. Samples 3 and 4 are called respectively Effluent I. and II.



It is to be noted therefore that while the samples of crude sewage in the main sewer may differ from the three later samples, these three are strictly comparable with each other. Some hours elapsed (12-24) before the samples were available for analysis.

#### GENERAL NATURE OF THE PROCESS OF PURIFICATION.

The organic matter which is present in the sewage is the substance towards the removal of which the various processes used in the purification of sewage are directed. This matter is the natural food of bacteria, or small animal organisms, and the effect of bacteria upon it leads to the ultimate transformation of it into chemical substances which no longer serve the purpose of a pabulum. This process in nature proceeds slowly, and the claim which is advanced in favour of contact beds, as a method of sewage purification, is that in them a rapid exhaustion of the pabulum takes place. The stages of exhaustion of this food supply are stages in the process of purification, at the perfection of which sewage treatment aims.

#### QUESTION TO BE ANSWERED BY BACTERIOLOGICAL ANALYSIS.

The practical problem takes this form:—"Is there any biological evidence that the contact beds produce exhaustion of the pabulum, and if this occurs, does it depend on bacteria or other organisms, or on both?"

The beds contain not only bacteria, but vegetable and animal organisms, such as fungi, infusoria, worms, and insects. These are present in enormous numbers. The study of these organisms may be called the biological investigation of sewage treatment, and this method has the advantage of being a direct study of the process which gives rise to the chemical changes in sewage purification. It has limitations, however, in the fact that, as regards microbes, for example, it is impossible to grow many of the forms by any known method of cultivation. The results of enumeration of microbes are therefore an index of the amount of bacterial growth rather than an absolute measure. The chief fact which can be made out is the ratio of the numbers observed at the successive stages in the sewage treatment. This we will see is the important matter in the interpretation of the process so far as bacteria are concerned.

## METHODS AND RESULTS.

The bacteria were enumerated by means of gelatine plates in the recognised manner. They were incubated at a temperature of 20°C., and the colonies were counted after 2-3 days' growth. The rule was followed of using, as far as possible, only those plates which contained not more than 20-30 colonies.

In the first of the following tables there are given nine observations on samples from the brick beds. The period of observation extends from January to April of this year. In a separate table I have placed the observations on three samples from the second set of beds, the upper of which was coke and the lower brick. These samples were taken during May and June.

The plates used for enumeration contained in all cases  $\frac{1}{1000000}$ th and  $\frac{1}{10000000}$ th of one c.c. respectively. Three plates were made of each dilution.

TABLE No. I.

Showing total number of bacteria per c.c. present in nine samples from brick beds :—

	Crude Sewage.	Screened and Sedimented.	Effluent from Upper Bed.	Effluent from Lower Bed.	Date of Sample.
1	2,500,000	6,600,000	7,600,000	6,500,000	Jan. 6
2	15,700,000	15,000,000	38,000,000	11,000,000	„ 20
3	5,250,000	13,100,000	5,750,000	3,400,000	„ 29
4	3,700,000	13,000,000	41,000,000	19,300,000	Feb. 5
5	2,150,000	186,000,000	143,000,000	87,000,000	„ 12
6	6,500,000	12,600,000	9,300,000	6,800,000	„ 19
7	3,300,000	2,000,000	4,300,000	1,600,000	Mar. 3
8	30,600,000	30,300,000	14,300,000	7,300,000	„ 10
9	27,600,000	112,300,000	35,300,000	19,600,000	„ 22
(A.) Average of first nine = brick beds :	10,811,111	43,433,333	33,172,222	18,055,555	



We may discuss Table No. I. in the first instance. The general conclusion which is evident is that the numbers of bacteria come out in a perfectly regular order. In the crude sewage the numbers are low on the average, and in this series it is only the average results which need be taken into account. In the screened and sedimented sewage the numbers are very considerably higher—on the average four times as high. The numbers in Effluent I. are slightly lower than those of the screened and sedimented sewage; while those of Effluent II. are less than half those present in the screened and sedimented. To this rule there is no exception. The effect of the contact is seen in each case in the reduction of the numbers of bacteria. Considering the amount of pabulum and the high temperature of the beds, it appears at first sight very remarkable that in such favourable conditions for multiplication of bacteria their numbers should have been reduced.

TABLE No. II.

Showing the total number of bacteria per c.c. present in three samples from coke beds:—

	Crude Sewage.	Screened and Sedimented.	Effluent from Upper Bed.	Effluent from Lower Bed.	Date of Sample.
10	168,000,000	120,000,000	103,000,000	117,000,000	May 17
11	53,000,000	125,000,000	75,000,000	79,600,000	„ 31
12	uncountable	70,000,000	41,000,000	40,000,000	June 7
Average of last	—————	—————	—————	—————	
(B.) three = coke					
beds =	110,500,000	105,000,000	73,000,000	78,866,666	
(C.) General aver-					
age—	28,936,363	58,825,000	43,129,166	33,258,333	

The numbers of bacteria follow the same rule in the observations on the combination of coke and brick beds; but much less clearly. The effect of the beds is to reduce the numbers, but this does not occur to such an extent nor with the same regularity as in the brick bed series. The total numbers also are very much higher than in the former series. This increase may be partly due to the fact that the samples were taken in May and June, when naturally bacterial growth was much more active than during the earlier months of the year. It might, however, be due to a lack of efficiency on the part of the combination of coke and brick

beds as compared with that of two brick beds. That this is so is proved by the results of the chemical analysis given in Professor Letts' report, which show that here the purification is, according to all the three standards applied, less complete than that of the brick beds. As regards the decrease in bacteria, we have here a fall of 26 per cent. as compared with 58 per cent. in the brick beds. The number of samples, however, is too small to give any decisive conclusion. This result, however, so far as it goes, points to the fact that the agency which brings about purification leads to a fall in the number of bacteria. As yet no standard has been agreed upon according to which the efficiency of the purification may be estimated by the effect of the contact in causing this fall. Nor has it ever been explained why with efficiency of purification there should be a fall in the number of bacteria. It might even seem desirable, as Dr. Houston suggests, that "an effluent partially purified should carry with it the bacteria which have been engaged in the work of purification, but experience has shown that the bacterial beds which yield the best chemical results yield also the best results bacteriologically."

I will later describe a prolonged series of experiments which I have carried out in the present investigation with a view to discovering the meaning of this anomaly. In the meantime it is of great interest to compare the results of the above tables with those of two other observers.

From one of the tables given by Dr. A. C. Houston in his Report to the London County Council (May 24th, 1900) I have compiled the following average:—

Crude Sewage.	Effluent from Primary Coarse Bed of Coke. SERIES A.	Effluent from Secondary Coarse Bed of Coke. SERIES A.	Effluent from Primary Coarse Bed of Coke. SERIES B.	Effluent from Secondary Fine Bed of Coke. SERIES B.
7,096,000	2,080,000	1,910,000	2,700,000	1,440,000

In some of Dr. Houston's other tables the results show a much less marked effect on the numbers of bacteria. For the purpose of comparison, however, I prefer to take the most successful amongst his series of experiments.



In Professor Boyce's Report on the action of the contact beds at West Derby, he gives the following average :—

Number of Bacteria in Crude Sewage. (Average of 29 Analyses.)	Number of Bacteria in the Effluent from the Final Contact Beds. (Average of 36 Analyses.)
1,090,726	614,158

The reduction in the numbers of bacteria in Dr. Houston's table is still more striking than that observed in the table I have given. The lowest figure which he obtained was a fall from 7,000,000 to 400,000, a decrease of 94 per cent. of the total number. The largest reduction in my table is that of March 22nd, when the numbers fell from 112 millions to 19·6 millions per c.c., implying a loss of over 80 per cent. In Professor Boyce's observations the fall on the average is 44 per cent. The total number of microbes in the Liverpool sewage is approximately 1 million, in the London sewage 7 millions, and in the Belfast sewage, after sedimentation, 43 millions.

The average fall in Houston's series implies a loss of about 80 per cent. of the total bacteria, and at the period corresponding to these observations the percentage purification as estimated by the oxygen absorbed test was on the average 85 per cent. (Clowes). In my series in Table I. the fall in bacteria implies a loss of nearly 60 per cent., and for this period the percentage purification according to the oxygen absorbed test was 69 per cent. (Letts).

There is not yet, however, a sufficient number of results published to show decisively in how far the reduction in bacteria may be regarded as a measure of the efficiency in purification by the contact beds. The results I have given show how the brick beds now examined compare with the coke beds examined for the London County Council, and the coke beds of the Liverpool Corporation, in regard to this point.

It is clear that to arrive at a true conception of the process which brings about purification, it is necessary to consider how far the decrease of bacteria, observed as a result of the contact, is due to the multiplication of bacteria themselves, and their demands for food supply. This might conceivably lead to an exhaustion of the pabulum, and subsequent decrease

in their numbers. That this hypothesis is problematical seems evident from the fact that in the second series of observations on the coke and brick beds the exhaustion of pabulum was less marked, while the bacteria consuming it were more numerous than in the samples in the first series. If bacteria are the sole agents which bring about the chemical changes in the sewage, one might expect that the greater the number of bacteria the more advanced would be the changes in which purification consists. The questions which arise out of this difficulty regarding rate of growth and rate of exhaustion of pabulum could be answered only by direct observations on the subject, and this I proceeded to carry out in the following way.

To a flask (A) containing 90 c.c. of sterile distilled water I added 10 c.c. of a broth culture of *bacillus coli communis* in active growth. From flask (A) 10 c.c. of the mixture were added to 90 c.c. of sterile distilled water in flask (B), and so on, forming a series in which each succeeding flask had the broth diluted to  $\frac{1}{10}$  of the strength of the flask before it. Thus A contained in each c.c.  $\frac{1}{10}$  of a c.c. of broth, B contained  $\frac{1}{100}$ , C  $\frac{1}{1000}$ , D  $\frac{1}{10000}$ , E  $\frac{1}{100000}$ , and F  $\frac{1}{1000000}$ .

Regular observations of the numbers present in these six flasks were made. The *bacillus coli communis* was selected because it is one of the commonest bacilli in sewage. It shows its power of disintegrating albuminous substances by breaking them up into indol. It is further also very easily cultivated. The plates were made with agar incubated at 37°C., and enumerated after 12-24 hours' growth. Four plates were planted for each enumeration. In the following table are given the results of these observations:—



TABLE III.

Showing the rate of growth of *b. coli* comm. in relation to the food supply present. The Nos. represent the No. of colonies per c.c.

	FLASK A	B	C	D	E	F
Jan. 16	9,100,000	910,000	91,000	9,100	910	91
„ 17	114,000,000	52,400,000	25,000	1,200	1,350	23
„ 18	120,250,000	209,000,000	22,930,000	20,700	30,500	64
„ 19	259,000,000	219,000,000	20,800,000	1,400,000	2,300,000	205
„ 20	430,000,000	230,000,000	40,000,000	10,600,000	1,800,000	4,400
„ 21	340,000,000	250,000,000	40,000,000	13,200,000	11,300,000	192,000
„ 22	595,000,000	240,000,000	85,000,000	20,000,000	26,000,000	3,100,000
„ 23	610,000,000	310,000,000	140,000,000	19,000,000	21,500,000	7,500,000
„ 24	480,000,000	490,000,000	380,000,000	50,000,000	38,000,000	19,000,000
„ 25	520,000,000	204,000,000	65,000,000	65,000,000	85,000,000	11,000,000
„ 26	542,000,000	2,400,000,000	45,000,000	96,000,000	80,000,000	11,500,000
„ 27	1,850,000,000	1,050,000,000	115,000,000	25,000,000	30,000,000	12,000,000
„ 28	...	1,500,000,000	450,000,000	30,000,000	55,000,000	35,000,000
Feb. 1	1,750,000,000	245,000,000	24,000,000	...	65,000,000	16,500,000
„ 2	4,700,000,000	1,100,000,000	65,000,000	25,000,000	35,000,000	20,000,000
„ 7	...	2,100,000,000	85,000,000	100,000,000	130,000,000	60,000,000
„ 16	5,600,000,000	1,150,000,000	135,000,000	60,000,000	40,000,000	35,000,000
„ 28	5,250,000,000	3,500,000,000	80,000,000	80,000,000	40,000,000	15,000,000
Mar. 9	2,700,000,000	250,000,000	85,000,000	50,000,000	8,000,000	too dilute
„ 23	5,400,000,000	290,000,000	63,000,000	6,500,000	1,500,000	too dilute

The experiment prolonged itself beyond all expectation, and even after two months there was little sign of exhaustion.

From this series of experiments it is clear that as regards exhaustion of a pabulum such as that present in meat broth, bacteria of the bacillus coli group have a very slight power. It is also clear that the reduction in the number of ordinary bacteria, seen after short periods of contact, must depend on other factors than their power of exhausting the pabulum. Manifestly also when the numbers reach a certain point variation is somewhat slowly brought about.

It is unnecessary to say that it would be desirable to continue this method of study of the rate of growth with other forms of bacteria, but the time and labour involved in this six-fold experiment were, as it came about, no small matter.

The conclusion was farther supported by a series of direct observations by chemical methods on the exhaustion of food by the bacteria of the sewage. As pointed out, the exhaustion of the food consists in the transformation or dispersion of the organic nitrogen. The food nitrogen exists in the form of unoxidized nitrogen, and the disappearance of this from a fluid medium in which bacteria are growing can easily be ascertained by the application of Kjeldahl's method.

In the first series of experiments I inoculated a broth diluted to  $\frac{1}{10}$ , the normal strength—i.e., containing on the average about 20 parts of nitrogen per 100,000—with a loopful of sewage, and left it to grow at room temperature for five days. Even after this long period the unoxidized nitrogen had never decreased below 86 per cent. of the original. It was frequently about 95 per cent., and often there was no appreciable loss. In the chemical section of this investigation, which is given in Professor Letts' Report, it is shown that in the contact beds the unoxidized nitrogen of the sewage is reduced to 5.7 per cent. of the original.

The experiments were carried out in a variety of ways. I inoculated the broth with a loopful of sewage, or made a mixture of equal parts of sewage and broth. The following are the details of some of the experiments:—



Experiment on the Disappearance of Unoxidized Nitrogen from 20 c.c.  
Broth Inoculated by a Loopful of Sewage Grown 5 days.

(1.) Inoculated from Crude Sewage	...	...	{ 6.9 per cent. loss 11.4 "
(2.) Inoculated from Screened and Sedimented	...	...	{ 9.6 " 8.6 "
(3.) Inoculated from Effluent I.	...	...	{ 5.5 " 5.8 "
(4.) Inoculated from Effluent II.	...	...	{ 10.3 " 9.4 "

Average loss of nitrogen 8.4 per cent.

In this experiment 20 c.c. of broth contained .04 grammes of unoxidized nitrogen.

The same experiment was repeated with broth of a similar composition, and a parallel series with broth diluted ten times with distilled water. The results were similar. The average loss in nitrogen was 12.2 per cent. after five days. This was the highest value reached in any experiment in this series. The results, however, were sometimes entirely negative, as may be seen from the following experiment:—

A mixture of broth and sewage in equal parts was kept for five days. The value in unoxidized nitrogen of 20 c.c. of the mixture was determined at the beginning of the experiment by double analysis. The results are expressed in c.c. of  $\frac{1}{10}$  normal ammonia, and were the following:—

		c.c. of $\frac{1}{10}$ $\text{NH}_3$	after 5 days' incubation	c.c. of $\frac{1}{10}$ $\text{NH}_3$
Crude Sewage	... 20 c.c. =	13.88	=	13.1
Screened and Settled	20 c.c. =	12.83	" "	= 12.9
Effluent I.	... 20 c.c. =	13.10	" "	= 13.0
Effluent II.	... 20 c.c. =	12.77	" "	= 12.0

It is to be noted here that there is no loss except in the case of the last, where a loss of about 5 per cent. has occurred.

This experiment was repeated with practically the same result. The conclusion was therefore that, though undoubtedly a certain amount of unoxidized nitrogen may disappear as a result of bacterial growth, after periods lasting five days, the highest average is not more than 12 per cent.

In view of the partly negative character of these results, I repeated the experiment in another form. I brought the broth into contact with brick taken from the brick beds, and with suitable specimens, *i.e.*, those from the lower layer of the beds, I found a very marked disappearance of the unoxidised nitrogen from the dilute broth.

The following experiment shows the kind of result which was obtained. The broth was again diluted with distilled water, so as to contain about 20 parts of unoxidised nitrogen per 100,000. It is important to note in connection with these observations on the disappearance of unoxidised nitrogen, that the chemical examination of the process as it takes place in the contact beds shows that the disappearance of nitrogen in the unoxidized form is not associated with its reappearance in the effluent in the form of oxides. (For the details see Prof. Letts' Report.)

TABLE IV.—Result of experiments to determine the loss of unoxidised nitrogen in dilute broth when in contact with the brick beds. Unoxidized nitrogen=20 parts per 100,000. Loss expressed in per centages of the original amount.

	In 22 hours.	46 hours	70 hours.
Upper bed, 6 inches deep	20%	23.7%	32.1%
„ 18 „	26.7%	34.8%	30.1% Some sediment.
Lower bed, 6 „	25%	28.2%	32.1%
„ 18 „	54.5%	63.2%	71.2%

Recharged after 4 days' rest

Upper bed 6 inches deep	19.1%	25%	23%
„ 18 „	24.72%	22%	27%
Lower bed 6 „	21.2%	14%	— Sediment.
„ 18 „	38.9%	56%	53%

The experiment was repeated several times, with the uniform result that a marked disappearance of unoxidised nitrogen from the solution took place.



The results of the chemical analysis of the sewage by Professor Letts are as follows.—

Percentage Purification as shown by Kjeldahl's method.

Highest result after 2nd contact	..	77% of Purification.
Average of all experiments	...	57% „
Brick beds      ...      ...	...	60% „
Coke beds      ...      ...	...	50% „

This set of results shows that we can in a measure locate the agency which has the strongest effect in causing the disappearance of unoxidised nitrogen. It is especially associated with the bricks of the deeper part of the lower bed. The agency, however, is here merely a degree or two more efficient than that associated with the other bricks. In each case there is a remarkable disappearance of unoxidised nitrogen. The only point about the more efficient bricks which I observed was that they were more richly covered with sediment consisting of the vegetable and animal organisms already referred to. In the vessels in which the bricks were placed for the purpose of testing their action on the dilute broth, a copious layer of sediment, similar to that on the bricks, formed at the bottom. This when analysed (Kjeldahl) showed large quantities of nitrogen in the unoxidised form. The following experiment gives the details of one such observation.

The broth after having been in contact with the bricks for six days was taken for analysis in two portions. One portion was filtered, and 50 c.c. of the filtrate were put in each of two flasks and analysed for nitrogen. The filtrate was found to contain only 35 per cent. of the unoxidized nitrogen of the original solution, or, to keep to our former method of expression, there had been a loss of 65 per cent. of the original unoxidized nitrogen. On the other hand, portions of the broth which were not filtered, and which purposely were made to contain a large amount of sediment yielded unoxidized nitrogen per c.c. amounting to more than 150 per cent. of that in the original diluted broth.

On these grounds, therefore, it seems clear that in the plant and animal life associated with the surface deposit on the bricks there is an agency which has the most striking power in causing the disappearance of unoxidized nitrogen from solution; whereas in the former experiments,

when dealing with inoculation of the broth with sewage bacteria, only 12 per cent. of the nitrogen disappeared, in contact with the bricks the broth shows a loss of a great proportion of its nitrogen, sometimes amounting to 70 per cent. The bacteria alone have at the best a moderate and more slowly acting power of dispersing the food nitrogen. The vegetable and animal organisms which occur along with the bacteria absorb organic matter directly into their own bodies, and also devour multitudes of bacteria. That this is the most reasonable way of explaining the disappearance of the bacteria in the sewage passing through the contact beds seems most probable when we consider the capacity for bacterial growth which the various samples of the sewage actually possess. To observe this it is necessary only to keep the samples at a favourable temperature, and make subsequent enumerations of the bacteria to be found in them. The following table gives the details of three observations of this kind. The flasks of sewage were kept at room temperature, i.e., about 20°C., sealed with sterilised cotton wool. The colonies were enumerated as before by means of gelatine plates.

TABLE No. V.

Showing total number of bacteria per c.c. growing in kept samples of Sewage.

## SAMPLE OF MAY 17TH.

		Crude Sewage.	Screened and Sedimented.	Effluent I.	Effluent II.
1. May 18	...	168,000,000	120,000,000	103,000,000	117,000,000
2. „ 20	...	uncountable	uncountable	uncountable	uncountable
3. „ 22	...	uncountable	uncountable	uncountable	uncountable
4. „ 25	...	uncountable	uncountable	3,500,000,000	1,250,000,000
5. „ 27	...	2,800,000,000	uncountable	500,000,000	650,000,000

## SAMPLE OF MAY 31ST.

1. June 1	...	53,000,000	125,000,000	75,000,000	79,600,000
2. „ 3	...	uncountable	uncountable	uncountable	uncountable
3. „ 5	...	uncountable	uncountable	uncountable	uncountable
4. „ 7	...	uncountable	uncountable	uncountable	uncountable



TABLE No. V. (Continued.)

## SAMPLE OF JUNE 7TH.

				Crude Sewage.	Screened and Sedimented.	Effluent. I.	Effluent II.
1.	June	8	..	uncountable	70,000 000	41,000,000	40,000,000
2.	"	10	...	uncountable	uncountable	uncountable	uncountable
3.	"	13	...	uncountable	uncountable	uncountable	uncountable
4.	"	15	...	uncountable	uncountable	uncountable	uncountable
5.	"	17	...	uncountable	3,500,000,000	2,500,000,000	1,400,000,000
6.	"	19	...	1,300,000,000	spoiled	200,000,000	100,000,000
7.	"	24	...	500,000,000	1,800,000,000	5,000,000	8,000,000

By the phrase "uncountable" I mean that when the sewage had been diluted 100 million times 1 c.c. of the liquid gave a growth of colonies too close to be enumerated with accuracy. It seemed to me to serve little purpose to continue the dilution to higher stages. The general conclusion is perfectly clear that the capacity for sustaining bacterial growth is in no way represented at its maximum by the numbers which are observed in the fresh samples of sewage. This series of observations further confirms the experiments on the power of bacteria alone in exhausting the food pabulum. Apparently in the case of sewage kept in this manner, a period of a week to a fortnight must pass before the number of bacteria show a sensible reduction, and the decrease is seen more especially in the samples of the effluents.

The agency, therefore, in the contact beds which brings about a reduction in the number of bacteria is largely something quite different from any general power of mutual destruction among bacteria, such as might arise from their overgrowth, or from their exhaustion of the food supply.

The question might be raised, "Are these changes not due to the growth of a special bacterium which cannot be cultivated on artificial media, and whose growth is such as to prevent the growth of other microbes?" This complex hypothesis suffers from the fact that it cannot

be tested. It would seem remarkable that the growth of a bacterium in the beds should suddenly not only arrest the increase of the other bacteria already in the sewage, but should actually cause a diminution to a very marked extent in their numbers. A further remarkable fact is that the same samples of sewage effluent in which we suppose this form of diminution has occurred, should, if kept in a flask in the laboratory, soon show unnumbered millions of bacteria per c.c. of the cultivable kind. It seems more natural to conclude that we have to deal with some agency other than bacteria which has the power of consuming them, and which has its habitat on the surface of the brick or coke fragments. The fact that the power of consuming nitrogen was so remarkably associated with the lower layers of the second beds gives confirmation to this idea.

It is well known that amongst the forms of life which occur in any solution which contains organic matter, and which is left exposed to the action of the small organisms commonly distributed in nature, a certain cycle is observed in the order of their appearance. One form succeeds and replaces another in a definite manner, those forms which have the most rapid power of multiplication becoming naturally predominant at first, and the more slowly multiplying appearing later. Amongst the first to appear are the bacteria, since they have specially rapid power of growth. Should the conditions favour, as they usually do, the growth of infusorians, these begin also to appear. Those forms which absorb their nourishment from food matter in solution occur along with the bacteria. Later, ciliated forms appear, which have the power of ingesting food particles, and, especially, which devour bacteria. As a result of their appearance in force, the numbers of bacteria are rapidly reduced. One form of infusorian succeeds another under the action of causes which are very little understood, but a definite order is maintained. As conditions become unfavourable for any of the forms, they become encysted and drop to the bottom of the fluid, forming a layer of sediment.

There is thus a certain cyclical order in which the forms of life appear, and one of the events which takes place early in this process is a more or less complete approach to extermination of the bacteria. That this well-known cycle of events occurs also in the purification of sewage seems



highly probable. On examining microscopically the sediment which is found at the bottom of the beds and on the surface of the bricks, we discover the bacteria and the active and encysted infusorians in large numbers including the forms which absorb their food in solution, and the ciliated forms which devour bacteria. There are also multitudes of oligochaeta and nematode worms which find their food and deposit their eggs in the sediment. Correlated to this microscopic evidence we have the outstanding fact that in the contact beds the bacterial forms are rapidly disappearing. We have, therefore, apparently this cycle of events. The food material in solution is first absorbed by bacteria and certain infusorians. These become in turn the food of higher forms which are able to ingest solid particles. If we are right in recognising these events as occurring in the contact beds, the great destruction of the bacteria which is always observed indicates that they constitute an important part in the changes which are taking place. In the present state of investigation it is impossible to go further in the interpretation of the process. The part which the infusorians, worms, &c., take demands further research; but in the time at my disposal it has not been possible to carry the work further than the point I have indicated. To complete the evidence which I have been gathering, I should have valued the opportunity of examining bacteriologically a contact bed in the condition described by sanitary engineers as "sick." In this condition the bed ceases to exercise its power of purification. It has an odour of putrefaction absent from an effective bed, and it further loses its swarms of insects (*podura aquatica*). The opportunity of making an examination of a bed in this condition has not offered itself. The interesting point would be to discover the relation of "sickness" to the cyclical appearance of animal forms which I have described.

## ANAEROBIC BACTERIA.

In addition to the enumeration of aërobic bacteria in the sewage, I made an enumeration of the anaërobes. The gelatine plates were made at the same time as those for the aërobes. They were transferred to a Bulloch's anaërobic chamber, in which a quantity of pyrogallate of potash was exposed in solution in an open vessel. A current of lighting gas was passed through the chamber, and it was then sealed, and placed in the incubating room, at 20°C. for about a week. The chief interest of these observations is to show that the decline in the numbers of bacteria which takes place in the sewage as it passes through the contact beds is not confined to the aërobes. In the Belfast sewage the number of anaërobes is relatively low. For example, in the screened and sedimented sewage the average number of aërobes is 43 millions per c.c., while the anaërobes number only 25 millions. A large number of aërobes are so-called "facultative anaërobes," i.e., they can live in the anaërobic conditions, and there must have been considerable overlapping in the numbers of the two kinds. It is further of great interest to observe that the same law holds in regard to the reduction of the numbers, and, indeed, an almost identical effect has occurred.

		Screened and Sedimented Bacteria per c.c.	Final Effluent Bacteria per c.c.	Loss.
Aerobic	...	43 millions	18 millions	58 per cent.
Anaerobic	...	25 millions	12 millions	52 per cent.

This identity of effect suggests that the chief part of the microbes appearing as anaërobes have already appeared as aërobes in the former enumeration, and that the number of microbes which are able to grow only in the anaërobic atmosphere is relatively small. Confirmation of this idea is further obtained by comparing the aërobic and anaërobic enumeration results for the three samples from the combination of coke and brick beds. The bacterial flora of the contact beds seems predominantly aërobic and facultatively anaërobic.



TABLE No. VI.,

Showing the number of Anaërobes per c.c. in the Samples of Sewage.

				Date.	Crude Sewage.	Screened and Settled.	Effluent I.	Effluent II.
BRICK BEDS.	1	Jan.	6	...	1,400,000	uncountable	3,200,000	6,000,000
	2	"	20	...	6,900,000	16,000,000	37,000,000	12,000,000
	3	"	29	...	3,800,000	4,500,000	19,000,000	13,000,000
	4	Feb.	5	...	7,000,000	62,000,000	27,000,000	34,000,000
	5	"	12	...	3,000,000	58,000,000	31,000,000	25,000,000
	6	Mar.	3	...	less than 100,000	700,000	4,000,000	2,500,000
	7	"	10	...	500,000	24,000,000	3,500,000	500,000
	8	"	22	...	6,500,000	36,500,000	8,500,000	3,500,000
Average				...	3,650,000	25,200,000	16,600,000	12,040,000
COKE BEDS.	1	May	17	...	4,500,000	5,500,000	1,000,000	2,000,000
	2	"	31	...	10,500,000	4,000,000	5,000,000	2,000,000
	3	June	7	...	uncountable	2,500,000	1,000,000	6,000,000
	Average				...	5,000,000	4,000,000	2,330,000
Average of Total				...	4,000,000	19,400,000	12,700,000	9,600,000

#### CONSIDERATIONS SUGGESTED BY THE FOREGOING OBSERVATIONS.

The bacteriological investigation of the process of purification in the contact beds shows that the agency at work differs essentially from a simple growth of bacteria in a fluid containing their food. The change which takes place in the sewage is not dependent simply on the growth of bacteria in the sewage, but occurs before the maximum of bacterial growth has been reached. From a study of the exhaustion of artificial media, it has been

shown that occasionally results may be obtained by bacterial growth, proving that unoxidized nitrogen has been dispersed. In this dispersion the nitrogen is doubtless in the gaseous condition. Data on this subject are furnished in the chemical report, showing that 12 per cent. of the nitrogen which is dispersed from the sewage could be accounted for in this way.

In the second place, it was found that, associated with the bricks which were covered with a sediment of plant and animal organisms, there was a great power of throwing nitrogen out of solution. The meaning of this, which I have suggested, is that the nitrogen is absorbed directly into the bodies of these organisms to form their tissues, or indirectly in the form of microbes, which are reduced in number in a very remarkable manner during the passage of the sewage through the beds. The nitrogen which disappears in this latter manner is partly stored up in the bed in the form of a deposit or sediment, and is partly carried off in the bodies of insects, worms, &c. In this form some of it no doubt may pass into the Lough, where ultimately its carriers become the food of their natural enemies. To have, therefore, as perfect as possible a form of contact bed, the conditions must be favourable to both agencies, the bacteria and the small plant and animal life. Since it has been found that the activity in bacterial destruction is an index of efficiency in purification, bacteriological analysis must in the meantime adopt this as the standard of efficiency. In the present investigation, if we compare the results of contact with the brick beds with those of the coke and brick beds, according to this standard we have another instance of the fact that where there is deficiency in purification, the reduction in the number of bacteria is smaller in amount.

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#### OBSERVATIONS ON THE PRESENCE OF LIQUEFYING BACTERIA.

The investigation of liquefying bacteria is one of the recognised methods which gives information as to a certain though varied type of bacteria. It is interesting in regard to liquefiers, as in regard to anaërobics to compare the ratios with those seen in the aërobic enumeration. In the enumeration of the total (aërobic) bacteria, it was seen that in passing through the beds there was a loss of 58 per cent. In this Table the fall is still greater, viz., from 3 millions to 1 million, implying a loss of about



70 per cent. In the coke and brick beds the loss was only 27 per cent. on the average, but the total numbers of liquefiers per c.c. of sewage were curiously reduced compared with those in the earlier (brick bed) series. It is worth while to have this comparison in tabular form.

Proportion of Liquefiers amongst the total (Aërobic) Bacteria  
in the Samples of Sewage.

	Crude Sewage.	Screened.	Effluent I.	Effluent II.
Brick Beds	14 per cent.	7.6 per cent.	5 per cent.	6 per cent.
Coke and Brick	under 1 „	1.4 „	4 „	3 „

From both series it is apparent that the greatest increase in the bacteria during the sedimentation of the sewage is not of the liquefying variety. In the first series it falls from 14 per cent. to 7.6 per cent. of the total. The increase has been chiefly of the non-liquefying variety. In agreement with this also we find the numbers of liquefiers per c.c. in the two series are much closer to each other than the total numbers.

TABLE VII.

Showing the number of liquefying organisms per c.c. present in  
samples of Sewage.

		Crude Sewage.	Screened and Settled.	Effluent I.	Effluent II.
Brick Beds.	1. Jan. 6 ...	—	1,300,000	600,000	1,300,000
	2. „ 20 ...	1,550,000	850,000	4,500,000	2,000,000
	3. Feb. 5 ..	50,000	180,000	165,000	70,000
	4. „ 12 ...	700,000	5,500,000	850,000	1,350,000
	5. „ 19 ...	2,000,000	4,000,000	2,500,000	2,000,000
	6. Mar. 3 ...	2,000,000	1,500,000	1,500,000	500,000
	7. „ 10 ...	7,000,000	11,000,000	500,000	500,000
	8. „ 22 ...	1,000,000	2,500,000	2,500,000	1,500,000
	Average ...	1,780,000	3,350,000	1,641,000	1,140,000
Coke & Brick	1. May 7 ...	100,000	150,000	150,000	300,000
	2. May 31 ...	1,500,000	2,500,000	3,500,000	1,000,000
	3. June 7 ...	—	2,000,000	2,000,000	2,000,000
	Average ...	800,000	1,550,000	1,850,000	1,100,000

It is interesting to find that there is no special increase of the liquefying bacteria. Should the bacterial growth in the beds be the sole cause of the disintegration and dispersion of nitrogenous bodies, one would expect to find that the liquefying bacteria would have increased out of proportion to the other forms. They are generally regarded as possessing to a pre-eminent degree the power of disintegrating albumins. Contrary to this view we find that the relative increase of the liquefiers is a good deal less than that of the non-liquefying variety. The liquefiers which are 14% of the bacteria in crude sewage (14.5% London sewage) are only 7.6% in the greatly multiplied numbers of the sedimented sewage. This result would point therefore to conditions in the sewage which favour the growth of bacterial forms other than the liquefying varieties. Confirmation of this view of the matter is found in the negative result of Houston's experiment on the effect of charging a contact bed with large quantities of a cultivation of sewage proteus. He found that this in no way added to the purifying power of the bed. (Report to London County Council, May 1900, page 75.)

On the other hand, the exterminating agency in the brick beds does not specially affect one species of bacteria more than another. The liquefiers remain in about the same proportion throughout the destructive period (5—7%).

Dr. Houston's results give the following ratios:—

Ratio of liquefiers to total number of bacteria aërobes.

Crude Sewage.	Primary Coke Bed Effluent (6ft.)	Secondary Coke Bed Effluent (6ft.)
14.5%	12%	20%

This curious rise to 20% indicates two possibilities. (1) There was increase in the numbers, or (2) the destructive agency was harmless to the liquefiers. The total number of bacteria fell to 60% in the second effluent, and 12—14% of the bacteria in the original sewage became 20% in the reduced numbers of the second effluent. The absolute number of liquefiers had remained stationary. On comparing these with my own results it would seem more probable that the liquefiers had increased at a special rate, and thereby counteracted the process of destruction. From this



line of observation we may expect to get information as to the mode of working of the bacteria destroying agency, and also on the question whether the bacteria which escape destruction continue multiplying at their usual rate. The conditions in the London experiments have obviously been in some way different from those I have investigated.

### AEROBIC SPORES.

TABLE VIII.

Number of Aërobic Spores per c.c. in Sewage samples.

Date.			Crude Sewage.	Sedimented Sewage.	Effluent I.	Effluent II.
Brick Beds.	1. Jan. 6	...	—	220	2	5
	2. „ 20	...	240	620	220	220
	3. „ 29	...	—	112	43	98
	4. Feb. 5	...	93	110	118	70
	5. „ 12	...	39	55	51	69
	6. Mar. 3	...	166	205	209	68
	7. „ 10	...	8	22	25	19
Average			109	192	95	78
Coke and Brick Beds.	1. May 7	...	8	35	31	50
	2. „ 31	...	41	41	44	24
	3. June 7	...	45	15	24	5
Average			31	30	33	26

There is not much information to be gathered from the results as regards spores. The total numbers are so small in the Belfast sewage that it would hardly be justifiable to draw any conclusions. Generally speaking, the formation of spores is supposed to take place in any medium in which

the conditions are unfavourable for a vegetative existence. The paucity of spores in the Belfast sewage, and the reduction of their number on the whole, as the sewage passes through the beds, indicates that the particular kind of unfavourable condition which conduces to spore formation has not been set up at any point. In the London raw sewage for one spore there were 21,000 bacteria in the vegetative form. In the Belfast crude sewage there is one spore to 100,000 bacteria, and in the sedimented sewage one in 200,000 bacteria, or thereabouts. The reduction on the average in the number of spores in Effluent II. as compared with those in the sedimented sewage implies a loss of 60 per cent. This is almost identical with the reduction in the total number of bacteria in these samples.

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#### BACILLUS COLI COMMUNIS.

The investigation of *b. coli communis* was carried out by Klein's method of planting the bacteria on the surface of phenolated gelatine. The dilutions used were 1 in 10,000 and 1 in 100,000. In the earlier period it was difficult to get evidence of the bacillus at this dilution. In January and the early part of February the numbers were invariably less than 1 in 10,000. A large number of colonies were planted out, but I failed to find bacilli which answered to the outstanding tests (indol, gas, milk clotting, &c.). Only once was there 100,000 per c.c., and the average, including some very doubtful forms, was in the positive observations about 50,000. Considering the paucity of these bacilli it is impossible to draw any conclusion as to the effect of the contact bed upon them. One important point remains to be noted. There was a distinct increase in the coli-form bacilli in the later samples. The following were observed in the sample of May 31st:—

Number of Bacillus Coli Communis per c.c.					
		Crude Sewage.	Sedimented.	Effluent I.	Effluent II.
May 31st	...	300,000	400,000	400,000	200,000

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#### BACILLUS ENTERITIDIS SPOROGENES.

Observations were made on each set of samples in regard to this bacillus also, which like *b. coli communis* is of intestinal origin. Klein's



method of isolating it was used, and the sewage was diluted according to the rule laid down by Houston. The evidence of its presence also was very scanty, and the results were parallel to those regarding *b. coli communis*. In the earlier observations there was occasionally some evidence in the milk tubes of imperfect clotting and whey formation; but it was not till late in the investigation, *i.e.*, in May and June, when evidence of *b. coli* became clear, that the milk tubes showed the typical ragged clot, and clear whey and gas, associated with the growth of *b. enteritidis sporogenes*. The following is the record of the observations:—

January 6th.—Nil.

„ 20th.—Nil.

„ 29th.—Imperfect clots only; no distinct separation of whey in tubes containing  $\cdot 1$  c.c. of sewage.

February 5th.—Indistinct clotting = negative.

„ 12th.—Nil.

„ 19th.—Clots with some watery whey, no gas, in  $\cdot 1$  c.c. tubes.

March 3rd.—Atypical clotting, &c., in  $\cdot 1$  c.c. tubes.

„ 10th.—Atypical.

„ 22nd.—Nil.

May 31st.—Crude Sewage,  $\cdot 01$  c.c., clot, &c., typical = 100 per c.c.

Sedimented Sewage,  $\cdot 1$  c.c., typical = 10 per c.c.

Effluent I.,  $\cdot 1$  c.c., typical = 10 per c.c.

Effluent II.  $\cdot 1$  c.c. and  $\cdot 01$  c.c. typical = 100 per c.c.

June 7th.—Crude Sewage ...  $\cdot 1$  c.c. and  $\cdot 01$  c.c. typical = 100 per c.c.

„ Sedimented Sewage  $\cdot 1$  c.c. and  $\cdot 01$  c.c. typical = 100 „

„ Effluent I. ...  $\cdot 1$  c.c. and  $\cdot 01$  c.c. typical = 100 „

„ Effluent II. ...  $\cdot 1$  c.c. typical = 10 „

It is of great interest that these bacteria of definite intestinal origin—*viz.*: *b. coli communis* and *b. enteritidis sporogenes*—should have increased so markedly at the beginning of June. It was unfortunately impossible to follow up the investigation of this increase. During April and the first half of May no samples were taken. It is curious, however, to note that about the period of this increase (June) an epidemic of typhoid fever had

broken out in Belfast. It seems highly probable that a continuous record of the occurrence of these two kinds of bacilli in the sewage would be of service in working out the origin of typhoid epidemics in Belfast. Generally speaking, the interest in investigating the occurrence of these microbes in sewage effluents is their connection with intestinal disease. The question has been carefully kept in view in other investigations as to whether the destructive action of the contact beds tells on these pathogenic forms more or less than on ordinary forms. These results are too negative to form the basis for a conclusion in regard to this problem. It is naturally a matter of minor importance in the case of Belfast compared to that of towns where the effluent passes into a river whose waters may be subsequently used for drinking. The question, however, does arise in connection with edible shellfish. Since Klein discovered the typhoid bacillus in the alimentary canal of an oyster it has been regarded as a matter of the greatest importance to prevent the introduction of shellfish into the market from beds situated in water polluted with sewage. It will, doubtless, be found necessary by the sanitary authorities to ascertain how far the cockle beds in the Lough, from which large supplies are drawn, are beyond suspicion in this matter.

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#### OBSERVATIONS ON THE BACTERIAL GROWTH IN A MIXTURE OF SEA WATER AND SEWAGE.

The sea water used in these experiments was taken from the area of the "slob land." The sewage was diluted with this 250 times. The results give some idea of the rate at which bacteria are reduced in number in a mixture similar to an average sample of the sewage-charged water of the Lough. After a period lasting from a week to a fortnight the bacteria become greatly reduced in numbers. The cause of this I have not had the opportunity of working out.



TABLE No. IX.

First Experiment started January 8th.

	Crude Sewage.	Screened and Sedimented.	Effluent from Upper Bed.	Effluent from Lower Bed.
January 10	—	150,000	75,000	87,500
„ 12	—	125,000	58,000	58,000
„ 13	—	25,750	16,250	spoilt.
„ 16	—	8,250	7,500	6,750
„ 18	—	1,250	1,750	1,925

Second Experiment started on March 23rd.

	150,000	450,000	190,000	125,000
March 28	26,300	43,200	15,700	17,500
April 1	1,700,000	800,000	600,000	100,000
„ 8	out.	out.	out.	out.

Third Experiment started June 1st.

June 1	2,300,000	5,000,000	3,300,000	3,500,000
„ 3	3,000,000	4,500,000	4,000,000	4,000,000
„ 11	out.	out.	out.	out.

Fourth Experiment on June 8th.

June 9	250,000	100,000	100,000	75,000
„ 11	310,000	10,000	160,000	50,000
„ 13	15,000	7,500	15,000	12,500
„ 15	10,000	12,500	40,000	75,000
„ 17	under 10,000	7,500	2,500	2,500
„ 19	1,250	250	nil.	500

## SUMMARY AND CONCLUSIONS.

The facts which have been established, even at the present incomplete stage of the investigation, yield some conclusions which are of value for the guidance of the Committee in their effort to obtain a satisfactory system of sewage purification. I have attempted throughout the report to compare the results I have obtained with those of other bacteriologists, and to correlate the chemical with the biological account of the "contact" process. The value of a large number of investigations on this subject, undertaken elsewhere, has been minimised by the fact that they did not include both chemical and biological observations. This is the more unfortunate as the major part of our knowledge of the working of "contact" beds is as yet experimental.

The first important consideration is to note the difference in the type of action which may be observed in the contact process according to different conditions. Every form of bed which is working efficiently causes a great transformation in the putrescible matter of the sewage, clearing away this substance to the extent of 80—90 per cent. of the whole. The nitrogen which enters into the composition of the putrescible matter is the constituent whose fate is carefully traced in the chemical study of the process. This nitrogen may appear in the effluent as ammonia, or as nitrites and nitrates, and, according to the analysis published, this, in certain instances, happens to a large extent, as, for example, in the case of the Manchester sewage beds. When we consider that in these substances (nitrates, &c.) the nitrogen is in the form most suitable for plant food, it is obvious that the effect of this kind of purification would be of no avail in diminishing the growth of the seaweed ("*ulva latissima*") whose decomposition on the foreshore causes a nuisance proportionate in magnitude to the amount of weed left to decompose. While in other respects the purification might be of a most excellent character, it would, in so far as it furnished these inorganic forms of nitrogen to the Lough, aid rather than diminish the growth of the weed. It is always to be remembered that we in Belfast have a problem of a very different kind from that before the Public Health Committees of many large towns, where these peculiar conditions caused by the existence of a great stretch of "slob land" do not occur.



The conversion of nitrogen into the forms of nitrate, &c., is generally believed to be due to the action of bacteria of a special kind, which require plentiful supplies of oxygen.

There is a second bacterial possibility of a somewhat similar nature. Bacteria have been described which possess the power of dispersing nitrogen from solution, in the form of gas. This would escape into the atmosphere as free nitrogen. I have in my report described a series of experiments in which I inoculated meat broth with the bacteria of sewage, and found a certain dispersion of nitrogen which I could not account for on any other supposition, and Professor Letts's study of the dissolved gases in the samples of sewage has led him to the conclusion that a considerable proportion of the nitrogen in the sewage disappears in this fashion. Should this dispersion of nitrogen depend, as we suppose, on the growth of particular species of microbes, a great deal might probably be done to further it.

These, as far as I know, are the only possible forms of a purely bacterial theory of "contact" action. The one mode of action is of no value for the problem before the Belfast Committee. The other is a perfectly satisfactory mode of action, inasmuch as it transforms nitrogen from a harmful to a harmless condition. Professor Letts has shown that, in the case of the brick beds, 12—20 per cent. of the nitrogen which disappears in the purification of the sewage can be recovered as free nitrogen in solution in the effluents. This change further seems to occur in the absence of the bacterial action which leads to the formation of nitrates and nitrites. The chemical analyses of the effluents from the brick beds show that, of the nitrogen which disappears from the putrescible form present in the raw sewage, practically none whatever re-appears in the effluent as nitrate or nitrite. There is, therefore, in these beds a constant process of a very different type from that at work in the beds of the Manchester sewage system.

Leaving this point aside for the present, we have to ask the following question—"If by this mode of action only 20 per cent. (to take the highest value) of the nitrogen disappearing from the putrescible form can be accounted for, what becomes of the remaining 80 per cent.?" It has vanished without leaving any traces of a chemical kind. To explain this most important aspect of the case, I have studied the other biological



factors which might come into play. I have found that connected with the sediment-covered bricks in the lower layer of the beds there is a most potent agency for abstracting nitrogen from solution in dilute meat broth. When the sediment is examined microscopically, it is found to consist of a variety of animal forms. In the appearance of the various animal forms in a decomposing organic fluid a certain cyclical order is preserved, and one form is succeeded by another; the later forms causing a more or less complete extermination of those appearing earlier in the series. The actual presence in the sediment of the forms which are known to take part in such a cycle can be demonstrated by the microscope. The question as to how great a share in the process of purification this cyclical change has, I have not been able to determine, and I need not say that only the great demand for haste on the part of the Committee induced me to intermit the investigation before I was able to answer this most important question. We have, however, some evidence to go upon. In this cycle of living forms, bacteria have an early place, owing, no doubt, to their power of rapid growth, and their turn to be exterminated comes correspondingly early also. In the contact beds a great process of extermination takes place, as is shown in the table of total bacteria per c.c. of sewage and effluent. That this extermination is on a large scale may be inferred not only from the observed reduction in numbers, but also conversely from the overwhelming increase in numbers which appears in all the samples if they are kept in conditions favourable for bacterial growth. The effluent then represents the typical organic fluid at the point in the cycle of events when the bacterial forms are being exterminated. It is easy on this hypothesis to understand why the ratio of extermination of the bacteria should be in direct relation to the percentage of purification. The bacteria, we may suppose, have absorbed into their bodies the greater proportion of the nitrogen available for food. They become in turn the food of infusorians which live in and form the sediment on the bricks, and so the change in forms of life proceeds. The cycle reached its final stage so far as I was able to observe with the introduction of worms (*oligochaeta*). These would ultimately pass out to the Lough and become the food of fishes. The nitrogen by this indirect means passes away from the beds in the form of animal tissue. All the nitrogen which can be diverted to this end vanishes from solution, and the sewage is purified in proportion. To



strike the cycle and measure the magnitude of one of its events is to measure the general capacity of this living economy for dealing with the available food nitrogen at any given stage, or indeed at all stages of its existence. It is to measure the quantity of nitrogen which has been diverted into the forms of animal life. To measure the ratio of extermination of bacteria, therefore, is to measure the percentage of purification. Hence the correspondence between the two ratios.

We are, however, merely on the threshold of the investigation, and it avails nothing to go beyond the most obvious inferences.

To return to the practical problem, it is clear that the beds we have been experimenting with are the only known biological type which is suitable for Belfast. The percentage purification, according to all tests, chemical and biological, is a good deal lower than we have reason to expect, and it would be necessary to take steps to secure better results in practice than we have had. The size of the material of which the beds are composed and the nature of it, the depth of the beds, the length of the period of contact and period of rest, &c., should all be carefully investigated to discover how we may obtain the maximum purification. It would be very desirable, if not necessary, to raise the percentage purification to over 80 per cent., and, if possible, to over 90 per cent., and until this has been fully tested the Committee should not, in my opinion, involve themselves in too large an undertaking. It will be further necessary to pay the strictest attention to the working of the beds. The chemical and biological investigation of the combination of coke and brick beds was enough to show that their efficiency was considerably below that of the two brick beds.

There has been discussed an addition to the beds, which might assist in the process of purification, and there are, to my mind, some very clear grounds for considering it as likely to be of substantial benefit. It is that the effluent should be allowed to flow into a pond of sea water in which a crop of "*ulva latissima*" and possibly other seaweeds could be grown. Two advantages would accrue in my opinion. (1) The weed during the season of growth would absorb the ammonia which had not been dispersed in the beds, and thereby raise the percentage of purification. (2) The particulate matter in suspension in the effluent has, when allowed to stand

a strong tendency to form a tough sediment. This, if it occurred in the proposed intercepting pond, would also increase the percentage of purification in the effluent before it reached the Lough. The weed would be available only in summer, but it is then that the foreshore nuisance is chiefly felt.

One other practical question requires mention. For the successful working of the scheme all other sources of organic nitrogen by which the Lough water may be enriched should be curtailed as far as possible. A certain amount of nitrate must be continually brought down by the land drainage in the Lagan area, and there seems no possibility of preventing this. On the other hand, the sewage and other discharges containing organic matter from the houses and villages round the side of the Lough should be prevented from reaching the water in a form suitable for acting as a manure to the weed. Further, the present and subsequent crops of the weed should be removed until the store of nitrogen already in the Lough becomes exhausted. If the present growth of weed is allowed to decompose, and pass back into the water, it will be available as manure to the subsequent crops.

In view of the facts which I have described in the report, I am convinced that the Committee is justified in proceeding in an experimental manner towards the establishment of a scheme of sewage purification similar in type to that exemplified in the present brick beds.

I cannot close this report without acknowledging the help which I had in carrying out the work from various members of my Laboratory, and in particular from Dr. Moorhead Beatty. Without his untiring assistance I should have been quite unable to undertake many of the most important lines of investigation.

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Queen's College,  
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