STUDIES ON DIRECT NESSLERIZATION OF KJELDAHL DIGESTATES IN SEWAGE ANALYSIS.

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Of late much has been written concerning the great advantage of direct readings of Kjeldahl digestates over the distillation method. The greater accuracy of the method, the more simple technique, the less bulky and bothersome apparatus, and the great saving of time are among the chief advantages claimed for the method. To test the truth of these claims, a series of parallel determinations were made at the Lawrence Experiment Station under the direction of Mr. H. W. Clark.

Previous Methods.—Several procedures have previously been advanced, each one claiming to obtain satisfactory results under local conditions. Kimberly and Roberts[1] determine the total unoxidized nitrogen by adding nitrogen-free sulphuric acid to a measured amount of sewage and digesting until colorless. The digestate is transferred to a 50 cc. flask, cooled, and made up to the mark. Twenty-five cc. of this mixture are transferred to a 100 cc. flask, a 25 per cent. caustic soda solution added nearly to neutralization, cooled and more caustic soda added until a flocculent precipitate appears, when 2 cc. of a 10 per cent. sodium carbonate solution are added to precipitate the calcium present. The whole is then made up to 100 cc., shaken thoroughly, and allowed to stand six hours, when a portion of the supernatant liquid is pipetted into a 50 cc. Nessler tube, made up to 50 cc., nesslerized and read. They found it necessary to use caustic soda free from organic matter because otherwise turbid tubes were obtained.

Whipple[2] altered the procedure by diluting the digestate to 250 cc., treating an aliquot portion of this solution with an equal amount of 5 per cent. caustic soda solution, and substituting filtering through filter paper washed free from ammonia in place of the long period of settling. He found ordinary "purified stick" caustic soda caused no trouble from turbid tubes.

The Lawrence sewage is a strong domestic sewage low in calcium and magnesium content. It was therefore not necessary to consider the calcium in our experiments so no sodium carbonate was used. The method of Whipple was tried but did not give satisfactory results. The amount of free sulphuric acid present in the Kjeldahl digestate varies so that it is impossible to take an aliquot portion after dilution, add an equal amount of 5 per cent. caustic soda solution, and have anywhere near the same excess of caustic soda present in the different digestates.

[1] Kimberly and Roberts, *Jour. Infect. Dis.*, 1906, 2, p. 109.

[2] Whipple, *Tech. Quart.*, 1907, 162.

As a result of this difficulty a large number of turbid tubes were obtained, due to the presence of too large an excess of caustic soda.

Method.

To overcome this difficulty the following method was devised:

Reagents.—The same as those used in ordinary Kjeldahl determinations of organic nitrogen, with the addition of a 5 per cent. solution of caustic soda.

Method.—Fifty cc. of the sample (or more if the nitrogen content is low) are put in a Kjeldahl flask, diluted sodium carbonate added, and boiled down to about 20 cc. to remove the free ammonia. Then 5 cc. of nitrogen-free sulphuric acid (1.84) is added and the sample digested until colorless. The digestate is transferred to a 250 cc. flask, diluted to about 100 cc., and a 50 per cent. caustic soda solution added almost to neutralization. After cooling, a 5 per cent. solution of caustic soda is added in slight excess, the sample made up to 250 cc. and mixed thoroughly. This is filtered through a filter paper washed free from ammonia, 10 cc. of the filtrate are pipetted into a Nessler tube, made up to 50 cc. with ammonia-free water, mixed by shaking, nesslerized, and read after fifteen minutes.

Discussion.

Turbidity.—The Lawrence sewage being very low in calcium and magnesium content, the chief difficulty experienced from turbid tubes was due to the presence of too great an excess of caustic soda in the neutralized digestate. All tubes having an excess of less than 0.05 gram caustic soda in 50 cc. gave turbid tubes due to incomplete precipitation of magnesium. Most tubes having an excess greater than 0.20 gram in 50 cc. were also turbid. The excess of caustic soda must therefore be between 0.05 and 0.20 gram per tube to obtain clear tubes. Kimberly and Roberts say that if potassium permanganate is used to complete the digestion, turbid tubes will be obtained. Since the use of that salt is not necessary for completing the digestion of ordinary sewages, it is not used at this laboratory. In this laboratory a high-grade commercial caustic soda costing six cents per pound is used for all work and no difficulty was experienced from turbid tubes unless the excess of caustic was outside the limits above mentioned. The neutralization of the whole digestate rather than a small portion thereof reduces somewhat the correction due to the blank and the smaller portion taken for nesslerization allows of a greater excess of caustic in the whole digestate without causing turbid tubes.

Accuracy.—Of 90 parallel determinations, 65 per cent. of the direct determinations are lower, 32 per cent. are higher, and 3 per cent. the same as the distilled determinations.

COMPARISON OF RESULTS BY DISTILLATION AND DIRECT METHODS.

Kjeldahl Nitrogen (Organic), Parts per 100,000.

	Regular sewage.		Settled sewage.		Septic sewage.		Contact and trickling effls.	Sewage + 50% city water.
	Unfilt.	Filt.	Unfilt.	Filt.	Unfilt.	Filt.		
Number of samples	13	11	13	13	6	5	24	5
Av. distilled	1.27	0.56	0.97	0.59	0.83	0.46	0.52	0.90
Av. direct	1.29	0.58	0.93	0.57	0.88	0.46	0.47	0.82
Max. difference	+0.22	+0.22	—0.30	+0.15	+0.10	+0.11	—0.19	—0.13
Min. difference	—0.01	+0.02	+0.01	0.00	0.00	—0.03	0.00	—0.02
Av. difference	+0.02	+0.02	—0.04	—0.02	+0.05	0.00	—0.05	—0.08
Av. % difference	1.6	3.6	4.1	3.4	6.2	0.0	9.6	8.9

Kimberly and Roberts, from 24 determinations, obtained results by the direct method, 41 per cent. of which were lower than the distilled, 33 per cent. higher, and 16 per cent. the same as the distilled determination.

Whipple in the same manner by the direct method, obtained 41 per cent. lower, 6 per cent. higher, and 53 per cent. the same as by the distillation method. From this it would seem as if the results obtained by the direct process are as a rule a little lower than they should be.

Time Necessary.—The chief advantage advanced for the direct method is the great saving of time accomplished by its use. That is to say, it can be done in less time by transferring the digestate to a flask, making up to a definite volume, mixing thoroughly, transferring an aliquot portion of this mixture to another flask, adding caustic soda almost to neutralization, cooling, then adding an excess of caustic soda, mixing thoroughly and either (1) allowing to stand for several hours, or (2) filtering through filter paper which must be washed free from ammonia, than by adding an excess of caustic soda and distilling two tubes.

In this laboratory there are eight stills available. It was found that if there were only four determinations to be made, the time required to distil the digestates was practically the same as by the direct method, but if there were more than four determinations to be made, by using the eight stills they could be done approximately twice as fast as by the direct method.

Furthermore, when the direct method is used, one's entire time must be directed to the digestates, whereas while distilling, something else may be done while the digestates are being distilled. This still further reduces the actual time of the distillation method as compared to the direct.

Apparatus and Technique.—While the direct method does away with the use of stills, on the other hand it makes necessary an increased handling of graduated flasks which are bothersome and bulky. It also calls for a large amount of nitrogen-free water which is a disadvantage, as

nitrogen-free water is not always available in large quantities in a sewage laboratory. In this laboratory two gallons and a half of nitrogen-free water can be made in about four and one-half hours. Starting with the water in the flasks cold, eight Kjeldahls can be distilled in about fifteen minutes. Figuring on this basis the cost of distilled water necessary for the direct method by Kimberly and Roberts' procedure is about one-fifth greater than by the distillation process, and by Whipple's procedure, about three times as great. If many determinations are to be made by the latter method the saving in the cost of water used would in a short time pay the cost of a still.

In the direct method the digestate has to be made up to volume at least twice and a definite amount measured twice, while in the distillation method but one measurement is necessary. The chance for error in manipulation is therefore four times as great by the direct as by the distillation method.

Conclusions.

The direct Kjeldahl method undoubtedly has its own place in sewage work, but it does not seem as if it should take the place of the distillation method in a permanent sewage laboratory handling many samples because of:

1. The greater amount of bothersome and bulky apparatus necessary.
2. The large amount of nitrogen-free water required.
3. The greater chance for error in manipulation.
4. The necessity of having the excess of caustic within narrow limits to avoid turbidity, this practically requiring a rough titration of each determination.
5. The greater length of time required for the determination.

The method, however, is without doubt an excellent substitute for the distillation method in a temporary laboratory where it is necessary to incur the least possible expense for apparatus or in a small laboratory where but a very few determinations are to be made daily.

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STUDIES OF INCUBATION TESTS.

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For the past seven years incubation tests have been made in the laboratories at the Lawrence Experiment Station to determine the stability of the effluents of contact and trickling filters. These studies have shown that the development of odor is, perhaps, the surest proof of putrescibility. Oxygen consumed and oxygen dissolved tests before and after incubation are of value but are sometimes contradictory. The so-called methylene-