THE FATE OF B. COLI AND B. AEROGENES IN SEWAGE PURIFICATION[1]

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Received for publication December 2, 1926

INTRODUCTION

One of the outstanding purposes of sewage treatment is to obtain a substantial reduction in the number of bacteria present. That the intestinal bacteria which constitute the bulk of the organisms in the incoming sewage are reduced considerably as a result of the treatment has long been known; but the factors influencing this reduction from the standpoint of practical operation of a sewage disposal plant have not been fully studied. Further it is conceivable and probable that even though these intestinal organisms are reduced in numbers, they may play a temporary and yet an important rôle in the process of purification itself. The following work was undertaken as an attempt to clarify some of these factors which operate in the reduction of *B. coli* and *B. aerogenes* in a sewage disposal plant and to determine their fate, and rôle.

The work will be reported in two parts, In the first part will be given the results of a survey of the distribution of these organisms in the different units of a sewage disposal plant. In the second part laboratory experiments will be reported on the fate of *B. coli* and *B. aerogenes* in fresh solids and in sludge to which a daily addition of fresh solids was made.

[1] Paper No. 325 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Sewage Disposal.

EXPERIMENTAL

Method

The numbers of *B. coli* and *B. aerogenes* were determined by the dilution method. One cubic centimeter portions of the various dilutions of the samples were inoculated into gentian violet lactose tubes. After forty-eight hours incubation at 37°C. these were streaked on the surface of solid gentian violet-eosin-methylene-bile agar. This is the ordinary standard agar used for this purpose as modified by Skinner and Murray (1924) with the addition of gentian violet at a concentration of 1:100,000 to repress the development of spreading colonies.

PART I

The numbers of *B. coli* and *B. aerogenes* were determined by the above method for a period of seven months in the different units of the sewage disposal plant at Plainfield. The sewage at this plant after passing through the Riensch-Wurl screen is treated in a series of Imhoff tanks, the first of which is used as a preliminary sedimentation tank. The effluent of the tanks is sprinkled intermittently over trickling filters. The effluent of the filter bed is further passed through a final settling tank.

Beginning with the middle of August 1925 up to the end of March 1926 samples were taken on Wednesday at 11 o'clock at which time the concentration of the sewage can be taken as an average for the rest of the day. Twenty minutes after the sampling of the screened influent a sample was taken from the effluent of tank 1 which was being used as a preliminary sedimentation tank. A sample of the sewage after going through the remainder of Imhoff tanks was collected forty minutes after. Half an hour was allowed before taking the next sample from the filter bed effluent and ten minutes after this, the effluent from the secondary sedimentation tank was collected. These intervals represent the average flow time through the different units. By this means comparative studies could be made as to the effect of the different units upon *B. coli* and *B. aerogenes* of what was initially the same sewage.

TABLE 1

The numbers of B. coli and B. aerogenes in the different units of a sewage disposal plant

	INFLUENT		TANK 1 EFFLUENT		UNIT EFFLUENT		FILTER EFFLUENT		FINAL EFFLUENT	
	Coli	Aerogenes	Coli	Aerogenes	Coli	Aerogenes	Coli	Aerogenes	Coli	Aerogenes
	thousands	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*
August 12	1,000	1,000			100	10	1	1	100	10
August 19	1,000	1,000	100	10	50	10	1	5	5	10
September 2	500	500	10	50	50	10	10	1	1	1
September 9	100	100	500	500	500	100	10	10	10	10
September 23	100	100	500	500	100	1,000	100	100	50	10
September 30	100	100	50	50	10	10	10	1	50	1
October 7	100	100	50	10	100	100	1	1	1	1
October 14	100	100	50	10	100	100	1	1	5	5
October 21	50	50	10	50	100	50	5	5	5	5
October 28	10	10	10	50	10	5	10	10	1	1
November 4	10	10	10	10	10	10	5	5	5	1
November 18	100	5	500	500	10	5	1	0.1	10	10
November 25	500	10	10	10	1	5	10	10	10	10
December 2	10	10	100	100	10	10	1	0.1	1	0.1
December 16	100	100	1	1	5	5	1	1	1	1
December 23	5	1	1	1	1	1	0.1	0.1	1	1
December 30	10	10	10	10	10	10	10	10	10	0.1
January 6	10	10	10	10	50	50	1	0.1	5	1
January 13	1	1	10	10	50	50	1	1	5	5
January 20	50	50	50	50	100	100	1	1	0.1	0.1
January 27	5	5	5	5	1	1	0.1	0.1	1	1
February 3	10	5	100	1	50	5	1	0.1	1	0.1
February 19	5	5	5	5	5	10	5	1	10	10
February 24	5	5	5	5	10	10	5	5	1	5
March 3	5	5	10	1	5	5	1	1	10	1
March 10	5	5			10	10	0.1	0.1	5	5
March 17	5	1			100	50	1	1	1	0.1
March 24	1	1			1	10	5	5	0.1	0.1
March 31	50	50			5	5	1	1	1	0.1

Discussion of results

The results are given in table 1 from an examination of which it can be seen that the fluctuations are great and that the results for *B. coli* and *B. aerogenes* follow each other quite closely. Hence the results for these two organisms were added and monthly averages made. These figures are given in tables 1 and 2, which contain also the monthly average of suspended solids in the units. As far as seasonal correlation is concerned it can be said in a general way that the numbers of *B. coli* and *B. aerogenes* in the incoming sewage were lower in winter months than in the fall, but the reduction in numbers due to treatment was not necessarily higher in the winter months. In fact in January and February the tank effluent showed a decided increase over the original numbers (see fig. 1).

TABLE 2

Monthly averages of B. coli and B. aerogenes and of suspended solids in the different units of the plant

	INFLUENT		TANK 1 EFFLUENT		UNIT EFFLUENT		BED EFFLUENT		FINAL EFFLUENT	
	Solids*	Bacteria†	Solids	Bacteria†	Solids	Bacteria†	Solids	Bacteria†	Solids	Bacteria†
	p.p.m.	*thousands*	*p.p.m.*	*thousands*	*p.p.m.*	*thousands*	*p.p.m.*	*thousands*	*p.p.m.*	*thousands*
August	251	2,000		110	62	85	32	1.7	25	62
September	189	400		540	59	445	82	60	46.4	33
October	231	130		60	45	141	28	8	39.0	6
November	186	212		347	63	14	44	10	25	15
December	189	61		56	64	13	67	6	43	3.3
January	229	34		37	81	100	65	1.3	44.8	4.5
February	164	12		40	88.8	30	50.4	5.7	47.4	9.0
March	186	25		11	82	40	32	3.2	23	4.7

* The figures on solids were obtained by the courtesy of Mr. Downes.

† Bacteria *B. coli* and *B. aerogenes*.

Table 3 gives the results calculated in terms of percentage reduction as compared with the incoming sewage for solids and for bacteria. On examining this table it can be seen that the greatest reduction in the bacteria took place after the sewage had passed through the filter beds. In the tanks the reduction was either inappreciable or altogether absent; in fact on a number of occasions there was a decided increase in the number of *B. coli* and *B. aerogenes*. The increase was most pronounced in January and February. An explanation of this will be offered below. With only one exception, once the sewage passed over

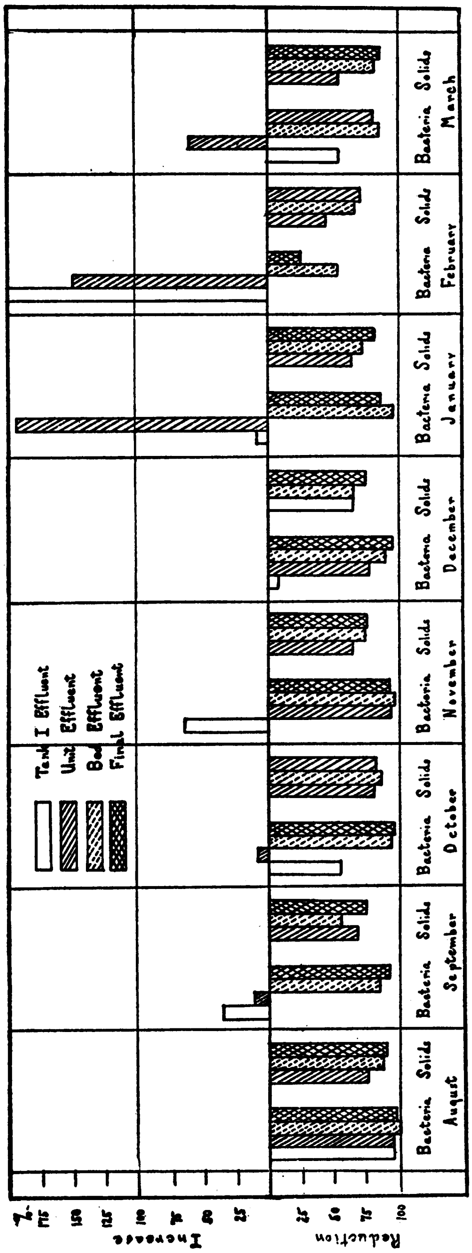

FIG. 1. MONTHLY AVERAGES OF THE REDUCTION OR INCREASE OF B. COLI AND B. AEROGENES AND OF SUSPENDED SOLIDS IN THE DIFFERENT UNITS OF A SEWAGE DISPOSAL PLANT

the beds, a very uniform reduction took place which averaged for the whole period about 89 per cent of the original numbers. The only apparent exception to this seems to be in February when only a 53 per cent reduction took place in the beds. However, on referring to the table it will be seen that the numbers of *B. coli* and *B. aerogenes* that went through the beds was low and that the percentage reduction was also low because the incoming solids in the month had low figures.

TABLE 3

Reduction of B. coli and B. aerogenes and of solids in the different units of the plant

	REDUCTION IN SETTLING TANK		REDUCTION IN IMHOFF TANKS		REDUCTION IN BEDS		REDUCTION IN FINAL TANK	
	Solids	Bacteria	Solids	Bacteria	Solids	Bacteria	Solids	Bacteria
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
August		−95	−75	−96	−88	−100	−90	−97
September		+35	−69	+11	−57	−85	−76	−92
October		−54	−81	+9	−88	−94	−83	−96
November		+63	−66	−94	−76	−96	−87	−93
December		−8	−66	−79	−65	−90	−77	−95
January		+9	−65	+194	−72	−96	−81	−87
February		+230	−46	+150	−69	−53	−71	−25
March		−56	−56	+60	−83	−87	−88	−81
Average		+15.5	−65	+190	−75	−89	−82	−83

* + is increase; − is decrease.

As far as reduction of the number of *B. coli* and *B. aerogenes* is concerned there is nothing to be gained from the final settling tank in fact there is usually a slight increase in the bacterial numbers due to this final treatment.

The suspended solids on the other hand were removed effectively in the tanks, an average of 65 per cent reduction of the original taking place for the whole period. The solids were reduced in the beds an additional 10 per cent. In the final settling tanks a further removal of 7 per cent over bed effluent took place. It thus becomes apparent that the reduction of the bacteria is not directly correlated with the reduction of solids. In the tanks where reduction of solids is most pronounced a bacterial reduction might either be small or there might even be an increase. On the other hand with a 10 per cent reduction of solids in the beds we get 89 per cent reduction of the bacteria. Furthermore while the solids are reduced further in the final settling tank there is no corresponding decrease in the bacteria. All these facts seem to indicate that the bacteria are not associated with the solid particles to a great extent. The determinations of solids were made by passing the material through Gooch crucibles. This would include all suspended solids except colloidal particles and dissolved solids. It would therefore seem

TABLE 4

Relation of fineness of particles to the distribution of bacteria

	SOLIDS	BACTERIA PER CUBIC CENTIMETER	INCREASE OR DECREASE OF SOLIDS OVER ORIGINAL	INCREASE OR DECREASE OF BACTERIA OVER ORIGINAL	BACTERIA PER GRAM DRY SOLIDS
	per cent	*millions*	*per cent*	*per cent*	*millions*
1 shaken	2.40	34.0			1,417
2 centrifuged a minute supernatant liquid	0.72	15.9	−70.0	−53.2	2,208
3 centrifuged 1 minute deposit	10.92	14.6	+355	−57.0	134
4 centrifuged 10 minute supernatant liquid	0.61	6.2	−74.6	−81.8	1,016
5 centrifuged 10 minute deposit	15.35	244.0	+539.6	+618.0	1,589
6 centrifuged 10 minute supernatant liquid filtered	0.52	1.2	−78.3	−96.4	234

that the bacteria are associated mainly with the fine colloidal particles.

The following experiments demonstrate that the bacteria are actually associated with the fine particles. Liquid from a tank was taken and different portions centrifuged for one to ten minutes and one portion filtered after centrifuging. Solids and the total number of bacteria were determined from the supernatant liquid and the deposit. The results as given in table 4 show that on centrifuging for one minute the percentage reduction of solids in the liquid was greater than that of bacteria. On centrifuging for ten minutes the reduction of bacteria in the liquid was greater than that of the solids. On the other hand the solids in the deposit on centrifuging for one minute showed a percentage increase while the bacteria decreased; and on centrifuging for ten minutes the increase in both cases was of the same order. In the filtered and centrifuged liquid, reduction in bacteria was of a greater order than that of solids. The bacteria per gram of dry solids were highest in the supernatant liquid of the one minute centrifuged material. They decreased on prolonged centrifuging. The bacterial content per gram of dry solids in the deposit of 1 minute centrifuged material was low and increased on prolonged centrifuging. All these facts would indicate that the bacteria are mostly associated with the finer particles in sewage.

In view of this conclusion an explanation for the actual increase in the numbers of *B. coli* and *B. aerogenes* in passing through the Imhoff tanks in the winter months can be offered. That it is not correlated with a corresponding increase in total solids is clear. To assume an actual multiplication of the organisms in the tanks during these months is untenable. On the other hand it is evident that rapid digestion does not take place in these months. Hence, solids are being added constantly to the digestion chamber of the tanks which are not being digested. These are accumulating constantly and naturally displacing the liquid. It is in this displaced liquid from the tanks that we have to look for an explanation of increased numbers of *B. coli* and *B. aerogenes* in the tank effluents. This liquid has a relatively high percentage of colloidal material and hence more bacteria. Furthermore lower temperatures in winter retard the rate of decrease of these organisms hence more of them are found in the displaced liquid than would be the case in summer months. This last statement is borne out by the temperature experiment in which the *B. coli* were observed to live over a longer period of time at lower temperatures than at high temperatures. This phase of the problem will be fully discussed in a later publication. Thus, in the winter months when digestion is slow not only is more of the liquid from the tanks displaced due to piling up of solids but the number of the intestinal organisms surviving in the tanks is greater due to the reduction in acid production by these organisms which is the dominating factor in reducing their number.

In view of the above conclusion, that the bacteria are mostly associated with the finer colloidal particles of sewage, it would seem to be a feasible plan to attempt to reduce their numbers by adding a coagulating agent to flock out these colloidal particles. The points to be considered in such a scheme would be: (1) The selection of a practical and efficient coagulating agent. (2) The addition of this material in the necessary concentrations to sewage which has undergone a preliminary sedimentation in order to remove most of the settable solids and thus reduce the concentration of the coagulant needed. (3) The detention period necessary for the settling of the coagulated material.

It would seem that such a scheme could be used in any one of the following ways:

1. Where no trickling filters or sand beds follow sedimentation and digestion tanks.
2. In addition to trickling filters where very pure effluents are required.
3. In combination with chlorination thereby reducing the amounts of chlorine required.
4. Instead of chlorination.

Such a procedure would have an advantage over chlorination in the fact that the colloidal and putrescible material in the sewage would be settled out and retained in the tanks, while in chlorinated sewage such substances decompose in the stream.

Summary and conclusions

A study was made over a period of seven months on the distribution of *B. coli* and *B. aerogenes* in the different units of a sewage disposal plant.

There were no significant differences between the relative numbers of *B. coli* and *B. aerogenes* at a given date for a given unit.

The weekly fluctuations were great but monthly averages show that the numbers in the incoming sewage were lower in the winter months.

Ordinarily in passing through the Imhoff tanks a material reduction of the numbers of *B. coli* and *B. aerogenes* does not take place.

A reliable and material reduction of these organisms invariably takes place in the filter beds, averaging to 89 per cent of the original numbers.

In the final settling tanks there is no further reduction, but sometimes an increase.

There is no correlation between solid removal and bacterial reduction in the different units. Whereas maximum removal of solids takes place in the tanks, maximum reduction of *B. coli* and *B. aerogenes* takes place in the beds, with no corresponding decrease in solids.

Bacteria are associated with the fine suspended particles which pass through the tanks and their numbers are reduced in the beds due to unfavorable conditions for continued viability.

There is an increase in the number of these organisms in the effluent from the tanks as compared with the incoming sewage in winter months due to more suspended solids passing out of the tanks. The lower temperature causes a low rate of digestion in the tank which induces (1) more of the liquid in the tanks to be displaced into the flow compartment and (2) retards the reduction of these organisms in the tank.

PART II

This part of the work concerned itself with the fate of *B. coli* and *B. aerogenes* in the fresh solids that enter the tanks continuously. As indicated in the previous discussion the majority of them pass on in the effluent of the tanks with the finely suspended solids to the filter beds where they are greatly reduced in numbers. However, considerable numbers of these bacteria are accumulating with the fresh solids in the digestion compartment of the tanks. They are always found present in these solids in considerable numbers especially when the tank is in operation and is receiving large quantities of solids. What is their fate and rôle here?

A series of laboratory experiments was started with the purpose of answering this question. The arrangement of the experiment was as follows:

1. Fresh solids diluted 1:3
2. Ripe sludge
3. Ripe sludge plus 2 per cent fresh solids
4. Ripe sludge plus 4 per cent fresh solids

To numbers 3 and 4 respectively 2 and 4 per cent of fresh solids (by volume) were added daily. The numbers of *B. coli* and

TABLE 5

The numbers of B. coli and B. aerogenes in the digestion of fresh solids alone and in combination with sludge

	I FRESH SOLIDS		II SLUDGE		III SLUDGE + 2 PER CENT FRESH SOLIDS		IV SLUDGE + 4 PER CENT FRESH SOLIDS	
	Coli	Aerogenes	Coli	Aerogenes	Coli	Aerogenes	Coli	Aerogenes
	thousands	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*
September 24	1,000	100	100	100				
September 25	1,000	500						
September 26	10,000	10,000	10	10	10	10	50	50
September 30	500	500	10	10	50	50	50	50
October 3			0.1	0.1	10	50	100	100
October 7	100	100	0	0	10	1	50	10
October 10	0.1	0.1	0	0	50	10	50	50
October 14	5	5	0.1	0.1	5	5	100	1
October 21	10	10	0	0	5	1	5	50
November 11	0.1	0	0	0	10	1	10	10
November 18					5	1	10	10

B. aerogenes were determined at intervals. The results are given in table 5. It can be seen at a glance that the numbers of *B. coli* and *B. aerogenes* follow each other closely. The number of these organisms in the fresh solids increased up to ten million after two days incubation and within a week they were back to their original numbers whereas after two weeks they fell to a very low level. If we compare the numbers of these organisms with total acidity production in the digestion of fresh solids as given by Rudolfs et al. (1924) we find a very close relationship. Acidity increases tremendously in the first two or three days after which it increases rather gradually and with fluctuations up to three weeks when it begins to drop. It is natural to ascribe this initial and sudden increase in the acidity to the activities of these intestinal organisms which have a very decided carbohydrate metabolism. The soluble carbohydrates of the fresh solids offer a good medium for their activities as a result of which large quantities of acids are produced. This high acid concentration in turn checks and represses their further growth. The reduction of the acidity can result from neutralization by alkaline material produced in the course of digestion or from the direct breaking down of organic acids by other groups of organisms. It is also possible that a combination of these two factors operate in the same direction.

In the second part of this experiment, where different amounts of fresh solids were added daily to ripe sludge, the tendency was similar except that the numbers did not go to as low a level. In the sludge bottle to which no fresh solids were added the numbers of *B. coli* and *B. aerogenes* were naturally reduced to a low level within a week. With 2 per cent fresh solid additions the maximum numbers occurred within the same period of time after which the numbers fluctuated with a declining tendency until an equilibrium was established. With 4 per cent fresh solids the maximum numbers occurred after ten days after which the numbers decreased with fluctuations until an equilibrium was established which was higher than with two per cent fresh solids.

The significant part of this experiment is that even with daily additions of fresh solids the numbers of *B. coli* and *B. aerogenes* do not keep on increasing but after reaching a maximum within a week or so they begin to decrease until they reach an equilibrium. The level of this equilibrium depends upon the amount of material added.

The conclusion reached as to the effect of materials produced in the course of digestion of fresh solids on the intestinal organisms applies in this case where fresh solids are added daily to ripe sludge. In fact each daily addition of fresh solids can be taken as a miniature portrait of what is taking place when fresh solids are digested alone. Each daily addition of fresh solids carries with it a number of the intestinal organisms which act on the available carbohydrates of the fresh material, resulting in an increase of acidity. Their numbers increase until the acidity reaches a certain point and then they are repressed in spite of the constant addition of fresh solids. The process in this case is complicated because of the action of ripe sludge in producing simultaneously alkaline material which over-balances the initial increase in acidity to a certain extent. The greater the addition of solids the greater the available food, hence the higher the peak attained will be. The numbers from this point on will be controlled by the balance between acid production of the neutralizing effect of sludge, which balance determines the point of equilibrium for these organisms.

Summary and conclusions

When fresh solids are digested without seeding the numbers of *B. coli* and *B. aerogenes* rise to a maximum within two days after which they fall rapidly to a low level. This coincides with the increase in acidity due to the attack of available carbohydrates. It is suggested that the *B. coli* group is mainly responsible for the decomposition of available carbohydrates in the beginning, giving rise to high acidity which in turn checks their own numbers.

With daily additions of fresh solids to ripe sludge the same relationship holds, namely, an increase in *B. coli* and *B. aerogenes* to a peak within a week or so, the level of the peak depending on the amount of fresh solids added. Their numbers decrease after this until an equilibrium is reached, the level of which is also controlled by the amount of material added.

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