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 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.

	Bio-chemical oxygen demand P. P. M.									
	Incubation at 20° C.					Incubation at 37° C.				
Serial No.	No. of days.	Glass stopper.	Cork stopper.	Bunsen seal.	Open bottle.	No. 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 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

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

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. Table IV. on the following page will show the relation obtained by me.

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

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