

A NEW METHOD FOR DETERMINING THE RELATIVE STABILITY OF A SEWAGE, EFFLUENT, OR POLLUTED RIVER WATER.*

ARTHUR LEDERER.

(From the Sanitary District of Chicago.)

In my paper entitled, "The Relation of the Nitrates to the Putrescibility of Sewages,"¹ I mentioned briefly the possibility of obtaining accurate information on the biologic-oxygen-consuming power of a sewage by means of the addition of definite amounts of saltpeter. The method has since been tried in the laboratory of the Sanitary District, as well as in field work, with very satisfactory results. The present paper is devoted to the discussion of the technic of the method, the results obtainable, the possibility of its application, and its advantage over other methods.

While comparing the effect upon stability of the dilution of the waters of the Drainage Canal with that produced by adding various amounts of saltpeter representing different amounts of oxygen, I found a very close agreement of the results obtained; i.e., a definite amount of atmospheric dissolved oxygen resulted in an improvement equivalent to the addition of the same amount of saltpeter oxygen as judged by the methylene blue putrescibility test. I assumed at the start that one saltpeter molecule gives off three oxygen atoms in the reduction process. However, on closer and more recent observations I have concluded that only two and one-half atoms are utilized. The amount of methylene blue employed was 0.4 c.c. of a 0.05 per cent aqueous solution per 150 c.c. bottle capacity. My former investigations showed that absolute figures could not be obtained with the methylene blue putrescibility test, because the quantity of methylene blue solution as recommended in the *Standard Methods of Water Analysis* retards decolorization by its germicidal property. When 0.4 c.c. of the coloring matter was added instead of 1.0 c.c., the end point coincided fairly closely

* Received for publication February 21, 1914.

¹ *Jour. Infect. Dis.*, 1913, 13, p. 236.

with the elimination of the total available oxygen in the original liquid. It is clear, therefore, that the relative stability figures assumed to designate the ratio between the available oxygen and the oxygen required for complete oxidation could not be identical where different amounts of methylene blue were employed. They will be lower the smaller the quantity of the dye. Just what the true relative stability figure would be in each individual case can be obtained only by actually determining analytically the amount of available oxygen present at the start and the additional amount of oxygen needed after incubation in a tightly stoppered bottle at 20° C. for 20 days, or at 37° C. for 10 days.

One method, most readily available for determining the biologic oxygen requirements, is the dilution method, which is as follows:

First determine the free oxygen, nitrite, and nitrate oxygen (it is possible that under certain conditions the oxygen available from other sources would have to be taken into account) in the original sewage or effluent. The sum of these constitutes the total available oxygen. Then carefully make different dilutions of the sewage with fresh water, saturated with atmospheric oxygen, in glass cylinders or other suitable vessels. This part of the procedure is tedious and requires great care, as there is danger of artificially aerating the mixture. With a stiff wire, spiral shaped at the bottom, such as I have employed in this method, no appreciable aeration occurs in the hands of a careful worker. Nevertheless, the fact remains that the dilutions require time and care and cannot be conveniently carried out in the field. The oxygen content of the fresh water employed for dilution purposes must be noted. After the dilutions have been made, the mixtures are carefully syphoned off into 5-ounce bottles containing 0.4 c.c. of a 0.05 per cent solution of methylene blue. The bottles are properly stoppered, tagged for identification, and shaken to obtain thorough distribution of the dye. We now know the initial available oxygen, as well as the oxygen which was added to the various bottles. After incubation at 20° C. for 20 days, a note is made of the dilution, which was just sufficient to retain the blue color. From this dilution can be calculated the total quantity of oxygen required per liter of sewage or effluent. The ratio of this amount and the oxygen available at the start will constitute the real relative stability. For practical purposes, it is not always necessary to incubate the samples for 20 days, except when the color appears pale during the time of incubation, as the biologic-oxygen-absorption curve is usually flattened out after 10 days to such an extent that the oxygen requirements of the following 10 days appear to be a comparatively small quantity. An experienced observer on routine samples can tell after 10 days' incubation whether or not the color is likely to disappear during the second half of the required incubation period. With a very putrescible sewage or trade-waste it is difficult to prepare high dilutions. A trade-waste may require a dilution of 100 to 500 times its volume of fresh water; in other words, 10-2 c.c. of sewage per 1,000 c.c. of fresh water. Often the character of the trade-waste makes it impossible to obtain a uniform suspension and one can readily see that with such a highly putrescible liquid this might make an appreciable difference in the result. Still

another difficulty is that the wastes of dye-houses, tanneries, gas works, etc., are very often so highly colored that it is impossible to observe the decolorization of the methylene blue. Again, the addition of some wastes or sewages will result in the absorption of the methylene blue by colloids. Provided there is no other coloring matter present, an experienced observer will have no difficulty in telling when the blue color in the sediment has disappeared. Very often, a bluish-green color precedes the formation of the colorless leukobase. Another drawback to this method is that even a skilled worker can hardly make more than four or five such tests during the working day of eight hours. In field work this becomes well-nigh impossible unless the laboratory is close by, altho a sample can be preserved at the start for the nitrite and nitrate examinations. The free oxygen should be determined on the spot, since a delay in this method incurs unreliable results.

Incubations of mixtures of sewage and fresh water for short periods to determine the biologic-oxygen-consumption serve a useful purpose in giving a fair approximation of the "strength" of a sewage, but they do not furnish concrete information on the complete oxygen requirements. Short-time incubation tests are convenient for those eager to obtain quick results, but unless the dilutions, time, and temperature of incubation are uniform, various workers will undoubtedly find it difficult to reconcile results. The rate of exhaustion during the first six hours may not continue during the next six hours or so. Yet there is no doubt that a knowledge of the rate of exhaustion during the first few hours, in connection with a knowledge of the total oxygen requirement of a sewage or stream, serves a valuable purpose. Both should be standardized as methods to furnish a definite basis for adjustment of stream pollution, as attempted by the English Royal Sewage Commission. Such standardization must be undertaken, however, with a view to suiting the conditions peculiar to the United States.

Altho incubations in glass bottles give comparative results, the actual changes in a river will only measure up relatively to the oxygen requirements obtained by such tests. The important factors in the absorption of atmospheric oxygen from a free surface and sunlight are necessarily omitted in the technic proposed by me. These two factors alone may, in many cases, easily account for a difference of 50 per cent in the results between the study under closed and under open conditions. Yet I believe it is better to exclude such variable factors in any attempt to standardize these tests for the sake of obtaining uniformly comparable results.

As previously noted, any method for the determination of the oxygen required for complete oxidation should be simple and convenient. The troublesome features of the dilution method can be largely eliminated by substituting saltpeter oxygen for atmospheric oxygen. A large number of tests, carried on in the laboratory of the Sanitary District and in the field, indicate the superiority of the use of the "saltpeter" method over the "dilution" method.

The technic of the method proposed by me is as follows:

To a number of 150 c.c. bottles containing 0.4 c.c. of a 0.05 per cent solution of methylene blue add varying quantities of saltpeter (a solution of sodium nitrate,

C.P.) representing definite oxygen equivalents. For instance, if the capacity of the bottles used is 150 c.c. and oxygen equivalents are required of 5, 10, 15, 20, and 25 p.p.m., it is best to prepare a stock solution containing 3.19 gm. of sodium nitrate per liter, 1 c.c. of which is equivalent to 1,500 milligrams of oxygen. One cubic centimeter of the solution to 150 c.c. capacity is equivalent to 10 p.p.m. of oxygen. The strength of the sodium nitrate solution can be varied to meet different conditions. When testing the oxygen-consuming capacity of a sewage which may utilize 100 p.p.m. of oxygen or more, it is desirable to employ a nitrate solution of 10 times this strength. If the capacity of the bottle is 150 c.c., the amount of nitrate solution 2 c.c., and the amount of methylene blue solution approximately 1 c.c., there are in the bottle actually only 147 c.c. of sewage, representing the quantity upon which to base calculations. The nitrate solution is fairly stable. Any marked deterioration can be recognized by testing for nitrites which are formed by bacterial reduction. At the time of the collection of a sample of sewage or river water, the nitrites and nitrates should be determined on the spot, or else a sample should be preserved for analysis. The dissolved oxygen is best determined at once. The sample of sewage or river water should be collected in a bottle or cylinder and syphoned off immediately into the various methylene blue bottles, which should be completely filled to prevent settling. The stoppered bottles are shaken to permit an efficient distribution of saltpeter and methylene blue solution, and then placed into the incubator for observation. The more bottles employed with a range of concentration, the better are the chances of accurately determining the biologic-oxygen-consumption or true relative stability. With a badly contaminated river water, it might be wise to add saltpeter in equivalents of 2, 4, 6, 8, 10, and 12 p.p.m. of oxygen, respectively. For a plain domestic sewage, 100, 130, 160, 190, 210, and 240 p.p.m. of oxygen would probably give the desired result. For a trade-waste, much higher dilutions may be required, and it becomes a question of using more bottles until working limits are defined. For colored trade-wastes this method is not suited, as the blue color is obscured. Another method, likewise based upon the principle of nitrate reduction and employed to advantage in such cases, will be described later. Frequently, the rate of oxygen exhaustion, after short-time incubation, may furnish a valuable clue to the approximate quantity of nitrate oxygen required in the "saltpeter" method. Clearly, the procedure of adding saltpeter in place of preparing dilutions with fresh water saves much time, and makes it easily possible to prepare four to five tests in place of one. This, again, means that the average "strength" of a sewage or effluent or stream, which may vary considerably during 24 hours, can be much more closely determined by the "saltpeter" method. The tests can be continued during the night, since even an unskilled man can do the preliminary work, provided the bottles containing the saltpeter solution are prepared in advance.

Table 1 (p. 486) shows the comparative results obtained with both methods on a water highly polluted.

One column was inserted showing the "relative stabilities" obtained when using 0.4 c.c. of 0.05 per cent methylene blue solution. It is clear, of course, that these relative stability figures cannot coincide with the figures in the *Standard Methods*, since in the original tests made by Phelps¹ a stronger concentration of dye was employed. Nevertheless it is of interest to note that the true relative stability as

¹ *Contr. Sanit. Res. Lab. and Sewage Exp. Sta., Mass. Inst. Tech.*, 5, p. 74.

obtained by the "saltpeter" method (average of 40 tests) was 15 per cent lower than the average of the relative stability according to the *Standard Methods*, and by the "dilution" method was 29 per cent lower than the average of the *Standard Methods*.

TABLE 1.
COMPARISON OF "SALTPETER" METHOD TO "DILUTION" METHOD ON POLLUTED RIVER WATER.

DATE, 1913			TOTAL OXYGEN REQUIRED P.P.M.		REL. STABILITY 0.4 C.C. METH. BLUE PER 150 C.C. CAPACITY	INITIAL AVAILABLE OXYGEN P.P.M.
Month	Day	Time	Dilution Method	Saltpeter Method*		
Aug.	4	2:00 P.M.	7.7	5.4	54	3.2
"	5	10:00 A.M.	6.2	4.4	44	2.2
"	5	3:00 P.M.	5.9	4.4	44	2.2
"	6	11:00 A.M.	8.4	6.4	36	2.0
"	7	9:00 "	9.0	6.8	25	1.3
"	11	2:00 P.M.	8.5	7.1	53	2.7
"	12	9:00 A.M.	6.4	4.5	44	2.3
"	13	10:00 "	9.0	6.7	44	1.3
"	15	8:00 "	7.8	5.8	26	1.4
"	20	10:00 "	6.8	5.1	47	1.8
"	20	2:00 P.M.	8.1	6.9	50	2.5
"	22	8:00 A.M.	9.1	6.9	35	1.4
"	25	9:00 "	7.1	5.7	59	3.5
"	25	11:00 "	5.7	5.9	64	4.2
"	25	2:00 P.M.	5.2	4.7	71	3.6
"	26	2:00 "	7.3	5.4	56	2.8
"	27	10:00 A.M.	7.0	5.6	44	1.7
"	27	2:00 P.M.	8.0	7.3	59	2.9
Sept.	4	3:00 "	5.1	5.7	66	3.5
"	5	2:00 "	6.8	5.0	44	2.8
"	7	8:00 A.M.	6.1	3.5	51	0.7
"	7	10:00 "	5.5	3.9	60	1.7
"	7	2:00 P.M.	4.7	4.3	60	2.1
"	8	9:00 A.M.	5.0	4.5	75	3.4
"	9	9:00 "	6.5	4.9	77	2.3
"	10	2:00 P.M.	5.4	5.5	75	3.3
"	11	2:00 "	5.9	5.5	71	3.2
"	12	9:00 A.M.	5.6	5.5	64	2.9
"	12	11:00 "	5.8	6.0	75	3.8
"	12	2:00 P.M.	6.5	5.6	71	3.4
"	13	9:00 A.M.	6.2	5.5	64	3.3
"	13	2:00 P.M.	6.4	5.8	75	3.6
"	15	9:00 A.M.	6.0	5.5	90	4.4
"	16	11:00 "	6.0	6.6	64	3.3
"	16	2:00 P.M.	6.3	6.4	77	4.2
"	17	9:00 A.M.	7.8	5.4	60	2.1
"	17	11:00 "	5.8	5.1	75	2.9
"	17	2:00 P.M.	6.9	5.8	71	3.6
"	18	9:00 A.M.	6.9	5.9	68	3.7
"	18	2:00 P.M.	7.0	6.1	75	3.9
Average			6.7	5.6	59	2.8

* Calculated on basis of N_2 equivalent to O_3 for the nitrates and N_2 equivalent to O_2 for the nitrites.

Average:	
Relative stability by Standard Methods	59
Relative stability by "Dilution" Method	42
Relative stability by "Saltpeter" Method	50

With river waters the application of this method is somewhat simpler than with sewages, as they contain comparatively little coarse suspended matter. In a sewage containing an appreciable quantity of dark sediment, the observer, particularly if unskilled, can note more easily the decolorization in "dilution" bottles than

in "saltpeter" bottles. To obviate this difficulty, I worked out a modification of the "saltpeter" method, which is given later.

In Table 2 is shown the relative oxygen consumption obtained by the two methods with the rather dilute domestic sewage of the Thirty-ninth Street sewerage area.

TABLE 2.
COMPARISON OF "SALTPETER" METHOD AND "DILUTION" METHOD ON A WEAK DOMESTIC SEWAGE.

Total Oxygen Required by "Dilution" Method P.P.M.	Total Oxygen Required by "Saltpeter" Method* P.P.M.
93	90
91	80
84	87
103	90
116	105
90	89
110	94
109	107
109	100
113	109
91	77
123	104
107	99
85	77
92	85
85	78
127	106
108	105
97	102
156	146
96	84
127	99
134	122
102	101
Average, 106	97

* Calculated on basis of N_2 equivalent to O_2 for nitrates and N_2 equivalent to O_2 for the nitrites.

The general agreement of the "saltpeter" and "dilution" method is somewhat surprising, considering that in making dilutions with fresh water in order to supply the free oxygen, the normal sewage flora is somewhat changed. Particularly in the higher dilutions will the mixture resemble polluted water rather than sewage. In choosing between the two averages, I prefer the "saltpeter" oxygen figure, because it represents the absorption in the original, undiluted sewage.

The fair agreement of the figures seems to indicate that one atom of nitrogen represents 2.5 atoms of oxygen, on which basis I prepared my stock solutions. It still remains to be seen, however, whether the combustion of the organic matter by the saltpeter oxygen will take place in other sewages with like precision, and whether this relation will always hold good. I realize that a biologic reaction, such as this, is likely to be subject to factors not met with in purely chemical reactions. The destruction of saltpeter in sewages has been investigated by various authors; in particular by Letts, Blake, and Totton,¹ who found that nitrogen is evolved, sometimes entirely as free nitrogen, sometimes partly as nitric oxid. Nitrous oxid may also be formed, but the authors' evidence on the point was not conclusive. "The general character of the change is that of a combustion, the oxygen of the nitrate appearing either partly or entirely in the form of carbonic anhydrid." Guth and Keim² found it difficult to show experimentally how the individual reactions take place. Their analysis of the gas formed in the saltpeter reduction showed 0.96 = nitrogen and the rest carbon dioxide and oxygen. That there is no ammonia formed during the process of nitrate reduction is amply demonstrated experimentally by Letts, Blake, and Totton,³ Glaser,⁴ Guth and Keim,⁵ and Bach.⁶ Rideal⁷ in his textbook allows 2.5 atoms of oxygen for one atom of nitrogen, and this was also the figure employed by C. B. Hoover in his work at the Columbus sewage testing station. As stated before, I assumed at first that one atom of nitrogen was equivalent to 3 of oxygen, and with the polluted water I obtained, as a matter of fact, a better check with the dilution method by accepting 3 rather than 2.5 atoms of oxygen for each molecule of saltpeter. The work on crude sewages and more detailed comparative tests with two different saltpeter stock solutions convinced me, however, that 2.5 atoms of oxygen for one saltpeter molecule should be allowed.

The question of the inhibiting effect of the larger quantities of saltpeter upon the normal bacterial water and sewage flora would naturally suggest itself. Guth and Keim⁸ have shown that even much higher concentrations of saltpeter than employed by me do not diminish the number of bacteria. Saltpeter in such quantities does not act as an antiseptic and the presence of bacteria is essential to the oxidation of the liquid. A few experiments carried on, in which 200 p.p.m. of saltpeter oxygen were added to an artificial emulsion in water, also showed in comparison with a "blank" no inhibiting effect upon the total number of the acid-forming bacteria during the 10 days of incubation at 20° C.

The active gas formation which takes place in a sewage on incubation with saltpeter is plainly visible. Soon the colloidal matter clumps together. The liquid becomes clear, and minute gas bubbles rise continuously to the surface. The continuous ebullition of gas makes the use of seals for the incubation tests highly desirable.

On previous occasions, I have observed, as has been pointed out in this paper at the beginning, that the methylene blue, when employed in quantities of 0.4 c.c. per 150 c.c. bottle capacity, showed no germicidal effect in a sewage. It seemed of interest to test these findings for sewages to which saltpeter had been added artificially. Table 3 shows the biologic-oxygen-consumption in sewage to which 200 p.p.m. of oxygen in the form of saltpeter has been added. The time of incubation was 11 days,

¹ *Chem. News*, 1903, 88, p. 182.

² *Gesundh. Ing.*, 1912, 35, p. 52.

³ *Ibid.*

⁴ *Arch. f. Hyg.*, 1913, 80, p. 165.

⁵ *Ibid.*

⁶ *Gesundh. Ing.*, 1912, 35, p. 341.

⁷ *Sewage*, third edition, 1906, p. 131.

⁸ *Gesundh. Ing.*, 1912, 35, p. 52.

the temperature 20° C. Methylene blue was added to one of the bottles, and the residual available oxygen determined in this, as well as the "blank" bottles, at the expiration of the incubation.

The small amount of dye added did not interfere with the biologic-oxygen-consumption.

TABLE 3.

BIOLOGIC-OXYGEN-CONSUMPTION OF SEWAGE AFTER THE ADDITION OF 200 PARTS PER MILLION SALTPETER OXYGEN DURING ELEVEN DAYS, WITH AND WITHOUT THE PRESENCE OF METHYLENE BLUE.

Experiment	Bottle	Oxygen Used
No. 1.	Blank	92.9
	Blue	94.5
No. 2.	Blank	128
	Blue	127

There remain two important questions to be solved: (1) What is the rate of absorption during the period of incubation? (2) Is the biologic-oxygen-consumption independent of the quantity of saltpeter present at the start?

For the purpose of solving the first question, a sewage was distributed into various bottles, a definite quantity of saltpeter added, the mixture incubated at 20° C., and the residual available oxygen determined at certain intervals. I have found on previous occasions¹ that the free oxygen will disappear during the incubation of a sewage independently of the quantity of saltpeter present; therefore, the sum of the residual nitrite and nitrate oxygen at the end of the incubation constituted the total available oxygen.

Table 4 (p. 490) shows the result obtained with crude sewage.

The most important conclusion which can be drawn from a study of this table is that an incubation period of 10 days at 20° C. is practically sufficient to accomplish the oxidation of the unstable matter in sewage. Altho I have made only a few tests to compare the oxygen consumed at 37° C., I judge that a 5-day period may suffice to complete the oxidation at that temperature.

Sewage containing an insufficient amount of saltpeter oxygen showed after incubation for a few days a black "septic" sediment, while the sediment assumed a brown humus-like color if an excess of saltpeter was present. Indeed, the color of the sediment proved such an unfailing indicator of the degree of oxidation that I could tell the approximate quantity of oxygen required, on incubating sewages with varying amounts of saltpeter, without the addition of methylene blue, and by merely selecting the sample the sediment of which did not turn black after 5-6 days incubation. It was apparent that the oxygen requirements of a sewage could be closely determined without any analytical tests, by merely noting the amount of saltpeter oxygen necessary

¹ *Op. cit.*

to prevent the black discoloration of the sediment on incubation. Still closer results could be obtained by determining analytically the residual nitrite-nitrate oxygen in the liquid which remained. The initial free oxygen, nitrate and nitrite oxygen in the average sewage is ordinarily so small that it forms a negligible quantity when compared to the total oxygen requirements.

TABLE 4.
BIOLOGIC OXYGEN REQUIREMENTS OF A SEWAGE ON INCUBATION WITH AN EXCESS OF SALTPETER.

Serial No.	Saltpeter Oxygen Added P.P.M.*	Total Available Oxygen P.P.M.	Days of Incubation	Total Biologic- Oxygen- Consumption P.P.M.	Percentage of Oxygen Consumed
I.....	200	204.7	0 1 2 3 5 10	 74.4 95.9 98.4 100 137	 54 70 72 73 100
II.....	200	204.0	0 1 3 5 10	 83.9 133 164 197	 43 68 83 100
III.....	200	204.4	0 11 20	 133 140	 95 100
IV.....	200	203.7	0 11 20	 191 196	 97 100
V.....	200	203.4	0 10 20	 149 152	 98 100
VI.....	200	203.0	0 10 20	 157 158	 99 100
VII.....	200	202.2	0 1 2 3	 142 150 181	
VIII.....	200	201.4	0 1 2 3	 121 171 183	
IX.....	200	207.7	1 2 3 5 10 20	 57.8 80.2 84.2 91.7 95.1 95.1	 61 84 88 96 100 100

* Calculation on basis of equivalents $N_2=O_5$ for nitrates and $N_2=O_3$ for nitrites.

In order to obtain more definite information concerning the time required for the blackening of the sediment in the bottles on incubation as compared to the decolorization of the methylene blue, a series of close observations were made. These observations are recorded in Table 5.

The results in Table 5 indicate that either the disappearance of the blue color or the darkening of the sediment may serve as a guide. While this undoubtedly will

hold good with most domestic sewages, it should be pointed out here that there may be exceptions. It is always desirable to run a series of comparative tests before adopting this method as a routine procedure. If fairly good checks can be obtained the observation of the darkening of the sediment in the bottles during incubation works out well, and is preferable when the blue color is obscured. As a rule, the sediment will begin to darken around the edge of the bottom. The darkening becomes more apparent when such a bottle is compared to the one containing the next higher concentration of saltpeter, by holding both against the light. The slightest darkening is coincident with the elimination of all of the available oxygen. Whenever the sediment turned black the liquid smelled putrid, thus furnishing an additional index of incomplete oxidation. When in doubt as to which bottle to take for the determination

TABLE 5.
COMPARISON OF TIME REQUIRED FOR APPEARANCE OF BLACK SEDIMENT AND DECOLORIZATION OF METHYLENE BLUE.

SAMPLE COLLECTED	TIME REQUIRED FOR SEDIMENT TO TURN		TIME OF DECOLORIZATION OF METHYLENE BLUE	SALTPETER OXYGEN ADDED IN P.P.M.
	Slightly Black	Noticeably Black		
Crude sewage		5 days	5½ days	75
" "	41 hours	47 hours	37 hours	75
" "		89 "	84 "	125
" "	6½ days		6½ days	125
" "	36 hours		36 hours	75
" "	4 days	4½ days	90 "	125
" "	7 "		7 days	75
" "	4 "		84 hours	75
" "		5½ days	5½ days	75
" "		5½ "	5½ "	175
" "		72 hours	72 hours	125
" "	52 hours		52 "	75
" "	8½ days		8½ days	175
" "	84 hours	5 days	84 hours	175
Settled		48 hours	48 "	50
" "	36 hours		36 "	50
" "	6½ days		6½ days	50
" "	36 hours		36 hours	50
" "		60 hours	60 "	100
" "	60 hours		60 "	100
" "		60 hours	60 "	50
" "	50 hours	65 "	60 "	50
Septic	5½ days	6 days	5½ days	50
" "	5½ "	6½ "	6½ "	50

of the residual nitrate oxygen where quantitative determinations are desirable, colorimetric determinations of the nitrites will often serve as a reliable guide. Where the nitrites in one bottle are absent, or present in traces, one can safely take the higher concentration for quantitative determination. In liquids in which the sediment is not turned black in spite of the elimination of the available oxygen, a rough nitrite determination will indicate the concentration to be selected. Sewages containing sufficient saltpeter oxygen had a distinct humus odor at the conclusion of the incubating period.

A method based upon this principle ought to be quite useful in sewage works which lack scientific laboratory control. It is also adaptable for sewages in which the decolorization of methylene blue is difficult to observe. The choice of methods to be employed for the determination of the biologic-oxygen-consumption will also depend

a good deal upon factors such as the iron content of a sewage or the presence of hydrogen sulfide and organic sulfur. A high inorganic iron content is helpful when the septicization of the sediment is to serve as an index of the oxygen requirements, since it is the ferrous sulfide which gives the black color to the sediment.

I have had a slaughter-house waste under observation, the sediment of which would not turn black on incubation, but remained as a grayish-brown floc. As yet I have made no investigation as to why the sediment failed to turn black. I believe that the sediment does not turn "septic" on account of the high sodium chlorid content of the waste, which often reaches 2,000 p.p.m. The sodium chlorid possibly acts as a preservative. I have had very good success with the trade-waste described, when adding methylene blue. The waste is yellowish, turning, after the addition of the dye, to a greenish color. The disappearance of the greenish color on incubation is very distinct.

Again, I have dealt with the effluent of a "biolytic" tank which was entirely free from colloidal matter, containing a little fine black sediment and large quantities of hydrogen sulfide. With this effluent, the total absorption of free oxygen is comparatively slight, since there is little oxidizable matter besides the hydrogen sulfide. The lack of suspended matter in the effluent led me to resort to the "methylene blue" modification of the method described herein. The blue color disappeared immediately in the bottles containing this effluent with various amounts of saltpeter, on account of the presence of hydrogen sulfide. The liquid in the bottles gradually became cloudy, free sulfur being precipitated. The blue color returned usually within an incubation period of 24 hours, to disappear again in the bottles containing the quantities of saltpeter insufficient to oxidize the effluent, altho it remained in the bottles containing the large quantities of saltpeter. The figures obtained with the consumption of oxygen in the "fresh-water" method and the "saltpeter" method compare well.

Having satisfied myself that the quantity of saltpeter oxygen consumed coincides fairly closely with the quantity of fresh-water oxygen, it became necessary to establish the ratio of exhaustion and the ultimate oxygen requirements of a sewage when employing an excess of saltpeter. Table 6 shows the results obtained.

The initial available oxygen, such as recorded in Table 4, has not been determined, as it is practically negligible when compared to the large quantities of saltpeter oxygen added previous to incubation. Considering the difficulties of accurately determining large quantities of residual nitrate in the liquid after incubation as a routine procedure, the figures obtained on adding various quantities of saltpeter to one and the same sewage are consistent. It may, at times, be sufficient to determine the biologic-oxygen-consumption of a sewage by incubating only one bottle with a considerable excess of saltpeter. This method seems attractive by its simplicity. However, I should not recommend it for the present, until more work has proven its reliability. I believe that more accurate figures can be obtained by employing various concentrations of saltpeter

and selecting the one closest to oxygen exhaustion after incubation for 10 days.

TABLE 6.
OXYGEN REQUIREMENTS OF SEWAGE ON INCUBATION WITH AN EXCESS OF SALTPETER.

Serial No.	Source	Total Available Oxygen P.P.M.	Days of Incubation	Total Biologic-Oxygen-Consumption in P.P.M.
I.....	Crude sewage	200	5	154
		300	5	160
		400	5	158
II.....	" "	200	5	171
		300	5	180
		400	5	191
III.....	" "	75	10	68
		125	10	76
IV.....	" "	125	10	98
		175	10	89
V.....	" "	125	10	124
		175	10	131
VI.....	Filtered "	300	9	180
		400	9	189
VII.....	" "	300	9	181
		400	9	191
VIII.....	" "	300	9	179
		400	9	203
IX.....	" "	300	9	180
		400	9	185
X.....	" "	200	5	90
		300	5	77
		400	5	109
XI.....	" "	200	5	79
		300	5	75
		400	5	89
XII.....	" "	200	5	83
		300	5	72
		400	5	80
XIII.....	Settled "	150	10	144
		200	10	139
XIV.....	" "	100	10	96
		150	10	109
XV.....	Septic "	150	10	135
		200	10	139
XVI.....	Settled "	100	10	82
		150	10	77
XVII.....	" "	150	10	108
		200	10	118
XVIII.....	Crude "	175	10	151
		225	10	151
XIX.....	" "	125	10	124
		175	10	122

We have still to consider the problem of obtaining the relative stability in colored trade-wastes where the decolorization of the

methylen blue cannot be observed. The method which would suggest itself is simple enough, considering the difficulties presented by the physical characteristics of the liquid.

Into a number of bottles containing the trade-waste, add varying quantities of saltpeter. The number of bottles will depend upon the accuracy desired for the determination of oxygen consumption. The same holds true for the various quantities of saltpeter to be added. The smaller the variations in the quantities, the more accurate are the determinations, the greater also is the possibility of missing the required concentration. The bottles are now incubated at 20° C. for 10 days, and the residual nitrate and nitrite in each tested qualitatively. The liquids which on rough quantitative or qualitative tests show the absence of nitrites or only traces are discarded immediately. The bottle giving a marked nitrite reaction is selected for determining the quantity of oxygen required for biologic-oxygen-consumption. Actual quantitative determinations of the nitrate and nitrite will result in a still closer figure.

I believe, however, that with trade-wastes more actual information can be gained by determining the oxygen consumption through many tests, rather than through a few very accurate tests, as wastes vary greatly within short intervals. The physical appearance of the trade-wastes often indicates to the experienced observer whether it is "strong" or "weak." I always prefer to make a few preliminary tests; for instance, with certain wastes the biologic "oxygen consumed" may vary between 400 and 1,000 p.p.m., and in a waste of that kind a figure to the nearest hundred is frequently close enough, whereas, with domestic sewage, the permissible limit may be the nearest 10 p.p.m. Ordinarily, it is not necessary to determine the available oxygen in a trade-waste, unless the relative stability is wanted, as well as the figure indicating the oxygen consumption in p.p.m. The relative stability is calculated in the usual way; assume that the available oxygen at the start (without the added saltpeter oxygen) is 10 p.p.m., and the total quantity of oxygen consumed (including saltpeter oxygen and initial available oxygen), 500 p.p.m., the relative stability would be 2, since 10 is 2 per cent of 500; hence, 98 per cent of oxygen is necessary, in addition, to obtain complete stability.

In conclusion, I wish to emphasize the necessity of controlling the effluent of sewage works by biologic-oxygen-consumption tests rather than by other routine determinations. The purification of sewage is undertaken in many cases to ameliorate or eradicate a live nuisance. A live nuisance from sewage in a fresh-water course is evident mainly in two ways: (1) by the development of putrid odors and (2) by the elimination of fish life. In both cases, the biologic consumption of oxygen is the all-important element. In general, a nuisance will not develop if some oxygen is present. Oxygen is essential to fish life. Of what use, then, are determinations of organic nitrogen, albuminoid ammonia, or solids in solution and suspension? It is common knowledge that with vegetable

matter and animal matter of the same organic nitrogen content the latter will have much greater biologic-oxygen-absorbing power than the former. Dissolved solids or suspended matter may be stable or, on the contrary, highly putrescible. The determination of the volatile suspended matter is, at best, only a rough indication of the putrescibility of the sediment. Of course, the quantity of suspended matter is required as a routine procedure to determine how much floating or settling matter must be removed. To predict definitely the time and extent of a nuisance, chemical determinations in general are of little value.

In studying sewage treatment, I am interested in but two questions: (1) To what extent has the treatment improved the biologic condition of the stream? and (2) What is the amount of suspended matter in the sewage effluent? The latter information is not always necessary. My belief is that the efficiency of a sewage-treatment plant should be judged primarily by the improvement in the biologic-oxygen-consuming power. Some uniform method of determination should be adopted. I am looking forward to the time when the "strength" of a sewage or effluent will be expressed in "milligram per liter biologic-oxygen-consumed," and when the responsibility for stream pollution by various communities in this country will be apportioned mainly on that basis. This process of standardization will be a tremendous task, not to be undertaken by one man alone, but by an organization of experts skilled in the survey of polluted streams.

SUMMARY.

1. Assuming that 5 oxygen atoms of 2 saltpeter molecules and 3 oxygen atoms of 2 sodium nitrite molecules are utilized, saltpeter oxygen added to a sewage or polluted water in bottles, containing methylene blue as an indicator, will improve the stability of the liquid in practically the same ratio as the free oxygen supplied in the "dilution" method.

2. The true relative stability of a sewage or polluted water can be obtained by the addition of varying quantities of saltpeter to bottles containing 0.4 c.c. of a 0.05 per cent aqueous methylene blue solution, and by recording the quantity of saltpeter oxygen in

the bottle which just retains its color. Ten days' incubation at 20° C. appears to be a sufficiently extended period for all practical purposes. Knowing the initial available oxygen and the total biologic "oxygen consumed," the percentage ratio of the two figures represents the true relative stability. This method is recommended for polluted waters and weak sewages. The quantities of oxygen in the form of saltpeter recommended for trial on polluted waters in general are: 2, 4, 6, 8, 10, and 12 p.p.m., respectively. Deviations from these figures may, of course, be necessary to suit the peculiar circumstances of each case. The use of seals on bottles to be incubated is desirable on account of the tendency of decolorized liquids for reaeration.

3. The biologic-oxygen-consuming power of sewages can also be determined without analytical test by the addition of various amounts of saltpeter (it is suggested that these amounts represent 100, 130, 160, 190, 210, and 240 p.p.m. of oxygen) to glass-stoppered bottles containing the sewage, and incubating the same for 5 days at 20° C. The bottles in which the sediment turns "septic" or in which a putrid odor develops should be discarded. The quantity of saltpeter oxygen in the bottle which contains sediment of a brown humus-like character indicates the oxygen required to obtain complete stability. Such a liquid will also possess a humus-like odor. The residual nitrite-nitrate oxygen may be determined in the liquid which just "held out" when greater accuracy is desired. This method is recommended for sewages containing much suspended matter, for sewage works without laboratory facilities, and as an optional method for the "saltpeter" methylene blue method previously described.

4. In a colored trade-waste, it is best to use various quantities of saltpeter (equivalent to 100, 200, 300, etc., p.p.m., up to 1,000 p.p.m. of oxygen), the amount depending upon the judgment, or the outcome of preliminary tests. Add these amounts to glass-stoppered bottles containing the waste. Then determine roughly the residual nitrite after 10 days' incubation at 20° C. Note the bottle, the liquid of which gave a decided nitrite reaction (qualitative or roughly quantitative). The quantity of saltpeter oxygen which this bottle contained at the start will indicate the oxygen-

consuming power of the trade-waste. The residual nitrite-nitrate oxygen may again be determined when greater accuracy is desirable.

5. The saltpeter-oxygen-consumption on incubation at 20° C. is practically complete after 10 days. Experiments with an incubation temperature of 37° C. have not been made, but it is believed that the time of incubation can be cut perhaps one-half without materially influencing the results. Comparative tests are needed, however, to verify this.

6. The method suggested in this paper should be applied to other sewages in order to determine whether saltpeter oxygen and fresh-water oxygen in equivalent quantities result in an equivalent improvement of the stability elsewhere.

7. It is hoped that in the near future a better understanding of the importance of biologic-oxygen-consumption tests will prevail among sewage chemists and engineers. It seems desirable to express the "strength" of a sewage or trade-waste on this basis, rather than on the basis of routine chemical tests in vogue at present.

Finally, I wish to acknowledge the friendly interest of Mr. Langdon Pearse, engineer in charge of sewage disposal investigations of the Sanitary District, in the progress of this work, and the analytical assistance of Messrs. Frank Bachmann and J. T. Meckstroth. I am particularly indebted to Mr. Bachmann for the compilations of the results and for helpful suggestions.