# NOTES ON THE PRACTICAL APPLICATION OF THE "SALTPETER METHOD" FOR DETERMINING THE STRENGTH OF SEWAGES.

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Since the publication of the saltpeter method of determining the biochemical oxygen demand of sewages and polluted waters* I have made a number of observations which throw more light on the reliability and practicability of the method. I have also simplified the procedure so as to make it more adaptable to working conditions. At the present time the operation of the sewage testing stations of the Sanitary District is entirely controlled by this method. It has also been employed in sanitary surveys of polluted streams.

The method is based upon the biochemical consumption of saltpeter oxygen by a sewage during an incubation period of ten days at 20° C. The initial minus the residual available oxygen expressed as parts per million oxygen indicates the biochemical oxygen demand. The original procedure consisted in the addition of varying quantities of saltpeter to the sewage in stoppered bottles, either with or without the addition of methylene blue. If no coloring matter was present, the "septization" of the sediment served as the index of the oxidation. The residual nitrite-nitrate oxygen was determined in the bottle in which the sediment did not turn septic after ten days' incubation. This method of employing varying quantities of saltpeter is necessary of course in preliminary tests when one deals with a sewage of unknown strength. There is, however, no additional labor involved, since only one bottle is selected for the final analysis. The other bottles are discarded. Once the approximate maximum strength of a certain sewage is established, a definite excess of saltpeter can be employed, thus reducing the number of bottles to one. I have found on various occasions that a reasonable excess of saltpeter does not result in an increased oxygen consumption. In this respect the method is vastly superior to all of the methods involving fresh water dilutions, such as required in the Modified English Incubation Test, accepted as a provisional procedure for the "Standard Methods of Water Analysis." It is true that in any dilution method, only free oxygen determinations are required which are much simpler than nitrate-nitrite determinations, from the analytical standpoint. However, the precautions and amount of preliminary work required in a dilution test such as the English Incubation

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*Lederer, J. Infect. Dis., Vol. 14, p. 482.

Test* are great and emphasize the difficulties of obtaining comparable results with such a test.

In order to have comparable results with the English Incubation Test, it is absolutely essential to prepare a dilution which is just sufficient to insure stability. The saltpeter method was originally developed on this basis. Various dilutions were made of the sewage with fresh water and methylene blue was added as an indicator. The dilution, just sufficient to retain the color, expressed in parts per million of oxygen, gave the same result as when an equivalent of saltpeter of oxygen was added to the undiluted sewage. The saltpeter solution was prepared on the basis of five oxygen atoms being released from two sodium nitrate molecules. It is obviously wrong to prepare *ad libitum* any kind of sewage-water mixture and compare the free-oxygen consumption thus obtained with the saltpeter-oxygen consumption. Matters are still worse if a dilution is incubated at one temperature and the saltpeter sewage at another. An intelligent comparison can only be drawn by adhering to the procedure indicated above. That a serious error can occur in comparing the free-oxygen consumption with the saltpeter-oxygen consumption, if the proper required concentration is not adhered to, was impressed on me during the coöperative work on the Modified English Incubation Test. In a number of the series of experiments I worked out the saltpeter-oxygen consumptions, as well as the free-oxygen consumptions in the various concentrations. Such a comparison is recorded in the following Table I, the results being expressed in parts per million.

The figures in parenthesis indicate the percentage of oxygen absorbed by the sewage after ten days' incubation. The influence of concentration upon oxygen absorption is strikingly illustrated in the table. However, when the per cent. of oxygen absorbed by the sewage approaches 50 or thereabouts the two absorption figures do not differ very greatly, making allowance for unavoidable working errors in either method.

One difficulty encountered by the sub-committee on the determination of the biochemical oxygen demand of the laboratory section of the American Public Health Association was the fluctuation of the oxygen content during the incubation of a sewage-water mixture from one day to the other. Such fluctuations have been noted by nearly all of the co-workers, and account for the unreliability of the method. The reasons for these irregularities has not been satisfactorily explained, but it is probably that the re-aëration through loose stoppers is only a minor factor. Nevertheless, mixtures in which the free-oxygen is measured after incubation must be guarded from contact with the air. Re-aëration constitutes an almost negligible source of error in the saltpeter method. A number of experiments have been

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* Lederer, J. Ind. Eng. Chem., Vol. 6, Nov., 1914.

## TABLE I.

## FREE-OXYGEN CONSUMPTIONS IN VARIOUS CONCENTRATIONS COMPARED TO SALTPETER-OXYGEN CONSUMPTIONS AT 20° C.

| Serial number. | Time of incubation in days. | Fresh water oxygen demand, P. P. M. | | | | | | | Saltpeter oxygen demand P. P. M. |
|---|---|---|---|---|---|---|---|---|---|
| | | Original concentration of sewage in per cent. | | | | | | | |
| | | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 4.0 | 5.0 | |
| 1 | 1 | 60 | 27 | | | 20 | | | 38 |
| | 5 | 180 | 113 | | | 97 | | | 71 |
| | 10 | 230 | 146 | | | 120 | | | 81 |
| | | (26) | (26) | | | (43) | | | |
| 2 | 1 | 50 | 33 | | | 33 | | | 18 |
| | 5 | 130 | 100 | | | 67 | | | 57 |
| | 10 | 180 | 126 | | | 90 | | | 81 |
| | | (20) | (22) | | | (31) | | | |
| 3 | 1 | 20 | | 15 | | | 20 | | 19 |
| | 5 | 150 | | 90 | | | 75 | | 78 |
| | 10 | 190 | | 135 | | | 92 | | 86 |
| | | (22) | | (32) | | | (44) | | |
| 4 | 1 | 50 | | 40 | | | 32 | | — |
| | 5 | 140 | | 95 | | | 75 | | 63 |
| | 10 | 210 | | 135 | | | 98 | | 80 |
| | | (24) | | (31) | | | (45) | | |
| 5 | 1 | 50 | | | 32 | | | 22 | 32 |
| | 5 | 110 | | | 84 | | | 66 | 66 |
| | 10 | 120 | | | 92 | | | 88 | 75 |
| | | (14) | | | (27) | | | (53) | |
| 6 | 1 | 30 | | | 24 | | | 22 | 30 |
| | 5 | 100 | | | 68 | | | 64 | 60 |
| | 10 | 140 | | | 100 | | | — | 71 |
| | | (16) | | | (30) | | | | |
| 7 | 1 | 70 | | | 32 | | | 32 | 24 |
| | 5 | 150 | | | 96 | | | 60 | 52 |
| | 10 | 180 | | | 104 | | | 76 | 66 |
| | | (20) | | | (30) | | | (45) | |
| 8 | 1 | 50 | | | 28 | | | 26 | 23 |
| | 5 | 130 | | | 96 | | | 80 | 66 |
| | 10 | 150 | | | 116 | | | 98 | 78 |
| | | (17) | | | (34) | | | (58) | |
| 9 | 1 | 70 | | | 36 | | | 36 | 27 |
| | 5 | 190 | | | 112 | | | 100 | 67 |
| | 10 | 240 | | | 124 | | | 108 | 89 |
| | | (28) | | | (36) | | | (65) | |

carried on incubating sewages with various stoppers, one seal,* and without a stopper at 20° C. and 37° C. The results are recorded in the following Table II:

### TABLE II.

### INFLUENCE OF EXPOSURE TO AIR UPON THE RESULTS OBTAINED BY THE SALTPETER METHOD.

| Serial number. | Bio-chemical oxygen demand, P. P. M. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Incubation at 20° C. | | | | | Incubation at 37° C. | | | | |
| | Number of days. | Glass stopper. | Cork stopper. | Bunsen seal. | Open bottle. | Number of days. | Glass stopper. | Cork stopper. | Bunsen seal. | Open bottle. |
| 1 | 9 | 94 | 94 | 94 | 85 | 5 | 94 | 96 | 93 | 86 |
| 2 | | | | | | 5 | 121 | 118 | 115 | 97 |
| 3 | 11 | 192 | 194 | 191 | 185 | 4 | 166 | 160 | 163 | 147 |
| 4 | 11 | 178 | 179 | 177 | 168 | 4 | 140 | 140 | 140 | 123 |
| 5 | 11 | 190 | 192 | 191 | 185 | 4 | 168 | 170 | 170 | 160 |
| 6 | 12 | 790 | 816 | 787 | 777 | 6 | 782 | 753 | 753 | 620 |

This table shows clearly that the precautions against re-aëration in the saltpeter method need by no means be rigid. A seal is not required, in spite of the appreciable quantity of gas accumulating beneath the stopper during incubation. Any kind of a stopper will do, even though the incubation be carried on at 37° C.

I wish to correct a statement made in the original article concerning the amount of free-ammonia formed during the nitrate reduction process. A number of observers, cited in the original article, assumed on the basis of experimental data that no free-ammonia is formed. Since this point has no actual bearing upon the availability of the oxygen present, it was not reinvestigated by me at that time. Mr. C. B. Hoover of Columbus, Ohio, found that free-ammonia is formed (not published), and this has since been substantiated by me in a number of experiments. Table III will show the results obtained.

The increase of free-ammonia appears appreciable.

Since the absorption of oxygen from the air in the saltpeter method seemed to be a negligible factor, it appeared likely that no error was introduced by incubating a large number of samples collected at frequent intervals and by mixing these individual samples after incubation. This would

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* Bachmann, J. Ind. Eng. Chem., Vol. 6, Sept., 1914, p. 764.

TABLE III.

FORMATION OF FREE-AMMONIA DURING SALTPETER-OXYGEN CONSUMPTION.

| Serial No. | Temperature of incubation, deg. C. | Time of incubation in days. | Free-ammonia in P. P. M. as N. | |
|---|---|---|---|---|
| | | | Before incubation, | After incubation. |
| 1 | 37 | 3 | 7.2 | 9.2 |
| 2 | 37 | 3 | 8.8 | 12.4 |
| 3 | 37 | 3 | 12.4 | 14.4 |
| 4 | 20 | 5 | 7.2 | 10.0 |
| 5 | 20 | 5 | 8.8 | 11.6 |
| 6 | 20 | 5 | 12.4 | 12.8 |
| 7 | 37 | 3 | 10.8 | 15.2 |
| 8 | 37 | 3 | 14.0 | 16.4 |
| 9 | 37 | 3 | 12.0 | 14.8 |
| 10 | 20 | 5 | 10.8 | 15.2 |
| 11 | 20 | 5 | 14.0 | 18.0 |
| 12 | 20 | 5 | 12.0 | 17.6 |

furnish a much better control of a sewage disposal plant, giving more precise information on the actual strength of a sewage or tank effluent than other available methods. The solution of this question hinged on whether, in mixing the samples, the nitrites would be oxidized, thus resulting in lower oxygen demand figures. A large number of samples were, therefore, incubated with saltpeter. At the end of the incubation period the bottles were opened and air passed through the liquid for ten minutes. Such a procedure would at once spoil any result based upon free oxygen consumptions. As a matter of fact, it did not alter the result of the saltpeter method at all. The quantities of free-ammonia, nitrites and nitrates were unchanged by the aëration. Composites, therefore, can be made from samples taken at frequent intervals. Large quantities of sewage may be collected in jugs or bottles, containing a sufficient excess of saltpeter, and the oxygen demand may be determined. The larger the size and number of samples, the closer the true figures can be obtained.

The next question of interest concerns the correctness of figures obtained by incubating at 37° C., instead of 20° C. While doubtless it is inconvenient to wait ten days for a result in the operation of a plant or in any particular investigation, it is often advisable to sacrifice accuracy to convenience, providing comparable figures can be obtained. The conclusions to be drawn from the following table should not be generalized. Each operator or laboratory should obtain his or its own temperature relation. It is probable that the relation in different places will not vary greatly with domestic sewages. When working with an unknown sewage or trade-waste, accurate figures can only be obtained by incubating at 20° C. for ten days, this consumption representing between 90 and 100 per cent. of the total. The following Table IV will show the relation obtained by me:

TABLE IV.

SALTPETER OXYGEN DEMAND OF SEWAGES AT 20° C. AND 37° C.

| Serial No. | 37° C. | | 20° C. | | Serial No. | 37° C. | | 20° C. | |
|---|---|---|---|---|---|---|---|---|---|
| | Incubation in days. | Oxygen demand P. P. M. | Incubation in days. | Oxygen demand P. P. M. | | Incubation in days. | Oxygen demand P. P. M. | Incubation in days. | Oxygen demand P. P. M. |
| 1 | 5 | 180 | 10 | 198 | 17 | 6 | 130 | 10 | 126 |
| 2 | 5 | 124 | 10 | 133 | 18 | 6 | 129 | 10 | 107 |
| 3 | 5 | 162 | 10 | 171 | 19 | 6 | 102 | 10 | 102 |
| 4 | 5 | 165 | 10 | 178 | 20 | 6 | 174 | 10 | 156 |
| 5 | 5 | 184 | 10 | 191 | 21 | 6 | 142 | 10 | 138 |
| 6 | 5 | 190 | 10 | 198 | 22 | 6 | 128 | 10 | 102 |
| 7 | 5 | 107 | 10 | 107 | 23 | 6 | 247 | 10 | 241 |
| 8 | 5 | 150 | 10 | 165 | 24 | 6 | 127 | 10 | 132 |
| 9 | 5 | 190 | 10 | 198 | 25 | 6 | 148 | 10 | 158 |
| 10 | 5 | 190 | 10 | 188 | 26 | 6 | 244 | 10 | 246 |
| 11 | 5 | 176 | 10 | 185 | 27 | 6 | 157 | 10 | 148 |
| 12 | 5 | 112 | 10 | 125 | 28 | 6 | 189 | 10 | 180 |
| 13 | 5 | 149 | 10 | 160 | 29 | 6 | 134 | 10 | 132 |
| 14 | 5 | 189 | 10 | 191 | 30 | 6 | 79 | 10 | 81 |
| 15 | 5 | 134 | 10 | 142 | 31 | 6 | 120 | 10 | 122 |
| 16 | 5 | 156 | 10 | 167 | 32 | 6 | 177 | 10 | 170 |
| Average | | 160 | | 168 | | | 152 | | 146 |

According to this table, no appreciable error is introduced by incubating the samples for five or six days at 37° C. instead of 10 days at 20° C. The figures for the five- and six-day incubations are both within 5 per cent. of the 20° C. figures. A maximum of 5 per cent. has been found to be the approximate working error of the method. After the fifth day of incubation at 37° C., the absorption of oxygen is very slight. I, therefore, resort to five-day incubations at 37° C. for rapid work with my particular sewage. The cut can be made shorter by ascertaining a rough relation between the first day and tenth day incubation figure. Personally, I do not advise such a short incubation period, on account of a possibility of serious error. In eight tests which I made, the one-day incubation at 20°

C. was approximately 33 per cent. of the ten-day figure. The figures fluctuated between 22 and 43 per cent., showing the inaccuracies of an extremely short incubation period. Very often it is highly desirable to obtain information on the twenty-four-hour and ten-day oxygen absorption as well, where this relation gives a clue to the deoxygenating quality of a sewage during the first stretch after discharge into a river.

The nitrate reduction does not seem to be influenced by the artificial addition of many common bacterial species ordinarily found in water and sewage. This was found true with B. fluorescens liquefaciens, B. acidi lactici, B. megatherium, B. subtilis, Str. pyogenes, B. enteritidis, B. lactis aerogenes, B. coli, B. cloaceae, B. vulgatus, and B. prodigiosus.

A word of caution is required to prevent the indiscriminate application of the method to tradewastes. All of the original observations have been made on domestic sewage and polluted river waters. The enormous variation in the character of tradewastes makes it impossible to recommend the method unqualifiedly without preliminary tests. My experience with the application of this method to tradewastes relates, so far, only to slaughtering housewaste, on which it is working very satisfactorily. A tradewaste should be alkaline and should contain bacteria, or this method is not applicable. An acid tradewaste or too alkaline a waste should not be taken without adjusting the reaction to a slightly alkaline. If sterile, the contents should be contaiminated artificially with sewage. Even this may not prove sufficient.

The residual nitrate-nitrite should be carefully determined to avoid the liability to error in making readings, due to the large dilutions required for the colorimetric determinations. The error in reading ought not exceed 5 per cent.

Lately I have carried on some work on the method discussed in this paper, employing, however, a sodium nitrite instead of sodium nitrate solution. The quantities employed are calculated, of course, on the availability of oxygen, five oxygen atoms being available from two saltpeter molecules and three oxygen atoms from two sodium nitrite molecules. The intention was to eliminate one determination at the end of the incubation, since only nitrites would have to be determined and not nitrates as well. A considerable number of results seems to indicate that an equivalent of sodium nitrite will give results such as are obtained by using saltpeter, but I withhold a definite opinion until I have investigated further.

The method in its present form should be applied as follows:

*For Sewages:* The initial available oxygen can ordinarily be disregarded, since it is very small compared with the total biochemical oxygen demand. The maximum oxygen consumption, once established by preliminary tests, may serve as a guide for future tests on that particular sewage. A solution of 26.56 grams of sodium nitrate, c.p., per liter should be employed. One c.c. of this solution in 250 c.c. sewage represents 50 p.p.m. of oxygen. The strength of the saltpeter solution may be varied to suit conditions. Incubate the sewage containing the saltpeter for ten days at 20° C. If 37° C. incubation is chosen, determine the relation of this incubation to that of 20° C. A seal is not required during the incubations. Composites may be made at the end of the incubation from samples taken at close time intervals. The appearance of a black sediment and the development of a putrid odor during incubation indicates that too little saltpeter oxygen has been added. Methylene blue may be added at the start to serve as an indicator during the incubations with saltpeter. Decolorization indicates the absence of sufficient oxygen. Domestic sewage usually varies in its oxygen demand from 100 to 300 p.p.m. At the end of the incubation determine the residual nitrite and nitrates, the latter by the aluminum reduction method. In order to convert the nitrogen into oxygen equivalents, multiply the nitrite nitrogen by 1.7 and the nitrate oxygen by 2.9. The initial available oxygen minus the residual oxygen gives the biochemical oxygen demand, which should be expressed in p.p.m.

*For Tradewastes:* Employ the same procedure with larger quantities of saltpeter. The reaction should be slightly alkaline. Acid reaction should be adjusted with sodium bicarbonate, and a too strong alkaline reaction with a weak hydrochloric acid solution. If the liquid is sterile, inoculate it with sewage, after adjusting the reaction. When working with a new waste, obtain checks with the dilution method.

For filter effluents and polluted river waters, this method can be supplanted by obtaining Phelps "relative stabilities" (Methylene Blue Method), provided colloidal matter is absent. .The initial available oxygen must be determined analytically. It is best to make up a stock solution of 1,0624 grams of sodium nitrate c.p. to one liter of distilled water. One c.c. of this solution in 250 c.c. liquid represents 2 p.p.m. of oxygen. Unless the river water is badly polluted it is hardly required to add more than 10 p.p.m. of saltpeter oxygen. If the methylene blue is absorbed by the colloidal matter of river waters, correct "relative stabilities" cannot be obtained. Filter effluents are ordinarily sufficiently devoid of colloidal matter, and there is no trouble in obtaining correct "relative stabilities" by the simple addition of methylene blue, followed by incubation at 20° C. for ten or twenty days. In waters carrying clay and other colloidal suspensions, the determination of correct "relative stabilities" necessitates the incubation of two samples, one to determine the residual free oxygen, the other to determine the residual nitrite-nitrate oxygen. This procedure is rather tedious but no substitute can be offered at present.

The analytical work connected with this investigation was carried on by Messrs F. Bachmann, J. T. Meckstroth, and F. L. Wurl, to whom I wish to express my appreciation for their valuable assistance.