

below a dormitory from which sewage was supplied by tapping a manhole in the main that served the building.

The installation consisted of three chambers, each of 2' x 3' x 4' dimensions with a capacity of 179.5 gallons. In effect there were three ~~separate tanks of~~ identical size constructed in one block. Sewage was diverted from the manhole by means of a dam that permitted approximately the desired quantity to flow and enter a receiving chamber from which it was apportioned to the three tanks in quantities that provided three different retention periods: the recommended 24 hours in one tank, 12 hours in another, and 8 hours in the third.

The first receiving chamber proved to have several faults of design and operation and it soon became obvious that changes would have to be made. About this time the State Department of Health engineer entered the Army and the Experiment Station took charge. Under the new management, the late Professor C. I. Gunness, Experiment Station engineer, designed a new receiving chamber which is shown in diagram II. The chamber was divided, by lengthwise partitions, into two equal compartments, one of which served the north tank (designated as N) and provided a quantity of sewage that would permit a retention period of 8 hours. The other half of the chamber was divided by a cross partition into two compartments that had a capacity ratio of 2 to 1. The larger of these served the middle tank (designated as M) and permitted a retention period of 12 hours, and the smaller compartment served the south tank (designated as S) and provided the conventional retention period of 24 hours. Thus the sewage volume flow ratio for the N, M, and S tanks was 3:2:1.

A tip-bucket extended the length of the receiving chamber with a partition lengthwise through its center which divided the tip-bucket into two sections of equal capacity. These respective sections emptied sewage into the comparable compartments of the receiving chamber. The tip-bucket was hinged at the bottom on an iron rod which ran its length, and was so arranged that when one side tipped to empty sewage into the chamber, the other was upright to receive sewage which was fed to the tip-bucket through a trough above it having a slit running lengthwise to distribute the incoming sewage as evenly as possible along the length of the tip-bucket. A cross section of the tip-bucket is shown in diagram II.

A mechanical counter was attached to the tip-bucket to record the number of tips. Since the capacity of the tanks and of the tip-bucket was known, it was possible to calculate the number of tips required each 24 hours to furnish the desired quantity of sewage to each tank.

A considerable trial-and-error period was necessary to arrive at the correct diversion of sewage from the manhole. At best, the supply of sewage to the tank was irregular. There were several peaks during the day: first, in the morning when the residents of the dormitory were getting up, then at noon when some of them were back at the dormitory for a time, and again in the evening. Another source of discrepancy, during the period of operation of the tanks, was the difference of personnel housed in the dormitory. During the first two seasons (fall and early winter, 1943-44 and 1944-5) Army Air Corps cadets occupied the building, and the third season (fall and early winter, 1945) it reverted to student use and was occupied by girls. The cadets were not in the dormitory during the morning or afternoon, while during the last season some of the girls were in at all times. There were two other sources of discrepancy: the dormitory was unoccupied from May to late September each year; and the heavy snow and hard freezing of midwinter made operation impracticable from January through March because the receiving chamber and collecting wells were not housed, with the result that control of flow and collection of samples were difficult at all times and frequently impossible.

Collection of effluent samples was made from wells provided on the disposal lines as indicated in diagram I. These wells were double "T" joints set with one arm vertical. The lower portion of each vertical arm was plugged with cement to provide a sump, while the upper portion extended up to the surface of the ground and was open at the top. A cross section of the collecting wells is shown in diagram II.

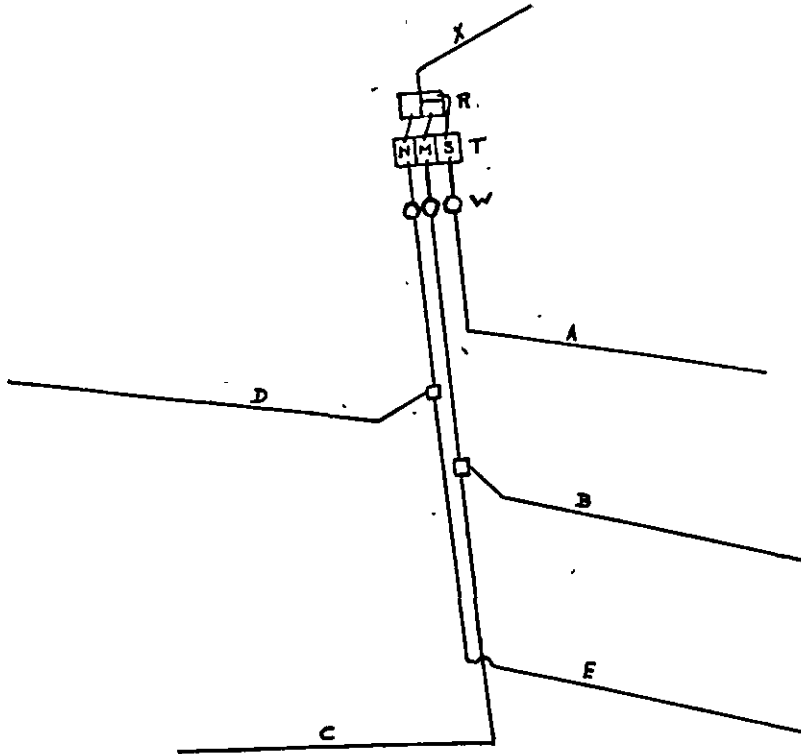


Diagram I. Plan of Septic Tank Installation and Disposal Field.

- X, line from sewer main.
- R, receiving chamber.
- T, septic tanks.
 - N, north tank, 8 hours retention.
 - M, middle tank, 12 hours retention.
 - S, south tank, 24 hours retention.
- W, collecting wells.
- A, B, C, D, E, disposal lines.

OPERATION OF TANKS

Operation of the tanks was begun in September, 1943, and was continued until hard freezing weather and snow in late December made it necessary to discontinue. No data from that period are included in this report because operation was in the experimental stage and a certain amount of trial-and-error procedure was required to detect faults in construction and to make plans to remedy them.

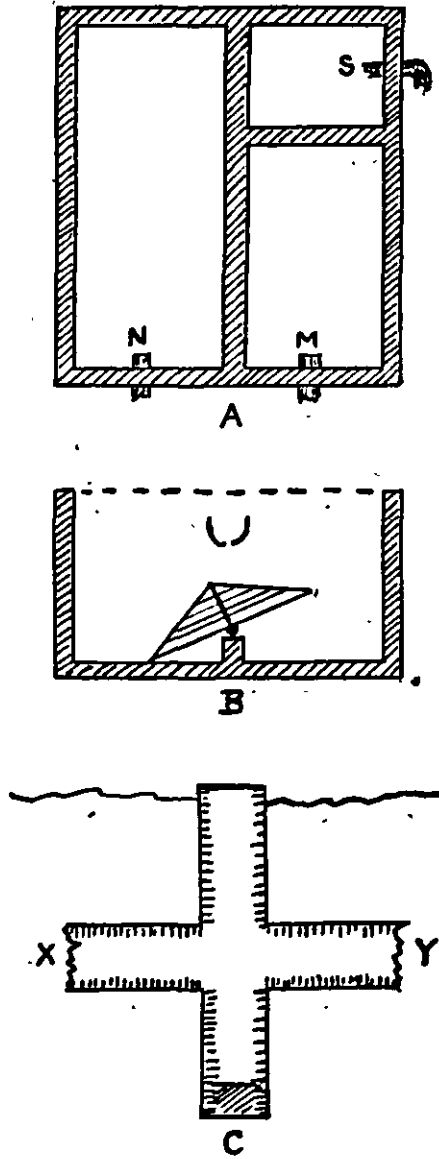


Diagram II. Details of Receiving Chamber, Tip Bucket, and Collecting Wells.

- A, Receiving chamber, viewed from above.
N, M, and S, outlets to north, middle, and south tanks.
- B, Cross-section of tip bucket.
U-shaped cross-section above is the supply trough from the sewer main.
- C, Vertical cross-section of a collecting well.
X-Y is line of flow from tank to disposal line.
Wavy line is ground level.
Top of vertical tile is open and bottom is plugged with cement to form well.

In the spring of 1944 the tanks were pumped out, the new receiving chamber with the tip-bucket was built, and operation was started as soon as the dormitory was occupied which was in the latter part of September. The installation required attention only about twice each day to record the amount of flow as indicated by the counter on the tip-bucket and to remove toilet paper that accumulated in the manhole on the dam that diverted the sewage to the line serving the tanks.

At the end of four weeks, the accumulation of scum in the north and middle tanks (8 and 12 hours retention respectively) was about 2 inches; in the south tank (24 hours retention), about 1 inch. By December 1, about two and one-half months after operation was started, so much scum had accumulated in the north tank that the outlet was plugged, and the middle tank was nearly in the same state. The south tank had only about $2\frac{1}{2}$ inches of scum. The outlet pipes had vertical "T" arms extending 5 inches below the outlet level, which will give an idea of the accumulation of scum in the north and middle tanks. An examination of the scum in these tanks revealed that it contained considerable quantities of undigested solid matter of the sewage.

Cold weather dictated the closing of the tanks for a time because cleaning was impracticable. In late March, 1945, the scum was removed and operation was resumed. At the end of April the cadets left the dormitory, so no further results were obtained. Some examinations of effluents were made and are reported later.

After the second closing of the tanks the contents were removed and the horizontal "T" arms of the inlet and outlet pipes in the north and middle tanks were exchanged for longer arms that reached 12 inches below the levels of the horizontal inlet and outlet pipes. The tanks were put in operation again in late September, 1945. The scum began to form promptly in all three tanks, and in six weeks was about 6 inches thick in the north tank, 5 inches in the middle tank, and about $1\frac{1}{2}$ inches in the south tank. By December 26, the thickness of the scum was 13 inches in the north tank, 11 inches in the middle tank, and about $2\frac{1}{2}$ inches in the south tank. Again, examination of the scum in the north and middle tanks revealed the presence of substantial amounts of undigested solid matter of the sewage, while there was practically none in the scum in the south tank.

This portion of the investigation gave results in favor of the conventional 24-hour retention period recommended for and commonly employed in septic-tank operation. The rapid accumulation of scum with its content of undigested solid matter in the north and middle tanks indicated that the rapid flow of sewage prevented efficient functioning in them as compared with the south tank which functioned in the usual manner of septic tanks, and the thickness of the scum in the north and middle tanks was all out of proportion to the capacity of the tanks.

DISPOSAL FIELD

The disposal field was laid out in anticipation of the volume of flow from the tanks. The south, or normal-flow, tank was provided with a lateral 50 feet long; the middle tank with two laterals 50 feet long each; and the north tank with two laterals 75 feet long each. Thus, the ratio of the lengths of the laterals was the inverse of the sewage-retention periods of the tanks. These lines are shown in diagram I. The soil of the field was light silty loam. The disposal lines were laid in trenches excavated to a depth of 20 inches. The width was 18 inches at the bottom and 22 inches at the top. The top of the drain tile was 11 inches below the surface of the ground. The trenches were filled with gravel to one inch above the tiles. The remainder of the fill was the soil taken from the trenches.

While the tanks were being operated the disposal lines were watched to observe any evidence of excessive drainage from the tanks. None was observed from the middle and south tanks but, mid-way through the October-to-December period of 1944, the soil about the laterals of the north tank was definitely damp and remained that way until the ground froze late in December. Around the end of the lower lateral, water was visible on the surface of the ground, particularly at the end of the line, and the ground was so soft that one stepping on it would sink in the mud to his shoe tops. The ground water table at the season was not unusually high. The situation during the second season (October to December, 1945) was similar except that the soil around the lower lateral from the north tank was not quite so wet and no water appeared on the surface.

Reference to the surveyor's notes indicated that the drop of the more distant line of the north tank was somewhat sharper than it should have been; but, even so, the results of these observations indicated that the flow from the tank with the 8-hour retention period was too heavy to be taken care of satisfactorily by the length of disposal line provided. If economy of size is sought in the construction of rural septic-tank installations, it would seem advisable to economize on the disposal line rather than on the tank, particularly if only a limited amount of space is available for disposal.

BACTERIOLOGICAL EXAMINATION OF EFFLUENTS FROM TANKS

Samples of the effluents were collected from the wells on the disposal lines previously described and shown in diagram I. The collection schedule is shown in table 1. Samples were collected in sterile glass dippers which were pyrex beakers of suitable size with long wire handles attached to permit them to be dipped into the wells. A separate dipper was employed for each well, and samples were collected into sterile, wide-mouth, glass-stoppered bottles with a capacity of 12 fluid ounces. Samples were taken directly to the laboratory, which was within a few hundred yards of the tanks, and setting up of the bacteriological tests was begun promptly and carried through as expeditiously as possible. In no instance were the samples stored before they were examined.

TABLE 1.— DATES OF COLLECTION OF SEWAGE EFFLUENTS FROM SEPTIC TANKS.

Period I	
1944:	October 17, 24, 30 November 8, 14, 20, 29
1945:	March 30 April 12, 18, 24
Period II	
1945:	October 5, 10, 17, 24 November 1, 7, 13, 20, 28 December 3, 5, 11, 13, 17*, 19

*Four samples were taken on December 17, at 9 and 11 a.m. and 1:30 and 3:30 p.m., to observe results through one day.

Plate Counts

Plate counts were made as directed in the Standard Methods of Water Analysis (2). Dilutions were made in sterile distilled water, and one ml. quantities were plated. Difco dehydrated nutrient agar was employed as the medium. In trial tests to determine the best time and temperature for incubation, the best

growth response was obtained in plates incubated at 30° C. for 48 hours, so this procedure was employed. All counts were made in duplicate from duplicate samples, and the related counts were averaged.

The counts represented the two collection periods: I — October 10 to November 29, 1944, and March 30 to April 24, 1945, a total of ten collections; II — October 10 to December 19, 1945, a total of seventeen collections (see table 1). The counts are summarized in table 2.

TABLE 2.—SUMMARY OF PLATE COUNTS FROM SEWAGE EFFLUENTS.

Counts as numbers per milliliter of effluent.

	South Tank	Middle Tank	North Tank
Period I			
Number of collections	10	10	10
Low count	1,200,000	1,500,000	500,000
High count	7,000,000	5,000,000	5,600,000
Average of counts	3,180,000	2,660,000	2,290,000
Number of counts:			
Under 1,000,000	0	0	1
Over 1,000,000 to 3,000,000	6	8	7
Over 3,000,000 to 5,000,000	3	2	1
Over 5,000,000 to 10,000,000	1	0	1
Over 10,000,000	0	0	0
Period II			
Number of collections	17	17	17
Low count	1,000,000	1,000,000	1,600,000
High count	10,000,000	16,000,000	50,000,000
Average of counts	5,100,000	4,794,000	9,312,000
Number of counts:			
Under 1,000,000	0	0	0
Over 1,000,000 to 3,000,000	6	5	5
Over 3,000,000 to 5,000,000	3	7	4
Over 5,000,000 to 10,000,000	8	2	4
Over 10,000,000	0	3	4

The author is of the opinion that the counts would have little significance in comparing the efficiency of the tanks unless there were marked differences in the results. No such differences appeared in either collection period. One low count from the north tank in Period I lowered the average for that tank for the season. In Period II there were three high counts for the north tank and one for the middle tank. Except for this one low count and the four high counts, the spread of the counts was similar for all three tanks. The high and low deviations just mentioned did not occur a sufficient number of times among the samples to justify drawing any conclusions from them. The results of the plate counts did not appear to afford any evidence to the advantage or disadvantage of any of the three tanks of the installation.

Most Probable Number of Coliform Bacteria in Septic Tank Effluents

Testing for *Escherichia coli* and related species of bacteria, collectively termed coliform bacteria, is the recognized procedure for detecting sewage pollution in water supplies. For that reason the procedure was applied to the effluents from the three tanks to determine the effect, if any, from the three different retention periods on the numbers and types of coliform bacteria present in the effluents.

The procedures employed were those outlined in the Standard Methods of Water Analysis. Difco dehydrated media were employed. Samples of the effluent, diluted suitably to obtain optimum results, were inoculated into fermentation tubes of lactose broth, five tubes for each dilution. After incubation at 37° C. for 48 hours the numbers of tubes showing positive gas production were recorded. Then plates of eosin-methylene blue agar were streaked from these tubes to indicate the types of coliform bacteria present, and all cultures were reinoculated from the plates into lactose-broth fermentation tubes to complete the tests. Cultures were isolated from plates representing the highest dilutions of the effluents giving positive gas tests, five cultures from each sample. These cultures were subjected to differential tests to determine the distribution of types within the coliform group of bacteria. This part of the study is discussed later.

It will be noted that the incubation temperature employed throughout for the various coliform tests was 37° C., while that for the plate counts was 30° C. The reason for the difference was that in isolating and differentiating the coliform bacteria the time required to complete the tests can be shortened considerably by incubation at 37°, while the intent of the plate counts is to obtain growth of as many types of bacteria as possible. Most types, including the coliform bacteria, develop satisfactorily at 30°, and some types require the lower incubation temperature for satisfactory development.

The numbers of gas-positive fermentation tubes were calculated to determine the M.P.N. (most probable number) of coliform bacteria by the "Standard Methods" procedure, with the results summarized in table 3. One result for each tank in each collecting period was much higher than any of the others for the respective tank, so two sets of calculations were made: one set including all results, and one with the extra high figures omitted.

The results show that for the entire set of calculations of period I the middle tank (12 hours retention) had the highest average M.P.N., the north tank (8 hours retention) was second, and the south tank (24 hours retention) had the

TABLE 3.—MOST PROBABLE NUMBER (M.P.N.) OF COLIFORM BACTERIA PER MILLILITER OF SEPTIC TANK EFFLUENT.

	South Tank	Middle Tank	North Tank
Period I			
Total number of tests	11	11	11
Low M.P.N.	490,000	790,000	700,000
High M.P.N.	9,200,000	92,000,000	16,000,000
Average M.P.N.:	2,997,000	11,450,000	5,273,000
Total tests minus one extra high*	10	10	10
Low M.P.N.	490,000	790,000	700,000
High M.P.N.	5,400,000	9,200,000	9,200,000
Average M.P.N.	2,377,000	3,399,000	4,200,000
Period II			
Total number of tests	18	18	18
Low M.P.N.	70,000	140,000	210,000
High M.P.N.	54,000,000	16,000,000	17,000,000
Average M.P.N.	4,360,000	3,500,000	3,590,000
Total tests minus one extra high*	17	17	17
Low M.P.N.	70,000	140,000	210,000
High M.P.N.	5,400,000	9,200,000	5,400,000
Average M.P.N.	1,420,000	2,770,000	2,240,000

* For each collection period, one M.P.N. value for each tank was much higher than other values for that tank for the same period. Calculations were made with and without these extra high values.

lowest average. For the set of calculations with the extra high figures omitted, the north and middle tanks changed places and the south tank again had the lowest average.

For period II, all tests included, the middle tank had the highest average M.P.N., the north tank second, while the south (normal) tank had the lowest average. The same order obtained for calculations that omitted the extra high M.P.N. figure for each tank.

The individual M.P.N. values from the three tanks were scored for each sampling date, allowing three points for the highest M.P.N., two for the middle value, and one for the lowest. On that basis, for the first operating season, the north tank scored 27, the middle tank 25, and the south tank 14 for eleven tests. The numbers of high, middle, and low scores for the three tanks were as follows:

	High Score	Tied for High Score	Middle Score	Low Score
North tank	5	3	2	1
Middle tank	2	3	6	0
South tank	1	1	0	9

For the second collection period, the scores for eighteen tests were: middle tank, 41; north tank, 38.5; south tank, 28.5. The numbers were larger than for the first period because there were more tests. The numbers of high, middle, and low values were:

	High Score	Tied for High Score	Middle Score	Low Score
North tank	5	1	9	3
Middle tank	8	2	4	4
South tank	3	1	3	11

In one instance all three values were the same and were counted as a tie for high. The results from the scoring procedure show that the south tank had the lowest M.P.N. values for both seasons, which agrees with the averages shown in table 3.

When all the data are considered, the evidence favors the south tank as being more efficient than the other two in eliminating coliform bacteria from sewage. It must be remembered, however, that the north tank received three times as much sewage as the south tank, and the middle tank twice as much. This gives a ratio of 3:2:1. The M.P.N. averages for the three tanks for either operating period are closer than that, which could be taken as an indication that the 8-hour and 12-hour retention periods were relatively as efficient as the normal of 24 hours. Even if that were true, however, the net result would be the liberation in 24 hours of fewer coliform bacteria in the effluent from the south tank than from either of the other two tanks.

Differential Distribution of Coliform Bacteria Isolated from Positive Fermentation Tests

The cultures included in this part of the study were isolated, as was mentioned previously, from eosin-methylene blue plates, five cultures from the highest dilutions of each sample that had given positive gas tests in lactose broth. The cultures were purified according to accepted methods (3) and then were put through the so-called "IMViC" tests (4). These are indol production (I), the methyl-red test (M), the Voges-Proskauer test (V) and growth in sodium citrate (C). The letter "i" is included to complete the word.

Indol is produced in tryptophane broth by *Escherichia coli* and related strains of the group and is considered to be a characteristic of coliform bacteria of sewage origin. The same is true of a positive methyl-red test which means that the coliform bacteria of sewage origin will produce sufficient acid in a medium containing dextrose to give a positive acid reaction with the methyl-red indicator. The Voges-Proskauer reaction depends on the production of a substance known as acetyl-methyl-carbinol from dextrose, and a positive test is characteristic of *Aerobacter aerogenes* and other coliform bacteria of non-sewage origin. The same is true of a positive sodium-citrate test which means the capacity of the bacteria to grow in a medium containing sodium citrate as the sole source of necessary carbon. The *Citrobacter* (5), closely related to *E. coli*, violates this rule because it gets its name from its ability to utilize sodium citrate. Of these tests, the methyl-red test is regarded by many authorities as the most dependable single test for sewage-type coliform bacteria. A positive methyl-red test may occur with any combination of reactions to the other three tests.

TABLE 4. — CLASSIFICATION OF COLIFORM BACTERIA FROM SEPTIC TANK EFFLUENTS EXPRESSED AS PERCENTAGES^S OF TOTAL CULTURES ISOLATED FROM THE RESPECTIVE TANKS.

	Percentages of Total Cultures		
	Period I	Period II	Both Seasons
South Tank			
<i>Escherichia coli</i>	36.7	75.4	56.0
<i>Citrobacter</i>	38.0	4.6	20.4
Methyl-red-positive intermediates	6.9	10.8	8.9
Total methyl-red-positive organisms	81.0	90.8	86.2
<i>Aerobacter aerogenes</i>	0	3.1	1.6
Methyl-red-negative intermediates	19.0	6.1	12.2
Middle Tank			
<i>Escherichia coli</i>	32.7	79.5	59.0
<i>Citrobacter</i>	36.5	1.5	16.7
Methyl-red-positive intermediates	15.4	8.8	11.7
Total methyl-red positive organisms	84.6	89.8	87.4
<i>Aerobacter aerogenes</i>	0	7.3	4.2
Methyl-red-negative intermediates	15.4	2.9	8.4
North Tank			
<i>Escherichia coli</i>	36.5	64.6	52.1
<i>Citrobacter</i>	36.5	3.1	18.0
Methyl-red-positive intermediates	5.8	7.7	6.8
Total methyl-red-positive organisms	78.8	75.4	76.9
<i>Aerobacter aerogene</i>	0	10.7	6.0
Methyl-red-negative intermediates	21.2	13.9	17.1

Results of the differential tests, as seen in table 4, are based on the following group reactions:

	Indol	Methyl-red	Voges-Proskauer	Sodium Citrate
<i>Escherichia coli</i>	+	+	-	-
<i>Citrobacter</i>	+	+	-	+
<i>Aerobacter aerogenes</i>	-	-	+	+

Organisms not conforming to the reactions in this tabulation are termed intermediates. For the purpose of this report the intermediates encountered were placed in two groups, methyl-red positive and methyl-red negative, to relate them to either *E. coli* or *A. aerogenes*.

It may be noted here that the term "Citrobacter" is employed by sanitary bacteriologists for the purposes of sanitary classification but is not recognized in biological classifications.

Reference to table 4 shows that more than 75 percent of all the cultures isolated were methyl-red positive, as was to be expected. Certain differences appeared between the results for periods I and II. In period I the Citrobacter percentages were considerably higher and those of *E. coli* were lower than for period II, but the totals of methyl-red positive cultures were similar for both periods. Also, no *A. aerogenes* cultures were isolated in period I, but they were encountered in period II. The total percentages of methyl-red negative cultures naturally were in accord with those of the methyl-red positives.

The result of the differential studies did not appear to favor any one of the tanks over the other two for either of the collection seasons or for the totals of the two.

CHEMICAL EXAMINATION OF EFFLUENTS FROM TANKS

The chemical examinations of the effluents, reported here, were made in the local laboratory of the Massachusetts Department of Public Health by Mr. Ernest A. Snow, Senior Chemist of the Division of Sanitary Engineering. The scope of the examinations was limited by the time at Mr. Snow's disposal for the work, so it was decided to make the tests that it was felt would give the best indication of the efficiency of the tanks in digesting the solid matter of the sewage. These tests were the determination of the biochemical oxygen demand, commonly called B.O.D., and of the free-ammonia nitrogen in the effluents from the three tanks. The tests were made on the samples collected for the bacteriological examinations of effluents reported in this paper.

Biochemical Oxygen Demand

The test for the biochemical oxygen demand depends upon the fact that oxygen in a sewage suspension is used up in the process of digesting suspended organic matter. Thus, the higher the B.O.D. value in a test, the greater is the amount of undigested matter in the sewage or, in this instance, the effluents from the tanks. The techniques employed for the tests were those of the Standard Methods for the Examination of Water and Sewage (2). Tests were made only in the second operating period, fall and early winter, 1945-6. Results are summarized in table 5.

On the basis of the averages and of the score (explained in note at bottom of table 5) the biochemical oxygen demand for the south tank was considerably less than for the other two tanks, which had values close together. These results indicate that the most nearly complete digestion of the sewage solids took place in the south tank which had the normal retention period; but it should be noted that the B.O.D. values and the scores for the three tanks were closer together than the 3:2:1 volume ratio of sewage passing through them. This could indicate that, considering greater volumes of sewage treated in the north and middle tanks, digestion of sewage solids was relatively more efficient in them than in the south tank.

TABLE 5.—SUMMARY OF BIOCHEMICAL OXYGEN DEMAND TESTS FROM SEPTIC TANK EFFLUENTS.

	South Tank	Middle Tank	North Tank
Number of tests	12*	13	13
Parts per million of oxygen			
Low	78	102	122
High	228	373	493
Average	124	211	221
Score*	15	27	30

* The number of tests represents the number of collection dates. There are 12 tests instead of 13 for the South Tank because the value for one of its tests was so much higher than any of the other values that it was considered a probable error and discarded. The average for this tank was based on the 12 tests. The scores were based on the same 12 tests for each tank: 3 points for the high value, 2 points for the middle value, and 1 point for the low value for each collection date.

The main point at issue, however, is the quality of the effluents, and in this the south tank would have to be considered superior on the basis of B.O.D. determinations. This decision agrees with the amounts of scum that accumulated in the three tanks and with the observation that the scum in the north and middle tanks contained considerable sewage solids which had disintegrated very little.

Free Ammonia Determinations

Free ammonia in the effluent results from the bacterial decomposition of the protein portion of the sewage solids. Consequently the amount of ammonia recovered in the tests and expressed as nitrogen would be interpreted as a direct indication of the relative activity of digestion of sewage solids in the tanks. If the total digestive efficiencies of the three tanks were in proportion to the 3:2:1 volume ratio of sewage handled by the tanks, then the south tank effluent should have shown much the smallest quantity of ammonia. Reference to table 6, however, shows that the averages for the three tanks were nearly the same and that the score for the south tank was only a little lower than the scores for the other two tanks. This agrees with the results of the B.O.D. determinations and with the presence of undigested sewage solids in the scum in the north and middle tanks.

TABLE 6.—SUMMARY OF DETERMINATIONS OF FREE-AMMONIA NITROGEN FROM SEPTIC TANK EFFLUENTS.

	South Tank	Middle Tank	North Tank
Number of tests*	15	18	18
Parts per million of nitrogen			
Low	18.0	13.0	10.0
High	61.0	48.0	50.0
Average	27.0	28.2	27.0
Score*	30.5	38.5	39.0

* The 18 tests represent 18 collection dates which include the dates represented by the B. O. D. tests of table 5. There are fewer B. O. D. tests than ammonia tests because facilities were not available for making the B. O. D. test on all of the collection dates. The scores are based on a comparison of values for the three tanks on each collection date: 3 points for the high value, 2 points for the middle value, and 1 point for the low value. The fractions are accounted for by the north and middle tanks having the same values on several dates.

BACTERIOLOGICAL EXAMINATION OF DISPOSAL FIELD SOILS

This examination was undertaken to determine the extent to which the coliform bacteria from the effluent had penetrated into the soil, and the permanence of the several species in the soil environment. Soil samples were taken at the points indicated as A, B, C, D, and E in diagram I. Samples were taken on both sides of each disposal line about midway of its length at the level of the tile and two feet from the line. In the first sampling season samples were taken also at two feet and five feet from the end of each line. In the second season samples were taken from both sides of each line as before, but not at the ends. The reason was that, in the cultivation of a field that surrounded the disposal area, the soil at the ends of lines C, D, and E had been plowed practically up to the tile ends and it was felt that the soil was so stirred up that representative samples could not be obtained. Line C was one of two from tank M (12 hours retention) and lines D and E were the two lines from tank N (8 hours retention). Control samples were taken from areas in the same field that received no effluent from the tanks.

The first sampling season was in May, 1945, after the tanks had been in operation through the previous fall and early winter and again in the spring; and the second season was in May and early June, 1946, after the tanks had been operated through the previous fall and early winter (table 1). Reasonable precautions were taken to collect the samples aseptically.

Samples were screened separately through ordinary window screen. Then portions were weighed, air-dried in the laboratory, and re-weighed to establish a basis for making dilutions of the moist soil in terms of dried soil. The samples were kept in closed containers to keep them as nearly as possible in the same condition as when they were taken from the field. Quantities of the samples sufficient to give 10-gram portions of air-dried soil were suspended in dilution bottles containing 100 ml. each of sterile distilled water, giving an initial 1 to 10 dilution. Further dilutions were made from these suspensions.

Since the main interest was in recovering coliform bacteria from the soils, the Standard-Methods procedure (2), as employed for the examination of water and sewage, was followed. This technique is the same as that employed for the study of coliform bacteria from the tank effluents as reported earlier in this paper.

Preliminary trials had indicated that dilutions of 10^1 , 10^2 , 10^3 , and 10^4 were best adapted for the purpose, so five fermentation tubes of lactose broth were inoculated from each of these dilutions of each soil, 1 ml. of suspension to each tube. The inoculated tubes were incubated at 37°C . and read daily for gas production. No tube was discarded as negative until after 72 hours of incubation. Eosin-methylene blue plates were streaked from all gas positive tubes and incubated at 37°C . Cultures were isolated from the plates on which growth occurred and were purified and held on nutrient agar slants for further study. Some of the gas-positive cultures were false-positive tests caused by organisms other than the coliform group. These cultures failed to give growth on the eosin-methylene blue plates. The agar-slant cultures were differentiated later on the basis of the "IMViC" tests.

Tables 7 and 8 give a summary of the results of the fermentation and differential tests. Table 9 is a statement of the results of the fermentation tests, from table 7, expressed as percentages of the numbers of fermentation tubes inoculated from the soil samples of a particular disposal line. Results from the several samples for each line respectively were so much alike that all six sets of results from the first series were combined, and all four from the second series, for each

line respectively. Table 10 shows the differential tests of table 8 expressed as percentages of the total numbers of coliform cultures isolated from the soil adjacent to particular disposal lines.

In evaluating the data of the tables the writer takes the position, stated earlier in this paper, that differences in the results obtained for the several disposal lines from the three tanks should not be considered significant unless they were pronounced. That was not true of the data obtained in this study. The results may be summarized as follows.

Gas-Positive Tests

For the first sampling season, the greatest number of positive tests (50 to 65 percent) was obtained at the 48-hour reading. The number of positive tests at 24 hours was about balanced with the number at 72 hours. This meant, therefore, that roughly three-fourths of the gas-positive tests were obtained at 48 hours or later. The control samples gave similar results. *Escherichia coli* from fresh sewage pollution usually gives a positive gas test in 24 hours or less, while the delayed reactions usually are given by non-sewage soil types or by false-positive reactions. On the other hand, the soil types may give positive results in 24 hours. All of this indicates that a majority of the coliform bacteria recovered from the soil were either of non-sewage origin; or that, if they were of sewage origin, their differential reactions had been altered by their sojourn in the soil. This latter possibility has been suggested by several investigators but never proved.

For the second sampling period the percentage of gas-positive results for 48 hours was again the largest; but it was lower and the 24-hour and 72-hour percentages were higher than for first sampling period. The majority of the gas-positive tests were obtained in 48 or 72 hours of incubation as in the first period. There was a considerable discrepancy between 48-hour results from the two lines of the middle tank, but its significance, if any, is not apparent.

The percentage of gas-negative tests was higher in the second period than in the first. In the first period the percentages for one line from the middle tank and one from the north tank were considerably higher than for the other lines. However, the high percentage for the middle tank was for the line nearest the tank, while the high percentage for the north tank was for the more distant line. The percentages of gas-negative tests from the control soils were similar to those from the several disposal lines except for the two high percentages just mentioned.

The percentages of false-positive tests were higher, in general, for the first sampling period than for the second. The distribution of values for the several lines does not lend itself to any particular interpretation. In the first period the highest value was for the control samples and the lowest, about half as great, was for the near line of the middle tank. The value for the other line of this tank, however, was nearly as large as that for the control. In the second period the value for the more distant line of the north tank was the lowest of the series, and that for the control samples was also low as compared to the high.

In general, the results throughout the two seasons were heterogeneous and did not indicate any preponderant values, either high or low, for any of the three tanks. The preponderance of gas-positive values at 48 and 72 hours incubation indicates that the cultures recovered were probably not from sewage but rather were native to the soil.

TABLE 7.—SUMMARY OF RESULTS OF FERMENTATION TESTS FOR COLIFORM BACTERIA IN DISPOSAL FIELD SOILS, 1945 AND 1946.

Disposal line	South Tank		Middle Tank		North Tank		Controls
	A	B	C	D	E		
Summer of 1945							
Number of tests	120	120	120	120	120	140	
Positive tests							
24 hours incubation	17	8	19	14	30	22	
48 hours incubation	65	65	40	39	44	54	
72 hours incubation	6	15	11	7	11	25	
Total positive tests	88	88	70	60	85	101	
Negative tests	32	32	50	60	35	39	
Analysis of positive tests							
Coliform positives	63	69	36	35	52	61	
False positives*	25	19	34	25	33	40	
Summer of 1946							
Number of tests	80	80	80	80	80	80	
Positive tests							
24 hours incubation	18	16	23	17	6	18	
48 hours incubation	27	43	22	29	26	25	
72 hours incubation	10	8	13	11	21	21	
Total positive tests	55	67	58	57	53	64	
Negative tests	25	13	22	23	27	16	
Analysis of positive tests							
Coliform positives	40	51	36	43	44	51	
False positives*	15	16	22	14	9	13	

* False positive tests produced by bacteria not of the coliform group.

Note: South tank, normal 24 hours retention; middle tank, 12 hours retention; north tank, 8 hours retention. Controls were soils from the same area that had not received sewage effluent.

TABLE 8.—ANALYSIS OF POSITIVE COLIFORM TESTS FROM TABLE 7.

Disposal line	South Tank		Middle Tank		North Tank		Controls
	A	B	C	D	E		
Summer of 1945							
Total coliform cultures	63	69	36	35	52	61	
Escherichia coli	2	1	0	1	1	1	
Citrobacter	0	1	0	0	0	0	
Aerobacter aerogenes	11	19	13	14	30	31	
Coliform intermediates							
Methyl-red positives	20	15	5	6	6	6	
Methyl-red negatives	30	33	18	14	15	23	
Summer of 1946							
Total coliform cultures	40	51	36	43	44	51	
Escherichia coli	0	1	0	1	0	0	
Citrobacter	0	1	1	4	0	0	
Aerobacter aerogenes	28	29	24	26	25	38	
Coliform intermediates							
Methyl-red positives	2	5	5	3	6	2	
Methyl-red negatives	10	15	6	9	13	11	

Note: South tank, normal 24 hours retention; middle tank, 12 hours retention; north tank, 8 hours retention. Controls were soils from the same area that had not received sewage effluent.

TABLE 9.—RESULTS OF FERMENTATION TESTS FROM TABLE 7, EXPRESSED AS PERCENTAGE; OF NUMBER OF TESTS MADE.

	South Tank		Middle Tank		North Tank		Controls
	A	B	C	D	E		
Disposal line							
Summer of 1945							
Number of tests	120	120	120	120	120		140
Positive tests							
24 hours incubation	14.3	6.7	15.8	11.7	25.0		15.7
48 hours incubation	54.0	54.0	33.4	32.5	36.6		38.6
72 Hours incubation	5.0	12.6	9.2	5.8	9.2		17.8
Total positive tests	73.3	73.3	58.4	50.0	70.8		72.1
Negative tests	26.7	26.7	41.6	50.0	29.2		27.9
Analysis of positive tests							
Coliform positives	52.5	57.5	30.0	29.2	43.3		44.5
False positives*	20.8	15.8	28.4	20.8	27.5		28.6
Summer of 1946							
Number of tests	80	80	80	80	80		90
Positive tests							
24 hours incubation	22.5	20.0	28.8	21.2	7.5		22.5
48 hours incubation	33.7	53.7	27.5	36.2	32.5		31.3
72 hours incubation	12.5	10.0	16.2	13.8	26.2		26.2
Total positive tests	68.7	83.7	72.5	71.2	66.2		80.0
Negative tests	31.3	16.3	27.5	28.8	33.8		20.0
Analysis of positive tests							
Coliform positives	50.0	63.7	45.0	53.7	55.0		63.7
False positives*	18.7	20.0	27.5	17.5	11.2		16.3

*False positive tests produced by bacteria not of the coliform group.

Note: South tank, normal 24 hours retention; middle tank, 12 hours retention; north tank, 8 hours retention. Controls were soils from the same area that had not received sewage effluent.

TABLE 10.—ANALYSIS OF POSITIVE COLIFORM TESTS OF TABLE 8, EXPRESSED AS PERCENTAGES OF NUMBER OF COLIFORM CULTURES RECOVERED.

	South Tank		Middle Tank		North Tank		Controls
	A	B	C	D	E		
Disposal line							
Summer of 1945							
Total coliform cultures	63	69	36	35	52		61
Escherichia coli	3.2	1.4	0	2.9	1.9		1.6
Citrobacter	0	1.4	0	0	0		0
Aerobacter aerogenes	17.4	27.5	36.2	40.0	57.7		51.0
Coliform intermediates							
Methyl-red positives	31.8	21.7	13.8	17.1	11.5		9.8
Methyl-red negatives	47.6	48.0	50.0	40.0	28.9		37.6
Summer of 1946							
Total coliform cultures	40	51	36	43	44		51
Escherichia coli	0	2.0	0	2.3	0		0
Citrobacter	0	2.0	2.8	9.3	0		0
Aerobacter aerogenes	70.0	57.0	66.5	60.5	57.0		74.6
Coliform intermediates							
Methyl-red positives	5.0	9.7	13.9	7.0	13.6		3.9
Methyl-red negatives	25.0	29.3	16.7	21.0	29.4		21.5

Note: South tank, normal 24 hours retention; middle tank, 12 hours retention; north tank, 8 hours retention. Controls were soils from the same area that had not received sewage effluent.

Differential Tests of Coliform Bacteria

A summary of the results of the differential tests on the cultures from the soils appears in table 8 and the differential tests of that table are expressed in table 10 as percentages of coliform organisms isolated from each tank effluent for each sampling season.

It is significant that, of the coliform bacteria recovered from the soils, very few were either *Escherichia coli* or their close relatives, *Citrobacter*, which table 4 shows were present in considerable numbers in the effluents and which are considered to be indicative of sewage pollution. The lack of *E. coli* and *Citrobacter* was observed in both sampling seasons and was consistent with the fact, already mentioned, that a preponderance of the gas-positive tests obtained required 48 or 72 hours of incubation. The percentages of methyl-red positive intermediates were generally lower for the second sampling season than for the first. The highest percentage of these positives in the first season was obtained from the south tank (24-hour retention) and the lowest percentage was from the controls. Accepting the opinion of certain authorities in the field of sanitation, this observation could argue in favor of the 8-hour and 12-hour retention as being more efficient than the normal of 24 hours in removing sewage types of coliform bacteria from the effluents. However, in the second sampling season the lowest percentages of methyl-red positive intermediates were obtained from samples adjacent to the south-tank line and from the control samples. *Aerobacter aerogenes* and methyl-red negative intermediates predominated for all samples and for both sampling seasons. There were no differences that the writer can call significant. In the second season there were more of the *A. aerogenes* cultures than of the methyl-red negative intermediates.

In general, it may be said that the data agree with those for the gas-positive tests and indicate that the bacteria recovered from the soil samples may well have been native to the soils rather than invaders from the septic-tank effluents. This raises a question: what becomes of the sewage-type coliform bacteria when they enter the soil? This will be the basis of a subsequent study.

SUMMARY.

The experimental results may be summarized as follows:

1. **Management.** As the tanks were being operated for two seasons it became evident that decidedly too much scum accumulated in the two tanks with retention period: of 8 and 12 hours respectively. The layer of scum in each of these tanks formed rapidly and became so thick that the inlet and outlet pipes to the tanks became plugged in about three months and use of the tanks had to be suspended. This was, in spite of special construction in the second season designed to permit much greater accumulation of scum than would be anticipated in normal tank operation. The scum in these two tanks contained much undigested solid matter of the sewage. A normal amount of scum accumulated in the tank with 24 hours retention.

The amount of effluent passing from the tank with 8 hours retention appeared to be greater than the capacity of the disposal field could take care of. The ground about the two lateral lines, particularly the more distant one, was water-logged and water stood on the surface part of the time. This effect may have been influenced in part by the grade drop of the lines.

Thus, from a management point of view, the shorter retention periods would seem to have limitations that would render them unsuitable for practical sewage disposal.