THE NUMBER OF BACTERIA IN SEWAGE AND SEWAGE EFFLUENTS DETERMINED BY PLATING UPON DIFFERENT MEDIA AND BY A NEW METHOD OF DIRECT MICROSCOPIC ENUMERATION.

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INTRODUCTION.

THE main object in sewage purification is the oxidation or removal of its organic constituents to such an extent that the effluent shall not be subject to putrefactive decomposition. The aim of the process is therefore chemical and its success is gauged by chemical methods. The bacteriology of sewage and sewage effluents is, however, of importance since a knowledge of the bacteria which are the chief agents in the destruction of organic matter must make it easier so to adjust conditions as to obtain the maximum possible results. The study of the nitrifying bacteria, for example, has already yielded valuable data as to the effect of various chemical and physical agents upon these organisms. The admirable papers by Boullanger and Massol (1903), and by Schultz-Schultzenstein (1903), the latter translated into English by Kimberly (1904), are models of thorough and accurate investigation. The third paper of recent date on the same subject, by Fremlin (1903), fails to carry the conviction that errors of manipulation have been successfully avoided.

The bacteria active in the newer processes of sewage purification, in the septic tank and in the contact and trickling filters, have as yet received almost no attention; and it is hoped that the work planned at the Sanitary Research Laboratory of the Massachusetts Institute of Technology may to some extent supply this deficiency during the next few years. Investigations should be

made which are at the same time quantitative and qualitative, and they ought ultimately to include the detailed study of all the forms isolated from a series of plate cultures. I have thought that the ground might be cleared to some extent by quantitative analyses alone, so carried out under different conditions as to measure roughly certain principal groups of bacteria. With this end in view the following preliminary studies were undertaken.

PREVIOUS QUANTITATIVE WORK ON SEWAGE BACTERIA.

Determinations of the total number of bacteria present in sewage were made nearly 20 years ago by Wahl (1886), who found from 1,686,000 to 5,248,000 bacteria per c.c. in the sewage of Essen. Miquel (1891), records 13,800,000 per c.c. in the sewage at Gennevilliers with an average of 7,475 in the effluent from the broad irrigation fields. E. Ray Lankester (1892), at Oxford, England, found an average of 3,170,000 bacteria in sewage and 40,000 per c.c. in the effluent from a sewage farm. The Franklands (1894), note the presence of 26,000,000 in the sewage outlet at Ballater in Scotland. Laws and Andrewes (1894), record from two to eleven million bacteria in various London sewages.

It is probable that certain of these early experimenters did not appreciate the necessity for planting samples promptly to forestall the enormous multiplication which takes place soon after collection. Fuller (1895), and Winslow and Belcher (1904), have shown that a tenfold increase may take place under such conditions in 24 hours. In recent years English investigators, as shown in Tables 3 and 4 have found from two to five million bacteria per c.c. In Germany, according to Bruch (1899), the sewage of Berlin showed, in one series of analyses, 1,600,000 bacteria per c.c. and in another from 600,000 to 3,800,000. Examinations at Charlottenburg quoted by Grünbaum (1900), showed 2,700,000 bacteria in crude sewage and 225,000 in contact effluents.

In America the reports of the Massachusetts State Board of Health show that the sewage of Lawrence taken from the Lawrence Street sewer contained on the average from 1894 to 1901, 2,800,000 bacteria per c.c. Each year the Lawrence Experiment Station furnishes an immense accumulation of valuable data published in less detail in the last two reports than formerly. The monthly analyses of Lawrence and Andover sewage are extracted from the Report for 1900 to form the first two columns of Table 1; and Table 2 is compiled from the same volume (Clark, 1901), to show the total number of bacteria present in effluents of various types.

The third column of Table 1 is from a report on the Worcester purification plant by Eddy (1902); the yearly averages reported by the same author are as follows: Sewage, 3,712,000; septic tank effluent, 2,539,000; sand filter effluent, 41,900.

Analyses of American sewage outside the Massachusetts Reports are, unfortunately, rare. At Plainfield, N. J., Le Clear (1902) records the following average numbers of bacteria per c.c.: Crude sewage, 1,321,000; septic tank effluent, 556,800; contact filter effluent, 171,500. At Ames, Iowa, Walker (1901) and Pammel (1902) have carried out somewhat exhaustive studies.

Their monthly results form the fourth column of Table 1; the general averages for the year 1901 are as follows: Sewage, 1,248,256; septic tank effluent, 991,298; sand filter effluent, 14,750.

TABLE 1.

BACTERIA IN SEWAGE. MONTHLY AVERAGES. BACTERIA PER C.C.

(Clark, Eddy, Walker.)

Month	Lawrence 1900	Andover 1900	Worcester 1901	Ames 1901
January	2,860,000	3,494,000	5,237,000	550,879
February	1,520,000	4,475,000	7,667,000	1,993,766
March	1,814,000	2,260,000	5,559,000	469,600
April	2,320,000	9,963,000	6,400,000	775,090
May	2,334,000	5,150,000	2,457,000	652,150
June	2,530,000	4,890,000	3,614,000	828,333
July	3,150,000	4,235,000	3,987,000	826,000
August	2,645,000	3,153,000	2,531,000	1,194,000
September	6,485,000	1,100,000	3,390,000	940,000
October	3,178,000	4,253,000	1,968,000	4,230,000
November	1,710,000	3,850,000	2,937,000	1,547,000
December	4,060,000	4,277,000		3,825,000

TABLE 2.

AVERAGE BACTERIAL ANALYSES OF EFFLUENTS FROM VARIOUS TYPES OF FILTERS AND SEPTIC TANKS DURING 1900, AT ANDOVER AND LAWRENCE, MASS. BACTERIA PER C.C.

Effluent from Septic Tanks	Effluent from Contact Filters	Effluent from Trickling Filters	Effluent from Sand Filters
1,209,500	94,500	74,200	73,900
1,929,000	486,200	69,700	1,175
	552,100		10,072
	386,400		1,243
	291,800		152
	543,900		10,300
	630,000		6,500
			16,300
			25,600
			1,485
			24,200
			252
			151
			23,600
			16,700
			4,700

So far only the total number of bacteria present has been considered; but in the various reports published by the Royal Sewage Commission of Great Britain and by the London County Council analyses are presented which go into greater detail. Woodhead (Rideal, 1899) found in Exeter

sewage, from 3,000,000 to 5,000,000 non-liquefying and 500,000 liquefying bacteria on aërobic plates and in anaërobic cultures, 300,000 liquefiers and 700,000 non-liquefiers. Klein and Houston (1899) report an average of 3,600,000 bacteria in sewage, of which 460,000 belonged to the B. coli group, with 1,300 anaërobic spore formers. Clowes (1898) gives the following figures for London sewage (Table 3).

TABLE 3.

BACTERIA IN LONDON SEWAGE. NO. PER C.C.

(Clowes.)

Source of Sample	Date	Total No. of Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	No. of Spores	Liquefiers	Spores B. subtilis B. mesentericus B. mycoides B. megatherium	B. Fluorescens Liquefaciens
Barking	Feb. 23 to May 4, '98	4,399,047	70,000	690	357,500	20	25,000
Crossness	Feb. 23 to May 2, '98	3,526,669	112,500	852	404,000	20	10,000

Rideal (1901) quotes figures for several other cities. At Exeter the crude sewage contained three to five million bacteria with 150,000 to 200,000 liquefiers, the tank effluent, one million with 300,000 to 400,000 liquefiers, and the final filter effluent, 900,000 bacteria with 100,000 liquefiers. At Chorley the crude sewage contained four million bacteria, the tank effluent 400,000, and the filter effluent, 46,000. At Leeds the crude sewage contained 2,500,000 to 3,000,000 bacteria per c.c. More exhaustive studies on London sewage and contact filter effluents were published by Clowes and Houston (1899 and 1903) in the second and third reports to the London County Council; these are summarized in Table 4.

TABLE 4.

BACTERIA IN LONDON SEWAGES AND EFFLUENTS. NO. PER C.C.

(Clowes and Houston.)

SEWAGE.

Source of Sample	Date	Total No. of Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	Anaërobic Spores (Agar 37°)	Aërobic Spores	Liquefiers	Total No. of Bacteria (Agar 37°)
Barking.....	Oct. 16, '99, to Jan. 17, '00.	7,096,666	770,000				
"	Mar. 16, '99.	10,000,000	600,000				
Crossness.....	May 11, '98, to Dec. 21, '98.	7,357,692	600,000		340	1,076,923	
"	Jan. 11 to Feb. 22, '99.			554			
"	Mar. 22 to Oct. 4, '99.	5,711,000	655,555				
"	Aug. 2 to Oct. 4, '99.	5,758,571					2,802,857

TABLE 4—*Continued.*

CONTACT BEDS.

Source of Sample	Date	Total No. Bacteria (Gelatin 20°)	B. Coli and Closely Allied Forms	Anaërobic Spores (Agar 37°)	Aërobic Spores	Liquefiers	Total No. of Bacteria (Agar 37°)
Barking	Oct. 16, '99, to Jan. 17, '00.						
Bed A Primary..		2,180,000	500,000				
Bed B Primary..		2,700,000	200,000				
Bed A Secondary		1,918,571	200,000				
Bed B Secondary		1,444,285	233,500				
Barking.....	Mar. 16, '99.						
Fine rag stone bed.....		4,000,000	500,000				
Fine coke bed.		1,800,000	300,000				
Crossness	May 11, '99, to Dec. 20, '00.						
4 ft. coke bed.		4,966,666	400,000		252	806,666	
6 ft. primary coke bed		6,787,500	600,000		256	837,500	
6 ft. secondary coke bed		4,300,000	100,000				
4 ft. coke bed..	Jan. 11, '99, to Feb. 23, '99.				320	833,333	
6 ft. primary coke bed.....				342			
6 ft. secondary coke bed.....				354 207			
13 ft. coke bed.	Mar. 22 to Oct. 4, '99.	5,364,000	411,111				
13 ft. coke bed.	Aug. 2 to Oct. 4, '99.	4,662,857					2,802,857

The sewage at West Derby and Walton, examined by Boyce (1900), showed the following results: Walton sewage, 13,400,000 bacteria per c.c.; West Derby sewage, 10,380,000; West Derby contact filter effluent, 614,000 (of which 10,000 were of the B. coli group and 100 anaërobic spore formers); West Derby sand filter effluent, 17,900 (of which 50 belonged to the B. coli group). Boyce, MacConkey, Grünbaum and Hill (1902) report the following results from the same locality (Table 5).

TABLE 5.

BACTERIA IN EFFLUENTS AT WEST DERBY. NO. PER C.C.

(Boyce, McConkey, Grünbaum, and Hill.)

Source of Sample	Total Bacteria	B. Coli Group	Anaërobic Spores
Contact Filter.....	838,000	9,800	10–100
Sand Filter 1.....	79,800	125	Less than 1
2.....	50,700	590	Less than 1
3.....	31,700	49	Less than 1
4.....	113,800	432	Less than 1

Lorrain Smith (1903) presented to the Royal Commission on Sewage Disposal analyses of sewage and effluents at Belfast which indicated considerably higher numbers than those elsewhere recorded. His chief results are averaged and brought together in Table 6.

TABLE 6.

BACTERIA IN BELFAST SEWAGE. NO. PER C.C.

(Smith.)

Source of Sample	Total No. Bacteria (Gelatin 20°)	Liquefiers	Anaërobes	Aërobic Spores	B. Coli
Crude sewage.....	15,300,000	1,510,000	4,000,000	86	300,000
Screened and sedimented sewage	47,280,000	2,860,000	19,000,000	143	400,000
Effluents from primary contact beds	35,660,000	1,700,000	12,700,000	76	400,000
Effluents from secondary contact beds	21,850,000	1,120,000	9,680,000	62	200,000

With regard to certain groups, of alleged significance in sanitary water analysis, further data are available. Klein and Houston (1898, 1899) report in crude sewage from 30 to 5,000 spore-bearing anaërobes per c.c., and 90,000 to 2,000,000 organisms of the B. coli group. According to Houston (1899, 1902) crude sewage contains over 10 million bacteria per c.c., as determined by plating on gelatin, between one and ten million on agar at 37°, 100,000 organisms of the B. coli group, at least 1,000 sewage streptococci, and 1,000 to 10,000 anaërobic spore-formers. A recent investigation by Belcher and the writer (Winslow and Belcher, 1904), indicated somewhat smaller numbers of all these groups in American sewage. Samples were taken from a small lateral of the Boston system receiving very fresh domestic sewage, and the results might thus be expected to differ from those obtained in the London experiments Anaërobic spores were found present in numbers less than 1,000 per c.c.; the B. coli group amounted to 28,000 per c.c. In these experiments all the colonies found upon dilute plates were fished and worked out in sufficient detail to place them in certain general groups whose characteristics are given in the original paper. The distribution in fresh sewage is shown in Table 7; the authors found in sewage stored in a glass-stoppered bottle that the bacteria of all groups multiplied tenfold within 24 hours, and then began to decrease.*

TABLE 7.

BACTERIA IN FRESH BOSTON SEWAGE.

(Winslow and Belcher.)

	BACTERIA PER C.C.									
	On Gelatin at 20°			Lactose Agar at 37°			Anaërobic Agar			
	1,240,000			151,000			140,000			
Group.....	II	IV	Va	VIII	X	XI	XII	XIII	XIV	XV
Type.....	Cocci	Chromogenes	B. subtilis	B. liquidus	B. coli	B. typhi	B. candicans	B. aërogenes	B. ubiquitus	B. rhinos. cleromatis
No. per c.c.	372,000	128,000	74,500	30,000	28,000	60,000	44,000	30,000	162,000	154,000

*For assistance in the collection of the foregoing references the author is indebted to Mr. G. C. Bunker.

SOURCE OF SAMPLES EXAMINED.

The Sanitary Research Laboratory of the Massachusetts Institute of Technology is situated near the junction of Albany street and Massachusetts avenue on the south side of Boston and on the line of the nine-foot main trunk sewer of the Boston Main Drainage Works. This is the principal vein of the South Metropolitan system receiving the sewage of Boston proper, Roxbury, Brighton, Allston, Newton, Brookline, Watertown, and Waltham, and its contributing population is over 300,000. At the station some 10,000 gallons a day are pumped from this sewer into three supply tanks $6 \times 4 \times 3$ ft. deep, from which it flows by gravity to the various experimental tanks and filters. As a rule, samples of the sewage were taken as it flowed from these supply tanks. Further statistics, with detailed monthly and hourly analyses of the station sewage will be found in another communication (Winslow and Phelps, 1905).

The septic tanks used in these experiments were cypress tanks, $6 \times 4 \times 4$ ft. deep. Four of them (5, 6, 8, 10) are closed tanks, and were first put in operation in June, 1903. Tank 6 is filled with 1½-inch broken stone. Tanks 7 and 9 are open tanks started in February, 1904. The contents of Tanks 5, 6, and 7 are changed once in 12 hours, of Tanks 9 and 10 once in 24 hours, and of Tank 8 once in 48 hours.

The contact filters studied are tanks $4 \times 4 \times 6$ ft. deep, or 4 ft. deep in the case of Nos. 17 to 20. No. 11 is filled with 2–3 in. coke; No. 12 with 1½-in. crushed stone; Nos. 13 and 16 with ½-in. crushed stone; No. 14 at first with 1-in.stone, later with tile-bricks arranged in regular open tiers. All these are single-contact filters receiving crude sewage in doses ranging from one to two million gallons per acre per day. Tanks 19 and 20 are primary contact filters of 1½-in. stone, and 17 and 18 are secondary beds of ½-in. stone. Seventeen and 19 receive septic sewage, 16 and 18 raw sewage at rates of one to two million gallons per acre per day for each bed. All were put in operation in June, 1903.

The trickling filters of the station are tanks $4 \times 4 \times 6$ ft. deep filled with 1½-in. crushed stone, and dosed by tipping buckets at a rate of 1,500,000 to 3,000,000 gallons per acre per day. Tank 15 takes sewage which has been septicized for 12 hours; Tank 23, septicized for 48 hours; and Tank 22, raw sewage. No. 15 was put in operation in July, 1903, and Nos. 22 and 23 in February, 1904.

The sand filters used are tanks $6 \times 4 \times 3$ ft. deep filled with 2 ins. of sand of effective size, .17 mm., resting on 6 ins. of coarser material. Tank 1 received 100,000 gallons of raw sewage per acre per day from June 30 to December, 1903, 200,000 gallons per acre per day for the first six months of 1904, and 400,000 gallons since June 24, 1904. Tanks 24 and 25 have received 400,000 gallons of septic sewage per acre per day since February, 1904. The sewage applied to No. 24 has been septicized for 24, that applied to 25, for 48 hours.

The bacteriological analyses were carried out in two series, one extending from July to December, 1903, and including examinations made twice a month, and the second comprising weekly analyses made in July and August,

1904. These are designated in the appended table as Series A and Series B respectively. In the analyses of Series A, I was assisted by Professor S. C. Prescott and Mr. E. B. Phelps, to whom I desire to express my thanks.

THE DIRECT MICROSCOPIC ENUMERATION OF BACTERIA.

Realizing that the ordinary culture methods reveal only a fraction of the bacteria present, I attempted to control them by a direct examination of sewage and effluents under the microscope, drying a measured volume upon a cover-slip of known area, and counting representative fields with a Sedgwick-Rafter micrometer such as is used for enumerating the larger micro-organisms in the examination of drinking water. In my first experiments I attempted to stain the bacteria in the liquid in which they were suspended by adding a few drops of methylene blue or gentian-violet to a one-ounce sample bottle of sewage. Methylene blue was soon discarded, because bacteria grew in the stain itself, and later carbol-fuchsin was substituted for gentian-violet because it was found to give larger counts. Finally the process of staining in the bottle was entirely abandoned. Five hundredths c.c. of the fresh sample was placed on the cover-slip, dried in the air, fixed in the flame, and stained with carbol-fuchsin by heating till steam appeared. The latter process stained the cells much more definitely and sharply, and gave higher and more constant results than those obtained by staining in the bottle. It is of interest to note that on many of the slides prepared by the earlier method some of the bacteria showed faint but unmistakable flagella, stained by the carbol-fuchsin without any mordant; and it is possible that some process by which staining reagents are added directly to liquid cultures in which bacteria are present in their most active state might give better results than the somewhat severe preliminary drying and fixing treatment to which they are usually subjected before staining.

The method of direct enumeration as finally developed offered no serious technical difficulties, and furnished constant and comparable results. Its accuracy and the significance of its results have been investigated more fully by Willcomb and myself in another communication (Winslow and Willcomb, 1905). It will there be shown that there are three main factors which might tend to make the microscopic count larger than the plate count; the inclusion of several bacteria in a single colony, the presence in the sample of dead bacteria in a stainable condition, and the presence of organisms which do not grow on our nutrient media. We shall show that with pure cultures of ordinary metatrophic bacteria the plate counts and microscopic counts closely correspond even when the number of bacteria present are rapidly decreasing. One hundred million cells per c.c. have disappeared in four hours without leaving any trace of stainable bacteria. It

appears, therefore, that the presence of dead cells introduces no serious error in the microscopic count—that its excess is due mainly to the presence of organisms which fail to appear upon our plates—and that it, therefore, furnishes a more accurate measure of the total number of bacteria present than do our ordinary methods.

If these conclusions are justified the results shown in Tables 8 to 12 represent the first attempt to determine the actual extent of the bacterial flora of sewage. The crude sewage itself contained on an average 29 million bacteria per c.c.; the septic tank effluents, 30 million; the contact filter effluents, 24 million; the trickling filter effluents, 17 million; and the sand effluents, 650,000. The ratio of the total number as determined by direct microscopic enumeration to the count upon gelatin plates was nearly 20 in the case of sewage, about 40 for the septic tanks and contact and trickling filters, and 70 for the sand filter effluents. This result is suggestive in view of the important rôle played in purification processes by the nitrifying organisms which are known not to develop upon our gelatin plates.

THE BACTERIA IN RAW SEWAGE.

The media used for these analyses included lactose gelatin, lactose agar and Nährstoff agar. The first two were made up according to the standard methods of the American Public Health Association, two per cent of lactose being added before the final filtration and the reaction adjusted to −.5 on Fuller's scale. The Nährstoff agar contained one per cent of agar and one per cent of Heyden's Nährstoff dissolved in water and filtered through cotton.

Anaërobic cultures were made first by the Wright method (Wright, 1901), later according to the admirable modification of Rickards (1904), by inverting a tube or Erlenmeyer flask containing the inoculated and solidified medium in a tumbler of pyrogalic acid and caustic solution. Cultures incubated at 37° were counted after 24 hours; gelatin plates and anaërobic cultures at 20° after 48 hours, and Nährstoff plates after seven days. Acid production was observed on plates to which litmus had been added in the usual manner, both agar and gelatin plates in Series A and gelatin plates in Series B.

The average results of the analyses of sewage are presented in Table 8 with the ratio which the count on each medium bears to the count on lactose gelatin at 20°.

TABLE 8.

BACTERIA IN BOSTON SEWAGE.

SERIES A.

No. of Samples	Bacteria per c.c.					
	On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
	Liquefiers	Acid Formers	Total	Acid Formers	Total	
56	365,000	1,670,000	5,430,000	1,670,000	3,760,000	2,440,000
Ratio to Gelatin Count.....	7	31	100	31	69	45

SERIES B.

No. of Samples	Bacteria per c.c.						
	Microscopic Count	Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
		Liquefiers	Acid Formers	Total			
25	29,000,000	149,000	429,000	1,690,000	1,400,000	2,930,000	850,000
Ratio to Gelatin Count..	1700	9	25	100	83	170	50

The total number of bacteria on lactose gelatin was three times as great in Series A as in Series B; this is due to the fact that the first series included the autumn months in which the number of bacteria in sewage reaches a maximum. In Table 9, the monthly values obtained in the first set of experiments show clearly this autumnal maximum which is also manifest in the Lawrence and Ames results of Table 1.

The average number of bacteria present in Boston sewage appears then to vary between 500,000 and 10,000,000 according to the season. These figures are, however, too high since they were obtained from samples collected during the daytime when the numbers are of course much higher that at night. Phelps and the writer have elsewhere (Winslow and Phelps, 1905), published

a series of hourly analyses which show the diurnal variation in the number of bacteria present to be very great. The numbers in Table 8 may, however, fairly be compared with those from other places which have generally been obtained in the same way by examination of day samples. They show that Boston sewage has the bacteriological composition which appears to be common to most of the European and American cities which have been examined. There is much less variation indeed than might have been expected. Of the localities for which bacteriological sewage

TABLE 9.

BACTERIA IN BOSTON SEWAGE. BY MONTHS. NO. PER C.C.

MONTH (1903)	ON LACTOSE GELATIN AT 20°			ON LACTOSE AGAR AT 37°		ON ANAËROBIC LACTOSE AGAR AT 20°
	Acid Formers	Liquefiers	Total	Acid Formers	Total	
July	445,900	314,300	2,995,000	420,000	1,864,300	3,480,000
August.....	318,750	150,000	4,263,600	1,133,000	2,688,900	1,461,500
September.....	4,021,900	850,000	11,487,500	3,268,750	8,504,400	4,557,500
October.....	635,000	95,700	3,693,000	1,298,300	1,407,000	639,300
November	530,400	15,000	587,100	530,600	551.300	605,100
December	655,000	8,700	712,000	762,000	814,000	696,000

analyses are quoted above, Essen, Berlin, Charlottenburg, Leeds, Exeter, Chorley, Oxford, Lawrence, Andover, Ames, Plainfield, Worcester, and Boston show results generally lying between 1 and 5 millions, London, Walton and W. Derby, figures varying from 2 to 10 millions, and Paris, Ballater and Belfast, over 10 millions. The accuracy of the latter results may be questioned since multiplication in storage so easily occurs.

In both series of analyses of Table 8 the ratios of the liquefiers and acid formers were remarkably constant. The liquefiers amounted to seven and nine per cent respectively of the total number of colonies on gelatin, a slightly lower figure than that obtained by English observers. Clowes (1898), found about 10 per cent of liquefiers; Woodhead at Exeter, about 10 per cent; Clowes and Houston (1903), 10 to 20 per cent; and Smith (1903), 1 to 15 per cent. The ratio of acid formers in Series A was 31 per cent both on gelatin at 20° and agar at 37°, and in Series B 25 per cent. It is significant to note the coincidence of these

figures which show that the acid forming organisms are in general so adapted to the body temperature that their counts at 37° are as large as at 20°, while the total number of bacteria on agar at 37° varies from 70 to 80 per cent of the total number on gelatin at 20°. Since in potable water the ratio of the 37 per cent count to the 20° count is generally under 10 per cent with acid formers absent, the application of the lactose agar plate to sanitary water analysis is apparent.

The anaërobic counts in Series A and B differ but little although made in one case on gelatin and in the other on agar. In each the anaërobic colonies were about half as numerous as the aërobic colonies on gelatin. In a few experiments with aërobic and anaërobic plates of Nährstoff the same general relation was found to hold.

Nährstoff agar incubated at 20° for seven days showed not quite twice as many bacteria as appeared on the gelatin plate in two days. This result is somewhat lower than that obtained by Gage and Phelps (1902), who report that with the count on Nährstoff agar as 100, that on gelatin was 34 after two days and 44 after three days when sewage was examined. With a sand effluent the gelatin count was less than 20 per cent and with river water, less than 10 per cent of the Nährstoff count.

BACTERIA IN SEPTIC TANK EFFLUENTS.

The analyses of the septic tanks studied, four in number in Series A, six in Series B, are summarized in Table 10; and for comparison the total numbers of bacteria on gelatin and the ratio to that number of the counts made in other ways are shown in Table 14 for all the various types of effluents examined.

The total number of bacteria shows a marked decrease after passing through the septic tanks, amounting to over 60 per cent in Series A and over 50 per cent in Series B. The Lawrence figures in Table 2 show a similar decrease compared with the sewage in Table 1; and at Worcester, Plainfield, and Ames the same phenomenon appears, the diminution amounting to about 50 per cent. At Exeter, Woodhead reported no such decrease. The ratios of the different groups to the total are almost identical with those obtained with crude sewage. Comparing the septic

TABLE 10.

BACTERIA IN SEPTIC TANK EFFLUENTS.

SERIES A.

	No. of Samples	Bacteria per c.c.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 5	15	1,650	291,000	665,000	298,000	454,000	700,900
Tank 6	14	180,000	525,000	1,660,000	550,000	1,300,000	810,000
Tank 8	15	202,000	360,000	1,244,000	484,000	730,000	900,000
Tank 10	12	290,000	880,000	3,830,000	1,210,000	1,840,000	1,390,000
Average	14	162,000	495,000	1,750,000	650,000	1,040,000	930,000
Ratio to Gelatin Count	...	9	28	100	35	59	53

SERIES B.

	No. of Samples	Bacteria per c.c.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 5	8	21,525,000	78,000	291,700	926,700	682,500	1,399,400	610,000
Tank 6	5	30,520,000	60,000	126,700	660,000	486,000	1,212,000	306,700
Tank 7	5	42,140,000	37,500	210,000	706,000	456,000	1,271,000	230,000
Tank 8	5	39,620,000	220,000	196,700	1,605,000	738,000	1,338,000	913,300
Tank 9	4	24,425,000	33,300	80,000	34,300	322,500	958,750	163,300
Tank 10	6	22,400,000	47,500	128,000	486,000	346,700	2,888,000	352,000
Average	5	30,105,000	79,400	172,200	787,800	505,300	1,511,190	429,200
Ratio to Gelatin Count	...	3,830	11	22	100	64	192	55

tank ratios with those of the sewage there is manifest in both series a relative decrease of the total on lactose agar at 37° and a very slight increase of anaërobes. The insignificance of the latter is somewhat surprising. A somewhat more marked increase is shown in the ratio of the Nährstoff count; and the ratio of the microscopic count to the gelatin count is doubled, the absolute value of the former being the same as in the case of the sewage.

These facts suggest that the decrease of 20° gelatin forms in passage through the septic tank may be balanced by a multiplication of other bacteria.

BACTERIA IN CONTACT FILTER EFFLUENTS.

The analyses of the contact filters, nine in Series A, and seven in Series B, are arranged in Table 11. They show in general that these effluents contained two-thirds as many bacteria as were found in the septic effluents and one-third to one-fifth as many as the crude sewage, or in absolute numbers 500,000 to 1,000,000. At Charlottenburg, Chorley, and Lawrence the numbers have varied from 100,000 to 600,000, while the effluents at Exeter showed one million and at London two to six millions. Smith at Belfast reports over 20 millions. In comparing the individual filters of Table 11 it may be noted that of the double contact beds, the primary pair, 19 and 20, show much higher numbers than the corresponding secondary beds, 17 and 18, 20, which takes septic sewage, being the highest of all.

The various groups of bacteria in contact effluents maintain the same relation as in the crude sewage, with two exceptions.

TABLE 11.

BACTERIA IN CONTACT FILTER EFFLUENTS.

SERIES A.

	No. of Samples	Bacteria per c.c.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaerobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 11	17	94,000	262,000	956,000	492,000	624,000	529,000
Tank 12	20	112,000	158,000	1,238,000	387,000	733,000	561,000
Tank 13	18	29,000	76,000	738,000	100,000	215,000	228,000
Tank 14	16	17,000	108,000	776,000	186,000	375,000	338,000
Tank 16	12	66,000	433,000	1,828,000	204,000	361,000	208,000
Tank 17	12	19,000	238,000	496,000	183,000	270,000	281,000
Tank 18	17	43,000	228,000	648,000	239,000	623,000	412,000
Tank 19	12	44,000	368,000	1,038,000	322,000	502,000	388,000
Tank 20	16	105,000	691,000	1,904,000	460,000	1,240,000	950,000
Average	15	60,000	270,000	1,060,000	290,000	570,000	440,000
Ratio to Gelatin Count	...	6	25	100	27	54	41

TABLE 11—*Continued.*

SERIES B.

	No. of Samples	Bacteria per c.c.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 11..	5	22,600,000	94,200	215,000	689,600	455,000	939,700	510,000
Tank 12..	4	40,875,000	125,000	23,000	897,000	1,045,000	1,263,700	255,000
Tank 13..	5	21,260,000	47,500	93,300	287,500	190,000	1,324,000	250,000
Tank 14..	4	32,525,000	55,000	35,000	665,000	602,500	397,500	935,000
Tank 16..	4	15,827,500	135,000	36,700	240,000	186,700	450,000	257,500
Tank 17..	4	8,625,000	8,100	87,500	185,000	120,000	612,500	80,000
Tank 18..	4	27,225,000	135,000	200,000	682,500	442,500	1,265,000	427,500
Average .	4	24,133,900	85,700	126,800	520,900	434,500	891,800	387,900
Ratio to Gelatin Count ..	...	4,610	16	24	100	83	171	74

The proportion of liquefiers was increased in Series B to 16 per cent of the total on gelatin. In Series A this did not occur, possibly because the beds had not been in operation long enough to exhibit their typical characteristics; but in connection with the results obtained with the trickling filters the figures of Series B suggest that in a loose stone filter there may be an increase in the proportion of liquefying bacteria present. Clowes and Houston (1903), obtained negative results in this regard.

In the second place the ratio of bacteria as determined by the microscopic method showed a very great increase, being nearly fiftyfold that for gelatin; its absolute value was 24,000,000 against 29,000,000 for sewage.

Bacteria in Trickling Filter Effluents.

The single trickling filter examined in Series A (Table 12), was not in thoroughly satisfactory operation and the analyses of its effluent are somewhat aberrant. Both series show a somewhat smaller total of bacteria than in the contact effluents, about one-quarter of the number present in raw sewage. The ratios of Series B, typical of the beds when in good working order, correspond almost exactly with those of the contact beds, except that they show a higher ratio of liquefiers, 30 per cent of

the total and nearly three times as many bacteria on Nährstoff as on gelatin. Apparently in the filters of these two latter types there is either a multiplication or a relative persistence of certain bacteria which do not grow on gelatin, and of the liquefying forms. Since nitrification and the dissolution of solid materials are among the most important functions of contact and trickling beds these phenomena are significant.

TABLE 12.

BACTERIA IN TRICKLING FILTER EFFLUENTS.

SERIES A.

	No. of Samples	Bacteria per c.c.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 15....	10	22,000	93,000	1,030,000	260,000	1,010,000	370,000
Ratio to Gelatin Count....		2	7	100	24	94	34

SERIES B.

	No. of Samples	Bacteria per c.c.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 15....	8	23,633,000	127,500	98,800	258,700	410,000	1,228,500	228,700
" 22....	5	20,080,000	192,000	120,000	678,000	676,000	1,739,000	370,000
" 23....	5	9,425,000	20,600	124,200	415,000	66,500	557,000	240,000
Average....		17,729,000	133,700	114,100	450,600	284,200	1,174,800	279,600
Ratio to Gelatin.....		3,940	30	25	100	63	260	62

BACTERIA IN SAND FILTER EFFLUENTS.

The bacterial analyses of sand filter effluents in Table 13 correspond with those obtained at Chorley, Oxford, Lawrence, Worcester, and Ames in showing numbers varying from 1,000 to 50,000 per c.c. The West Derby and Exeter figures are somewhat higher.

TABLE 13.

BACTERIA IN SAND FILTER EFFLUENTS.

SERIES A.

	No. Samples	Bacteria per c.c.					
		On Lactose Gelatin at 20°			On Lactose Agar at 37°		On Anaërobic Agar
		Liquefiers	Acid Formers	Total	Acid Formers	Total	
Tank 1.....	14	120	470	1,220	330	630	320
Ratio to Gelatin Count.....		10	38	100	26	50	26

SERIES B.

	No. Samples	Bacteria per c.c.						
		Microscopic Count	On Lactose Gelatin at 20°			Lactose Agar at 37°	Nährstoff	Anaërobic Gelatin
			Liquefiers	Acid Formers	Total			
Tank 1.....	5	1,320,000	155	400	7,900	17,000	66,000	770
" 24.....	5	340,000	680	2,600	15,600	14,400	44,000	700
" 25.....	5	300,000	670	1,100	3,970	2,900	21,000	2,160
Average.....		650,000	500	1,360	9,160	11,400	43,600	1,210
Ratio to Gelatin Count.....		7,100	5	15	100	120	480	13

With regard to ratios on different media, three points are noticeable. The proportion of anaërobes in each series is distinctly lower than in any other type of effluent, being only half that of the sewage in Series A and less than a third in Series B. The ratio of the Nährstoff count and the microscopic count on the other hand is higher for the sand filter effluents than for any others, three times that of sewage for the Nährstoff count and four times that of sewage for the microscopic count.

CONCLUSIONS.

The results of this preliminary study may be briefly summarized as follows:

1. The day flow of Boston sewage contains on an average from one to five million bacteria per c.c. as determined by plating on

TABLE 14.

RATIOS OF COUNTS BY MICROSCOPIC METHOD AND BY PLATING ON VARIOUS MEDIA TO THE COUNT ON LACTOSE GELATIN AT 20°. SEWAGES AND EFFLUENTS.

SERIES A.

SOURCE OF SAMPLE	AVERAGE NO. OF BACTERIA PER C.C. LACTOSE GELATIN AT 20°	RATIO OF NO. OF BACTERIA TO THE NO. ON LACTOSE GELATIN								
		Microscopic Count	On Lactose Gelatin at 20°			On Lactose Agar at 37°		At 20°		
			Liquefiers	Acid Formers	Total	Acid Formers	Total	On Anaërobic Gelatin	On Anaërobic Agar	On Nährstoff
Sewage.....	5,430,000		7	31	100	31	69		45	
Septic Tanks.....	1,750,000		9	28	100	35	59		53	
Contact Filters...	1,060,000		6	25	100	27	54		41	
Trickling Filters.	1,030,000		2	7	100	24	94		34	
Sand Filters.....	1,250		10	38	100	26	50		26	

SERIES B.

Sewage..... ...	1,690,000	1,700	9	25	100		83	50		170
Septic Tanks.....	787,000	3,830	11	22	100		64	55		192
Contact Filters...	520,900	4,610	16	24	100		83	74		171
Trickling Filters.	450,600	3,940	30	25	100		63	62		260
Sand Filters.....	9,160	7,100	5	15	100		120	13		480

lactose gelatin at 20°. Of these 7 to 9 per cent are liquefiers and 25 to 30 per cent acid formers.

2. Lactose agar counts at 37° show 70 to 80 per cent as many colonies as on gelatin at 20° with the same absolute number of acid formers as at the lower temperature.

3. Anaërobic cultures show about one-half as many colonies as corresponding cultures under aërobic conditions.

4. Nährstoff agar gives counts not quite twice as high as those obtained by the use of lactose gelatin.

5. Direct microscopic enumeration shows nearly 20 times as many bacteria as appear upon gelatin plates.

6. The bacteria in Boston sewage exhibit a marked seasonal variation with a maximum in September and a minimum during the winter months.

7. In passage through the septic tank the number of bacteria on gelatin falls off one-half, while 37° counts show a slightly less decrease. The microscopic count remains unchanged.

8. In passing through contact and trickling filters the number of bacteria on gelatin is reduced to one-third of its sewage value, or less. The proportion of liquefiers is doubled or trebled. The microscopic count is only from one- to two-fifths less than in the case of sewage.

9. Sand filter effluents contain about one-half of one per cent as many bacteria as raw sewage on the gelatin plate, over one per cent measured on Nährstoff agar and about two per cent as shown by the microscopic count. The anaërobes are more markedly decreased than the aërobes.

10. The count upon the gelatin plate, in sewage purification as elsewhere, appears to correspond well with the amount of decomposable organic matter. The number of bacteria as determined in this way, decreases with the amount of purification effected and may furnish an indirect measure of it.

11. The total number of bacteria as determined by direct microscopical enumeration does not decrease directly with the gelatin count. Its ratio to the latter is highest in the purest effluents. In view of the fact that the nitrifying bacteria do not appear on the gelatin plate this result is somewhat significant.

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