RAPID CHEMICAL AND PHYSICAL
ANALYTICAL TECHNIQUES IN
WATER AND SEWAGE ANALYSIS

By

George W. Reid

And

Robert S. Ingols

Project No. 117

Prepared for

U. S. Public Health Service
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ANNUAL SCIENTIFIC PROGRESS REPORT  
(October, 1946-October, 1947)  

PROJECT NO. 117  

RAPID CHEMICAL AND PHYSICAL  
ANALYTICAL TECHNIQUES IN  
WATER AND SEWAGE ANALYSIS  

Prepared for  
U. S. PUBLIC HEALTH SERVICE  
Grant in Aid No. 1937  

By  
GEORGE W. REID  

SEPTEMBER 6, 1947
I. SUMMARY

Since October 1, 1946, the project has developed a research laboratory, completed a 3,000-abstract literature search, assembled research personnel, developed and published a new calcium technique, and studied the use of a micro-Kjeldahl technique, infra-red ray drying, a new chemical BOD (oxygen consumed) method, photometric determination of hardness, an electronic endpoint to the relative stability test, several new magnesium determinations, and the standard fluoride determination.

II. INTRODUCTION

Upon receipt of a grant in aid from the Surgeon General, U. S. Public Health Service, for the period October 1, 1946-September 30, 1947, this project was initiated to conduct a study of "Rapid Chemical and Physical Analytical Techniques in Water and Sewage Analysis."

The primary objective was to ascertain what can be accomplished with micro-methods, rapid semiquantitative procedures and physical methods of analysis.

When the grant was received, a search was immediately undertaken for personnel. As indicated in the project contract, the burden of research was to be rightfully on a water and sewage chemist with guidance and consultation from engineers. A trip was made to Cincinnati to discuss possible personnel for the project with C. C. Ruchhoff of the U. S. Public Health Service. On the basis of his recommendation, Georgia School of Technology was able to obtain the services of R. S. Ingols, Ph.D., as Chief Chemist on the project. Because of prior commitments, R. S. Ingols was not able to leave his former position until February, 1947, but between the proceeding and that time he used his holidays from this position at the University of Michigan in assisting us. Conferences were held with colla-
Laboratories at Johns Hopkins and several other trips were made in accordance with the original request to orient the project with the problems in the field. Priorities for tests to be undertaken were developed through these meetings and were adhered to so far as available equipment would permit. When Dr. Ingels joined the project in February on a full time basis, a special laboratory had been completed, but much of the equipment was still back-ordered, and it was not until April that actual laboratory operations began.

III. PERSONNEL

The present staff members for the project are*:

George W. Reid, Director, SM, Sanitary Engineering
R. S. Ingels, Chief Chemist, Ph.D., Sanitary Chemistry
E. H. Shaw, Chemical Engineer, M.S., Chemical Engineering
P. E. Murray, Research Assistant, B.S., Chemistry
2 Graduate Assistants
C. E. Renn, Johns Hopkins Consultant

IV. LITERATURE SEARCH

An extensive literature search was carried out by a graduate chemist technician, P. E. Murray, and student assistants, aided by the Technical Information Staff of the Engineering Experiment Station, and was completed before the special laboratory facilities were ready for use. The search has proved very useful, and it is expected that it will be published by the Engineering Experiment Station. The search covered the period from 1907-1917, and the material was taken primarily from Chemical Abstracts, Public Health Engineering Abstracts, American Water Works Association Journal, and Federation Sewage Works Association Journal. Many other

*Viva Curricula and publications attached to U. S. Public Health Service request forms.
sources were consulted. There were approximately 3,000 abstracts cataloged in terms of standard test and techniques.

V. LABORATORY FACILITIES

The Georgie School of Technology has made available to the project, at no cost to the project, a special laboratory in which much of the developmental work on new procedures has been carried out. The laboratory was equipped with practically all of the standard laboratory equipment found in analytical and physical laboratories. The work involving the use of certain special or rare equipment, such as spectrophotometer, is being carried out elsewhere on the campus. Among the special items furnished the project by the School is a $600 Brown strip recorder and a 9' x 16' BOD incubator room. A pilot plant on the campus is also planned.

VI. EXPERIMENTAL PROGRESS ON ANALYTICAL TECHNIQUES

In reviewing the progress to date, it is well to remember that the order of undertakings was first dictated by the availability of personnel, and then by equipment. As personnel arrived, they were assigned tasks that could be accomplished with the available facilities; as new equipment arrived, work was expanded to a full research program.

A. Calcium Determination

As work in the laboratory began, it was necessary to study those problems that could be handled with limited supplies and equipment. During this time, a new calcium determination was developed, which is more accurate than the one in Standard Methods and is about twice as fast. (Attached is a paper describing this work which was presented at the 1947 Fall meeting of the American Chemical Society.)
The biochemical oxygen demand test is the standard method for measuring the strength of sewage samples, and probably no purely chemical method can ever completely supplant it. However, it is frequently necessary to know the quantity of bacterial food in industrial waste samples that are toxic to bacterial activity, or to have the strength of a sewage sample in less time than the five days required for the routine B.O.D. test. The present permanganate oxygen consumed test is the only available procedure that even partially fulfills these requirements. Recently, a sewage plant chemist published some preliminary directions for a so-called Chemical B.O.D.\(^1\) which is an oxygen-consumed method using chromate instead of permanganate. Additional work on this method was carried out, noting the difficulties encountered by Rhame and as a result, satisfactory control measures have been developed. This led to a test which, as finally outlined, uses only simple laboratory apparatus, requires a small sample (10 cc) of material to be tested, is rapid (requires less than one hour for several simultaneous determinations), and gives values for many known substances such as starch and peptone which agree perfectly with their true B.O.D. values. The values on raw sewage samples run somewhat high because paper in the sewage is attacked by the reagents used while it is not a factor in biological measurements. On settled sewages and secondary treatment unit effluents, the agreements are very good because the sampling errors are reduced and there is no paper in those samples. It is believed that the results of this study of the new oxygen consumed method will comprise the material of the next published work.

C. Fluoride Determination

Early in the year, some fundamental studies were made on the colorimetric determination of fluorides using the spectrophotometer. The results of this work indicated that extensive changes would be needed in the fluoride procedure to accomplish any improvement in the analytical results. This test was particularly suggested for study by C. C. Ruchhoff, U. S. Public Health Service, and others. Mr. E. H. Shaw, a new member of the staff, is now working on this problem.

D. Magnesium Determination

A rapid gravimetric or volumetric procedure for the magnesium ion is definitely needed in the water field. A new method was applied to a pure solution of magnesium sulfate with good results; however, when tried on natural waters, it was found necessary to include some modification. This modification required laboratory exhaust equipment which has not yet been installed, but the preliminary results are very promising. The present standard colorimetric technique for the magnesium ion determination has such a limited range that studies are in progress on the application of a different color reagent.

E. Micro-Kjeldahl Procedure

The study of the micro-Kjeldahl procedure for total nitrogen in sewage, a study specifically proposed in our prospectus was delayed until June because of the slow deliveries of the special equipment required. To prevent further delay, arrangements were made with the Clayton Sewage Treatment Plant (Atlanta) to furnish the total nitrogen values of their composite samples by the regular Kjeldahl technique. A portion of their composite samples has been delivered to our laboratory daily for us to run the Hengar micro-Kjeldahl procedure for total nitrogen. The Hengar tech-
nique was chosen through consultation with Dr. C. E. Ronn of Johns Hopkins University. To date, we have completed 50 analyses, and the results indicate that the micro technique can be substituted for the regular method. This particular micro technique, however, requires almost as much time and attention as the regular technique. Some changes in equipment have been made which have proved helpful, and work has just been started on additional modifications which should reduce the time and attention required. On the other hand, the micro apparatus is much less expensive and occupies less space, and may well serve a useful purpose in small sewage treatment plant laboratories.

F. Relative Stability Determination

Early in June, an idea for the accurate timing of the endpoint of the Relative Stability test was conceived. The Georgia School of Technology purchased a 4-point electronic recording microammeter for this and other problems, and, by using this with a photoelectric cell, it is expected that an exact endpoint for the relative stability test can be studied. This test requires less time than that needed for a regular BOD of raw sewage and should give results which are essentially correct. The composite samples used for the total nitrogen test will be used for the relative stability studies so that these results can be compared with the other analyses in our laboratory as well as with the values obtained by the Clayton Sewage Treatment Plant personnel. For use in a small sewage plant laboratory, it will be necessary to eliminate the expensive electronic recording device; therefore, a relay has been designed, working from the photoelectric cell which will control a time accumulator. All of the equipment is now available for the initial studies.
C. Hardness Determination

One of the student assistants requested the opportunity of adapting the photocolorimeter in our laboratory to total hardness test. The analyses of a large number of known solutions under varying conditions soon indicated that there were too many factors that could not be adequately controlled. Plans are now under way to use an amperometric titration for hardness. A rotating platinum electrode has been built, and, as mentioned, it is to be used in conjunction with the recording device described above.

H. Infra-Red Drier

The infra-red ray drier, pictured in Figures 1 and 2, was designed and built at Georgia Tech. It has been found that more rapid evaporation are possible with this unit than when using the usual heating methods. The removal of the water during the micro-Kjeldahl determination is accomplished readily with this apparatus. When using open vessels, splashing is not eliminated because of the very rapid rate of evaporation. Thus, although the drier has proved very useful, it does have definite limitations which need to be eliminated through the design of necessary accessories. We are in the process of applying a rheostat to the infra-red lamps to control the heat for use in Kjeldahl digestion.

VII. DISCUSSION AND FUTURE WORK

Currently, it is realized that some of the determinations studied are not used in present routines in small plants, but it is felt that work on these tests which involved speeding up and simplifying the present standard determinations, was justified on the basis that a new improved technique may well find such use. For example, the calcium determination should, once established, find ready use in small softening plants.
Also, it was understood that the principal investigator has freedom to deviate if he considers it advisable. In many instances, it appeared that chemical techniques approached the physical ones in speed and simplicity. It is the desire of Dr. R. S. Ingols (and I support him) to broaden the purview of the project to permit study of tests which may, at present, not be adaptable to a single apparatus, but which may prove superior to existing ones, with the hope that they will lead to other developments more readily adaptable. It is with this idea in mind that the study of some tests has been continued even though they may not fit exactly the original request. Through acquaintance with a large number of tests and techniques, simplified apparatus and procedures common to all may be developed. When applicable, several techniques will be tried on all tests. Thus, though it is too early to point to results, this should proceed, until a single set of procedures is developed based on one or several units of equipment within the financial reach of the small plant and designed for simple manipulation. One concrete example of our work to date is the general usefulness and simplicity of infra-red drying.

It will be noted that most of the apparatus and equipment has now been received, and it is hoped that we may receive the necessary financial support to complete the work now in progress and also that still to be initiated on the original proposal. It has been very gratifying to receive favorable and critical comments from the engineers and chemists in the Atlanta area as well as those of national stature during the technical meetings attended by members of the staff. These relationships
with the plants in the area will assure us of field testing of our techniques when we are ready. It is hoped that our second application (which was made in March, before much actual work had been initiated and just after Dr. Ingle had arrived) may be considered with this fact in mind.

Respectfully submitted,

George W. Reid
Project Director

Approved:

Gerald L. Rosselot, Director
Figure 2 - Side view of Infra-red Drier
The determination of the calcium ion as calcium oxalate is a technique that is well established and has not been modified appreciably for fifty years. However, Willard and Furman (2) give a newer method for precipitating calcium oxalate, and, on a recent lecture tour of the local sections of the American Chemical Society, Willard presented some of the basic material indicating the advantages to be gained from using this newer method. By the older method as given in Standard Methods (1) and in Willard and Furman (2), the precipitate is formed during the addition of the ammonium oxalate. The pH is then raised upon the addition of ammonium hydroxide. In the new method, acid is added to the sample to produce pH 1.0; then, ammonium oxalate and urea are added. The solution must remain clear until the sample is boiled to hydrolyze the urea and raise the pH to the point of calcium oxalate precipitation, a step which may require 10-15 minutes. It is evident from Figure 1, that the crystals precipitated from the hot solution by the hydrolysis of urea are enough larger than those formed by the old technique that they are ready for filtration within a short time after their formation. This eliminates much of the digestion period needed by the older method and permits a technician to speed up greatly the test for calcium.

It is the purpose of this paper to report on experiments designed to test the accuracy of the analysis for calcium by this newer method in comparison with the standard technique, especially in the presence of possible interfering ions.

*Delivered before the Division of the Water, Sewage and Sanitation Chemistry of the American Chemical Society, September 16, 1947, in New York City.
METHOD

An aliquot (100 ml.) of the sample is placed in an Erlenmeyer flask. A few drops of methyl red are added, then 2.4 ml. of a 1:1 hydrochloric acid solution; this develops a pH of 1.0 which is needed to prevent precipitation of calcium oxalate until desired. Following this, 15 ml. of a saturated ammonium oxalate solution and 10 g. urea are added and mixed. The solution must remain clear. The urea must be added dry or as a fresh solution, since even a 24-hour solution contains sufficient ammonium hydroxide to cause precipitation upon addition. The solution is heated on a hot plate (generally for 15 minutes) until the methyl red changes color at pH 5.0; it is then ready for filtration. A coarse qualitative paper is used as the filter medium and returned to the original flask after adequate washing. The calcium oxalate precipitate is dissolved with hot sulfuric acid solution and titrated immediately with a standard permanganate solution.

A large solution (5 gallons) of calcium chloride of known concentration was made up and used as the test solution. Various amounts of magnesium and aluminum sulfates were added to portions of this stock test solution, and at least ten determinations were made on every portion by the standard and the new techniques.

RESULTS

Averages of ten determinations on each solution by each technique were calculated and the probable error computed. These values are recorded in Table I. It can be seen that when the following standard method is followed or when the urea modification is used, there is no significant difference in the concentration of calcium ion concentration when no other cations are present originally. This is also true for the five natural water samples tested. When 40 p.p.m. Mg ion (400 mg. MgSO₄·7H₂O per liter)
was added to the stock solution and the determinations repeated, the urea method of precipitation showed only a slight increase (statistically insignificant) in the apparent calcium ion concentration. However, the apparent calcium ion concentration showed a precise 3.1 p.p.m. (3.9%) increase when the calcium oxalate was precipitated by the standard procedure. When the concentration of magnesium ion was doubled to 80 p.p.m., the standard method of precipitation showed another significant increase, and the values were very much more scattered, as indicated by the large increase in the probable error. The urea precipitation also produces an apparent calcium ion increase from 88.1 to 89.1 with 80 p.p.m. magnesium ion, but there was no loss of precision in the higher apparent calcium value as shown by the small probable error value.

In the presence of 1.0 p.p.m. aluminum, the calcium ion concentration by the standard method of precipitation is higher each time by the amount which is equivalent to the extra aluminum ion present. When the urea method is used on 30 samples containing some aluminum ion, there are two distinct sets of results: two sets of ten were as low as the control, while a third set of ten was higher than the control by an amount equivalent to the extra aluminum ion. When tartrate ion is added to the sample and it is analyzed by standard methods, the results are high. However, when the newer urea method is used, there is no apparent calcium increase if 30 minutes is allowed for the aluminum and tartrate reaction.

**DISCUSSION**

The separation of two similar ions in a determination of one of them by precipitation has always been a difficult problem because of the tendency to coprecipitate. To minimize coprecipitation, the precipitate must be formed from a hot solution where it is most soluble, and then the crystals
must be digested to develop them to a size that can be filtered. With the newer technique of precipitating calcium oxalate by using urea hydrolysis, the crystals which form are so large that much coprecipitation would seem likely according to the older concepts. At the higher magnesium concentrations used, the product of the concentrations of the magnesium and oxalate ions is in excess of the solubility product for this salt at 100°C. The solubility product is $K = 1.2 \times 10^{-2}$ (at 100°C.), while the value in the laboratory solutions is $\text{Mg}^{2+} (5.7 \times 10^{-3}) \times \text{C}_2\text{O}_4^{-} (8.0 \times 10^{-2}) = 4.5 \times 10^{-4}$.

In view of the larger ion product over the solubility product, it is obvious that some coprecipitation can be expected to occur in spite of the tendency for magnesium to form a supersaturated solution. With the standard procedure, the average error amounted to 6.0% while the maximum amounted to 13.0%. However, by using the urea hydrolysis method, the average error amounted to 1.1% while the maximum error was 1.5%. These results indicate that the standard procedure is not only slower and more cumbersome but also less accurate in the presence of magnesium sulfate.

Even at the lower magnesium concentration, the product of the concentration of the magnesium and oxalate ions is slightly exceeded. However, the use of the urea hydrolysis method yields an error that is not statistically significant. Coprecipitation, therefore, appears to be the result of the local superconcentration of reagents induced by the addition of a second solution which produces the precipitates and not a factor of the size of the crystal at its formation.

It is apparent from the data that the precipitation of calcium oxalate in the presence of the aluminum ion cannot be carried out without obtaining aluminum oxalate as well as calcium oxalate when standard methods are used even in the presence of tartrate ions. However, when the urea hydrolysis
method of changing pH was used in these experiments, the aluminum ion was apparently changed to a non-reactive form in 20 out of 30 precipitations without tartrate and in every precipitation after the aluminum tartrate reaction.

It can be concluded that the calcium ion can be determined more rapidly and accurately by using the technique of Willard and Furman (2). This calls for precipitating the calcium oxalate from a hot acid solution while raising the pH to the precipitating value by the hydrolysis of urea.

SUMMARY

The use of the hydrolysis of urea for raising the pH of a solution containing both the calcium and oxalate ions permits the formation of large, readily-filtered crystals of calcium oxalate. These are less contaminated by magnesium or aluminum oxalate than the small crystals formed by the standard methods, while the overall time for a set of duplicate determinations for calcium is reduced, by the use of urea, from 90 minutes to 40-50 minutes.

ACKNOWLEDGEMENT

The authors acknowledge the donation of a grant-in-aid by the National Institute of Health of the United States Public Health Service to support the work of this paper.
TABLE I

A COMPARISON OF TWO METHODS OF PRECIPITATING CALCIUM OXALATE FOR THE VOLUMETRIC DETERMINATION OF CALCIUM

<table>
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<th>Concentration of other cations</th>
<th>Average of ten calcium ion values with probable error</th>
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<tr>
<td></td>
<td>Standard methods</td>
</tr>
<tr>
<td></td>
<td>P.p.m.</td>
</tr>
<tr>
<td>No cations</td>
<td>87.9 ± 0.05</td>
</tr>
<tr>
<td>Magnesium 40 p.p.m.</td>
<td>92.0 ± 0.14</td>
</tr>
<tr>
<td>Magnesium p.p.m.</td>
<td>93.3 ± 0.35</td>
</tr>
<tr>
<td>Aluminum 1.0 p.p.m.</td>
<td>90.0 ± 0.50</td>
</tr>
<tr>
<td>Aluminum 1.0 p.p.m.</td>
<td>91.5 ± 0.50</td>
</tr>
<tr>
<td>Aluminum 0.9 p.p.m.</td>
<td>90.7 ± 0.20</td>
</tr>
<tr>
<td>Aluminum 1.0 p.p.m. plus tartrate</td>
<td>91.5 ± 0.35</td>
</tr>
<tr>
<td>Natural waters</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>43.8 ± 0.15</td>
</tr>
<tr>
<td>2 Average of three</td>
<td>8.5</td>
</tr>
<tr>
<td>3 Average of three</td>
<td>5.1</td>
</tr>
<tr>
<td>4 Average of three</td>
<td>8.5</td>
</tr>
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</table>
LITERATURE CITED


ANNUAL SCIENTIFIC REPORT NO. 2

PROJECT NO. 117

GRANT-IN-AID RG264C

RAPID CHEMICAL AND PHYSICAL ANALYTICAL TECHNIQUES
IN WATER AND SEWAGE ANALYSIS

Prepared for

UNITED STATES PUBLIC HEALTH SERVICE

By

GEORGE W. REID and ROBERT S. INGOLS

OCTOBER, 1947--AUGUST, 1948
ANNUAL SCIENTIFIC REPORT NO. 2

PROJECT NO. 117

GRANT-IN-AID RG264C

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OCTOBER, 1947--AUGUST, 1948
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I. SUMMARY

Since October, 1947, the special laboratory facilities of the project have been completed and the personnel requirements filled. Arrangements have been made for publication by the Engineering Experiment Station of the Bibliography on Water and Sewage Analysis. A paper on a modified "oxygen consumed" method which is considerably more accurate than the standard method was published as a result of project studies. The previously reported calcium technique, which was described in the 1946-47 Annual Report, has had considerably more work done on it, and the paper describing it has been accordingly revised, expanded, and accepted for publication by the American Chemical Society. A fundamental study on several fluoride ion determinations was undertaken, and the ferric thiocyanate determination was developed sufficiently for use in the testing of natural waters. Studies on total nitrogen determination by the microkjeldahl procedure have been completed, and studies on chromium, nitrate, and sulfate ion determinations have been inaugurated. Some very fundamental studies on the entire B. O. D. problem, including development of a photometric end-point method for the relative stability tests, have established patterns for further studies on B. O. D., including B. O. D. tests with blended sewages and with the addition of artificial surfaces, as well as direct B. O. D. studies with the Sierp apparatus.

II. INTRODUCTION

Since the 1946-47 Annual Report, those laboratory facilities which were then incomplete have been finished; a full staff has been assembled; and laboratory work has been continued. The initial grant for the period
October 1, 1946--September 30, 1947, was extended to December 31, 1947, and an application for a continued grant-in-aid from the National Institute of Health was approved for the period January 1, 1948--December 31, 1948.

In the project's work, priorities for the study of specific tests have continued to be established through conferences with collaborators as well as with U.S. Public Health Service personnel. Real assistance in this respect has been obtained locally by the establishment of two advisory groups: (1) a Sanitary Engineering Research Advisory Council, and (2) a less formal Chemistry Advisory Group. The first group is constituted as follows:

H. G. Hanson, USPHS
W. H. Weir, GDPH
P. Enloe, FSWA
N. Singleton, Consulting Engineer
G. Rosselot, Georgia Institute of Technology
G. W. Reid, Georgia Institute of Technology
P. Weir, AWWA
J. Hickland, Consulting Engineer
F. Eidness, Manufacturer's Agent
G. Henley, Manufacturer's Agent

This council was established for the purpose of evaluating practical trends from an engineering standpoint and because of its value in suggesting new ideas.

The second group's membership has fluctuated widely but, in general, has consisted of interested chemists from the Georgia State Department of Health, water and sewage plant operations, various institutions, and industry. Frequent meetings have been held, and current work has been reviewed with particular reference to analytical problems. The project's staff has been included in these meetings.

Personnel has continued to be somewhat of a problem; Mrs. Murray and Mr. Shaw both resigned, and replacements for them have been made. Much non-project-supported assistance has been received from students working for their Masters' degree.
Dr. W. D. Hatfield, Chairman of the Standard Methods Committee of the Sewage Works Federation, was consulted during the year, and Dr. Ingols attended a symposium sponsored by the Sanitation Study Section of the National Institute of Health, held in Cincinnati in June, 1948, at which he presented a preliminary report on the progress of the project on methylene blue B.O.D. and the blending of raw sewage.

Dr. Ingols has been awarded the 1948 Georgia Tech Sigma Xi faculty research prize of $300 for the paper by Ingols and Murray on "An Oxygen Consumed Technique."

III. PERSONNEL

The project staff for the period reported has included:

George W. Reid, Project Director, S. M., Sanitary Engineer
R. S. Ingols, Chief Chemist, Ph. D., Sanitary Chemist
E. H. Shaw, Chemical Engineer, M. S. Ch. E., Chemical Engineer (resigned July 1, 1948)
P. E. Murray, Chemist, B. S., Chemist (resigned April 1, 1948)
J. C. Hildebrand, Chemist, B. S., Chemist and Biologist (began April 1, 1948)
H. L. Lawless, Chemist, B. S., Chemist (began July 1, 1948)
1 Undergraduate Assistant (Part-time)

IV. LITERATURE SEARCH

The bibliography of literature and patents on water and sewage analysis, completed early in 1947 and reported in the 1946-47 report, has been brought up to date by the Engineering Experiment Station's Technical Information Division, as a Station-sponsored project. Work is now under way on the publication of this bibliography in book form, also as a Station-sponsored project. This book will be board bound and will contain over 200 pages; the references contained are being listed under 36 material
classes and cross-referenced by methods of analysis. Preparation of copy for this photo-offset printed book should be completed by August 15, 1948, and the book should be published before November 1, 1948.

V. LABORATORY FACILITIES

The special laboratory facilities reported as incomplete in the 1946-47 Annual Report are now fairly complete. Other facilities of the Georgia Institute of Technology, both research instruments and the services of the machine shops, have been used regularly by the project. The machine shop fabricated a shaking machine for the Sierp apparatus, an electrode assembly for the physical-chemical measurement of dissolved oxygen, and several other special pieces of apparatus. The spectrophotometer of the Chemistry Department was employed in the fluoride ion studies.

The Georgia Institute of Technology has increased its laboratory facilities, including a larger sink and a new water still.

The B.O.D. incubator room has been reinsulated, and a chemical and glassware storage space has been provided. Attached is a diagram of the laboratory facilities.

VI. PROGRESS ON EXPERIMENTAL ANALYTICAL TECHNIQUES

A. Calcium Determination

The calcium determination paper by Ingols and Murray, reported in the 1946-47 Annual Report and presented before the Water, Sewage, and Sanitation Section of the American Chemical Society, has been accepted by the Society for publication in Analytical Chemistry. The early manuscript which was included in last year's report has been expanded in order to meet
Bird's Eye View
Sanitary Engineering Laboratory
reviewers' criticisms, but has not been basically changed. Reprints will be submitted as soon as received.

B. Oxygen Consumed Test

Work was completed on the study of a new oxygen consumed method. An article entitled "An Oxygen Consumed Test," by Ingols and Murray, which appeared in the March issue of Water and Sewage Works, describes a rapid acid dichromate procedure which was developed for analyzing sewage or industrial wastes. This method is also much more accurate than the standard method. A reprint is attached as an appendix to this report.

C. Fluoride Ion Determination

A very extensive survey and study of the present techniques for determining the fluoride ion was undertaken, and indications have been obtained that the ferric thiocyanate test might well yield a rapid, sensitive, reproducible, single color test for fluorides. A detailed description of this work is included in the appendix. The fundamental study which has been completed will be augmented by ample field data, and an account of the work will be prepared for publication.

D. Magnesium Ion Determination

Work on this subject was delayed, and, since a recent article in Analytical Chemistry* provides a very similar approach and solution, it has not been continued.

E. Microkjeldahl Procedure

The study of the microkjeldahl technique for total nitrogen was continued, including work on sampling and modification of the apparatus. It has been determined that by using special sized Hengar tubes (21 mm, instead

of 15 mm. diameter) to fit a 24/40 standard taper outer joint, one can digest with no special vacuum equipment even in macorkjeldahl flasks with standard taper joints. Several Pyrex kjeldahl units with a 29/42 fitting were handled readily for occasional student use by using the 21 mm. diameter Hengar adsorption tube with a standard taper bushing (which reduces to 24/40) in the digestion flask. This technique should be especially useful for laboratories with limited space and only occasional need for total nitrogen values. Analysis was completed on a wide variety of samples, including samples prepared by blending, but publication of results is being withheld pending developments on L. C. McMurray's work using this technique on biological samples at Johns Hopkins University.

F. Relative Stability Determination

A large number of records have been obtained which show the color of methylene blue during incubation of sewage samples with oxygen from either nitrates or the dissolved gas. For convenience sake, most of the early work was done with sewage samples from one plant. Results indicated that close agreement can be obtained between the B. O. D. values obtained by dilution and the value obtained from noting the time required for the dye to decolorize. After developing what seemed an acceptable technique, the method was used on four different sewages (from the original source and three others) simultaneously. Only the sewage with which the early work had been done gave reasonable results in comparison with the dilution B. O. D. values on each of the four samples. The only significant conclusion that can be drawn from the work would seem to be the fact that similar B. O. D. values from the same amount of organic matter do not indicate a similarity in the initial flora of the sewages, for even the shape of the decolorization curves of the
methylene blue were completely different for the four samples. Studies indicate, at present, that the method cannot be used as a standard technique in sewage analysis but might be applicable to certain situations, and the work has established a pattern for much additional work on the B. O. D. Publication of a description of this method is contemplated as part of a larger paper on B. O. D. or as a brief unit in a series of papers on the B. O. D. test. A more detailed summary of this work is included in the appendix.

G. Amperometric Titration for Hardness and Chlorine

The use of the rotating platinum electrode for the determination of hardness was studied. It was found that this electrode, however, is not responsive to the oxalate ion. Studies on the amperometric titration for chlorine are under way.

H. Chromium Determination

The importance of accurate chromium determinations in work on industrial wastes and stream pollution and the complete inability on the part of students to obtain results with the technique outlined in Standard Methods has prompted an investigation into a possible modification of this method. It is too early to report the findings, but theoretical considerations have led to the use of an alkaline medium and a catalyst for destroying the excess of the hydrogen peroxide used as the oxidant. The alkali is neutralized, and the color is developed under strict pH control. The test requires less time than does the Standard Methods technique, with 100 per cent recovery of the trivalent chromium.

I. Nitrate Determination with Brucine

Based on the excellent work that has been done on determination of
nitrates with brucine, for boiler water control, it is believed that a good technique can be developed for the determination of nitrates in surface water and sewage effluent. *

J. Sulfate Ion Determination with Benzidine

The accuracy of the determination of the sulfate ion with benzidine is limited both by the rather high solubility of the benzidine sulfate precipitate formed and by the adsorption of the excess benzidine dihydrochloride on the filter medium. According to Standard Methods, there are both upper and lower limits to the sulfate ion concentration that should be determined by this technique. A study has been undertaken here to develop reasonable accuracy at lower sulfate ion concentrations. This study has made use: (1) of a buffer to utilize the pH of minimum solubility for precipitation of the benzidine sulfate, and (2) of a small filter paper disc (2.1 cm. diameter) supported by a Gooch filter crucible in order to reduce the quantity of paper that will adsorb the excess benzidine reagent. A vacuum is used with this small filter area, and this change has necessitated a reexamination to determine the best amount of wash water. When the small filter was first used, values obtained were irregular, because of the small sized crystals formed in some samples while using the precipitation technique of Standard Methods. An uniform precipitate or "batch" of crystals is obtained by adding the benzidine reagent to the sample after it is heated to 90°-100° C. and then cooling the mixture with slow steady stirring. A slightly greater accuracy is obtained by chilling the sample and precipitate in ice water just before filtering. This technique is in the testing stage, and a description of it should be forthcoming for publication.

*This work is being undertaken under project direction but free of fiscal support by a graduate student who is working for his M. S. in Chemistry.
K. B. O. D. Studies

Work here was not originally intended to include a study of fundamental factors involved in the B. O. D. test, but rather to determine whether it would be possible to blend a raw sewage before sampling it for use in the routine dilution B. O. D. technique. Results soon indicated, however, that even 0.5-minute blending caused a sharp fall in the B. O. D. value of the blended sewage. This indicated that blending could not be used in the routine B. O. D. determinations with raw sewages; the question arose, however: "Why not?"

Several possible answers have been considered, but the most probable one would appear to be the fact that the blending reduces the discrete surfaces and therefore the rate of bacteriological activity. It has been shown by Zobell in sea water and by Heukelekian with a pure culture in laboratory media that the numbers of bacteria and the oxygen demand of samples with low food concentration is markedly affected by the presence or absence of discrete surfaces in the incubated sample.* When an excess of oxygen is present in a five-day B. O. D. sample, the initial concentration of organic matter is less than 10 p.p.m. This organic matter concentration is typical of sea water and is within the range of food concentration wherein Heukelekian has shown the importance of surface on bacterial numbers. Our studies indicate that the addition of discrete surface in the form of pyrex glass wool increases the B. O. D. values when it is added to the incubation bottles containing settled or blended sewages in which discrete surfaces would otherwise be absent. A summary of the work on this fundamental factor in the

*See more detailed report on this subject in the appendix.
B. O. D. procedure is included in the appendix. It is contemplated that a description of this work may be published as a part of a large paper on B. O. D. or as a unit in a series on this subject.

L. Direct B. O. D. Studies

Sierp apparatus for direct gas adsorption studies of B. O. D. has been obtained, and tests have been run on many samples to aid in interpreting results. The development of modified apparatus to follow the dissolved oxygen values with the dropping mercury electrode is in progress. This electrode system can be attached to our very sensitive recorder for studies of the effect of different D. O. levels upon the rate of oxygen demand and of the differences in the B. O. D. curves of various sewages.

VII. DISCUSSION AND FUTURE WORK

As pointed out in the Annual Report for 1946-47, the work of the project has deviated from its original program only in that it has involved much more fundamental research on unit methods than was anticipated at the time of the initial application. This was done primarily because much of the published data on methods of analysis have been found to be inadequate for our purposes; for example, the concentration ranges have been considerably different. The program's goal remains the same, but more time is therefore needed to accumulate fundamental data on methods, and, through this fundamental program, to develop simplified apparatus and procedures common to all. Thus, this program should develop, unit by unit, a single set of procedures based on one or several units of equipment, designed for simple manipulation, which will fall within the financial reach of the small plant.
Moreover, each fundamental study in itself constitutes a useful contribution; for example, the present calcium and oxygen consumed publications.

It has been gratifying to the staff to receive the continued support of the U. S. Public Health Service and the helpful comments from other engineers and chemists.

Respectfully submitted,

George W. Reid  
Project Director

Robert S. Ingols  
Chief Chemist

Approved:

Gerald A. Rotholot  
Director
DETERMINATION OF THE FLUORIDE ION

The advent of the control of fluoride concentration in municipal water supplies has increased the demand for a rapid method of analysis for fluorides in water. The recognized methods of analysis (1, 2, 3) are considered unsuitable for control-type testing because they require either too long a period of time, or equipment and technique too elaborate for use by the laboratories which will perform the analyses.

This report describes briefly the findings of investigations made to determine the possibility of modifying one of several existing colorimetric methods of analysis for the determination of the fluoride ion in the concentration range 0-1 p.p.m.

A photoelectric filter photometer* was employed for the measurement of color-concentration relationships.

Investigation of The Standard Methods Procedure (3)

It was found that the zirconium-alizarin lake prepared according to the Standard Methods procedure increased rapidly in color density over a period of three to four hours after preparation and continuing to increase slowly over a period of at least one week. The color development was found not to be affected by light. 1 p.p.m. of fluoride ion affected a decrease in color density equivalent to 30% transmission at a wave length of 530 millimicrons, a scale deflection which would provide for accurate estimation of fluoride ion concentration. Reproducibility, however, was found to be poor. Standard color solutions prepared identically were found to have, after equal intervals of time had elapsed following preparation, transmissions which varied

considerably. In some cases, the variation corresponded to 0.25 p.p.m. of fluoride ions.

It was hoped to find the color development of sufficient uniformity to permit the determination of fluoride ion concentration by obtaining the per cent transmission of solutions at measured time intervals after preparation of the color solutions and then referring to a graph of transmission vs. time elapsed for a series of fluoride ion concentrations. Rapid and irregular initial fading of solutions and poor reproducibility, however, discouraged this approach.

Investigation of The Ferron-Iron Procedure

Attempts were made to modify the method of Fahey (4) to make it suitable for the determination of lower concentrations of fluoride ions. By systematic variation of the concentrations of ferron and iron over a wide pH range, the maximum bleaching effect of 1 p.p.m. of fluoride ion upon the ferron-iron color solutions was found to be very small, approximately 6% transmission at a wave length of incident light of 650 millimicrons. It was concluded that this test could not be made sufficiently sensitive for the photometric determination of fluoride ion concentration below 1 p.p.m.

Investigation of The Ferric Thiocyanate Procedure

Attempts were made to adapt the Foster method (5) to the photometer, but it was found that the optical density of the color solutions produced was too great for direct photometric examination with the instrument used. During the course of the work performed to modify the method for the photometer, interesting observations were made which indicate a good possibility for the final development of a satisfactory test for the fluoride ion.

The effect of fluoride ions upon color solutions of ferric thiocyanate
formed by the reaction of ferric perchlorate and ammonium thiocyanate was studied with the idea of eliminating the interference of ions such as chloride and sulfate which form complex ions with the ferric ion and would obscure the effect of the fluoride ion.

All work was performed with solutions at 20°C, using proper volumetric techniques.

To a standard fluoride solution in a photometer tube, proper amounts of the following were added in order: perchloric acid for pH control, ferric perchlorate solution, and ammonium thiocyanate solution, with small rinses of distilled water between additions to prevent contamination of pipettes. The volume was brought to 100 ml, with distilled water, the resulting solution was well mixed, and the light path through the liquid was adjusted to 150 mm.; measurements were then made with the photometer.

Sunlight, incandescent light, and fluorescent light were found to cause rapid fading of the color solutions. Light of the wave length 490 millimicrons, corresponding to the wave length of maximum absorption by the solutions, was found to cause very rapid fading of the solutions. Light of the wave length 650 millimicrons, corresponding to a wave length of minimum absorption of the solutions, was found to cause the least fading. Fading of color solutions was suspended by preparing them under a red safety light and keeping them in complete darkness. By making transmission readings within two minutes after addition of thiocyanate to the solutions, values for per cent transmission of identically prepared solutions were obtained which were well within the limits of accuracy of the photometer. Series of a dozen solutions each were prepared by adding thiocyanate to the solutions individually under a dim incandescent light and also a dim red safety light.
and storing the photometer tubes immediately in metal-foil light-tight containers. At various intervals up to several hours, the photometer tubes were removed, transmission readings made, and the agreement was found to be excellent.

No study was made to determine the exact effect of temperature. Decreasing the temperature of the solutions to 10 °C. increased the color density and retarded fading. Increasing the solution temperature to 60 °C. decreased the color density and hastened fading.

A number of experiments at various liquid levels, incident light intensities, and concentrations of solutes showed the ferric thiocyanate solutions to be in excellent agreement with the Beer-Lambert law for colored solutions at incident light of wave length 490 millimicrons and constant ferric thiocyanate concentration. These experiments indicated that it is possible to adjust incident light intensity, galvanometer sensitivity, and liquid light path for a particular photometer to realize the greatest change in transmission of a ferric thiocyanate solution effected by a given concentration of fluoride ions.

By spectrum analysis it was established that the effect of fluoride ions could be best observed at the wave length 490 millimicrons.

Color density of ferric thiocyanate solutions increased from very low values at pH 3 and above to a maximum at about pH 2 and again decreased below pH 2. High concentrations showed maxima slightly above pH 2, and low concentrations showed maxima slightly below pH 2.

Considerable work was done to enable the selection of optimum concentrations of ferric iron and thiocyanate ions. Numerous combinations of concentrations of each were tried over the pH range 1 - 4 for the maximum
bleaching effect of 1 p.p.m. of fluoride ions. The concentrations selected for use in developing the test were 1.5 p.p.m. of iron ions and 100 p.p.m. of thiocyanate ions. A lower ion concentration of iron would make the test more sensitive to traces of ferric iron in waters to be examined by the test.

Because of the marked effect of pH upon the color density of the solutions and the difficulty of preparing random water samples to an exact pH for the test, a procedure was followed which would permit adjustment of color solutions to an approximate pH value before making photometric measurements and afterwards obtaining the pH to within ±0.05 pH unit. The results of this procedure, shown in Figure 1, indicate the effect of 1 p.p.m. of fluoride ions on color density over the pH range 1-3. The curves were drawn from values obtained by preparing solutions individually to the ion concentrations 1.5 p.p.m. of iron, 100 p.p.m. of thiocyanate, varying concentrations of fluoride ions, and varying amounts of perchloric acid (for pH control). Transmission readings were made within two minutes after addition of thiocyanate to the solutions, using incident light of the wave length 490 millimicrons. The greatest effect of fluoride ions was observed at pH 2.5 to pH 2.7, but best reproducibility was found at pH 2. Below pH 2, the effect of fluoride ions diminished until the transmission values converged, as shown in the curves of Figure 2. An expanded section of these curves is shown in Figure 3. Through use of Figure 3, several workers obtained reproducible results within ±0.05 p.p.m. of fluoride ion.

When fresh iron and thiocyanate solutions were prepared to make a greater number of determinations to establish with finer accuracy the location of the curves in Figure 3, it was found that the family of curves had shifted upwards by seven per cent transmission. New solutions were
Figure 1.

The effect of the fluoride ion on the ferric thiocyanate complex at varying pH values (adjusted with perchloric acid).
Comparison of the effect of 1.0 p.p.m. of fluoride ions on ferric thiocyanate in chloride and perchlorate media.

<table>
<thead>
<tr>
<th>Curve</th>
<th>F (p.p.m.)</th>
<th>FeCl$_3$ (p.p.m.)</th>
<th>Fe(10$_4$)$_3$ (p.p.m.)</th>
<th>NH$_4$CNS (p.p.m.)</th>
<th>Acid for pH Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
<td>100</td>
<td>Hydrochloric</td>
</tr>
<tr>
<td>a'</td>
<td>1.0</td>
<td>1.5</td>
<td>0</td>
<td>100</td>
<td>Hydrochloric</td>
</tr>
<tr>
<td>b</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>100</td>
<td>Perchloric</td>
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<tr>
<td>b'</td>
<td>1.0</td>
<td>0</td>
<td>1.5</td>
<td>100</td>
<td>Perchloric</td>
</tr>
</tbody>
</table>
Figure 3.

Constant ferric and thiocyanate ion concentrations with amounts of fluoride ions (expansion of section from Figure 1.)
again prepared, and the shifting was confirmed; this could not be explained by variation of technique of preparation or concentrations of the reagents. Although not proved, it is suspected that this system suffers the same disadvantage as other colorimetric techniques. Since any photometric-colorimetric procedure is empirical, variation of conditions from one set of reagents to the next, one day to the next, or one series of determinations to the next is liable to lead to an irregular, unpredictable, and extremely-difficultly-controlled variation in color-concentration relationships. This would necessitate the preparation of several standard color solutions, with each series of unknown solutions being tested to be used either to establish the position of the family of curves in Figure 3 or to adjust the photometer to give transmission readings corresponding to those of Figure 3.

There are certain ions which might be present in natural waters which would interfere with the test for the fluoride ion. Ferric ions present would intensify the color and would have to be compensated for by using less ferric iron reagent, or by removing the extraneous ferric ions by basic treatment. Chloride, sulfate, nitrate, phosphate, borate, and other less frequently occurring ions which form complex ions with the iron, thiocyanate, or fluoride ions would decrease the color intensity to give the effect, usually, of higher fluoride ion concentrations and would have to be accounted for. An effective procedure for compensation would be to depress the activity of the interfering ions to a negligible amount. The perchloric acid used previously for pH control could be replaced by a mixture of acids containing the negative ions mentioned, and this would greatly depress the interference of those ions if present in the water to be tested. For unusually high concentrations of interfering ions, a series of graphs would have
to be prepared after experiment to show the correction of the apparent fluoride ion concentration to be made for known concentrations of specific ions. In any event, the analysis for fluoride ion would have to be performed after the analysis for the other ions, so that the proper corrections could be applied.

The effect of chloride ions is illustrated in Figure 2. One set of curves shows the effect of fluoride ions on the ferric thiocyanate complex in a perchlorate medium, and the other set shows the effect of fluoride ions on the ferric thiocyanate complex in a chloride medium. It may be seen that the change from perchlorate ions to chloride ions had small effect on the color density at pH 2, the pH most favorable for the test. Thus the effect of extraneous chloride ions could be practically eliminated.

The effect of chloride ions is typical of the effect of using sulfuric acid, nitric acid, etc., in the acid for pH control. It will still be necessary to select the most favorable acid mixture and to establish the limits of concentrations of interfering ions beyond which fluoride ion concentration corrections are necessary.

The consideration of other ions depends on the accuracy that is required in the fluoride ion determination.

The results of the work done on this procedure are by all means encouraging enough to warrant the further refinements necessary to complete the procedure for fluoride ion analysis since such a test is urgently needed.
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DISCUSSION OF METHYLENE BLUE B. O. D. TEST

In the relative stability test outlined in Standard Methods, methylene blue is used to indicate the oxidation-reduction potential of the sample. As long as dissolved oxygen is present, the redox potential remains high and the methylene blue retains its color. After the dissolved oxygen has disappeared, the oxygen combined in nitrites or nitrates is utilized at a redox potential high enough to retain the methylene blue color. When the nitrite and nitrate oxygen has been utilized, the methylene blue is then decolorized, before the combined oxygen in sulfates is attacked.

The nitrate B. O. D. method proposed by Loderer in 1915 (1) was discarded as a means of determining sewage strength primarily because the analyses for nitrate and nitrite present at the end of the incubation period were more cumbersome than the determination of the D. O. in the excess oxygen (dilution) method. However, by amalgamating the nitrate and methylene blue methods, it was hoped here to adapt the use of methylene blue to serve as an indicator of the time required to use up all of the available nitrate oxygen.

Some special apparatus was developed for this study. Windows were cut in two small tin cans; one was left right side-up and the other turned upside down, then both were soldered together (side to side) in such a manner that light could pass through the window of the first one to a photoelectric cell under the second (inverted) can. All the inside surfaces were painted a dead black. The top of the sample bottle was fitted with a small tin cap, an arrangement shown in Figure 1. The leads from four of these cells were connected to the four sets of leads of an electronic-strip-chart recording galvanometer. Each point recorded once every two minutes; i.e., there was
Figure 1. The Light Source, a Photoelectric Cell, Covers and Sample Bottle Used with Methylene Blue Studies.
a point printed each 0.5 minute. This recording galvanometer was the slowest period recorder manufactured by the Brown Instrument Company, and it has proven satisfactory for this work.

Each sample was placed in a regular D. O. bottle, then one ml. of the 0.5 per cent methylene blue solution was added and one or two ml. of a nitrate solution containing five mg. of nitrate-oxygen per ml. The stopper was replaced, and the bottle was then shaken and placed in the upright tin can in front of the photocoelctric tube. With the light on and the recorder operating, the only work required was the reading of the chart on the next day, then the calculation of the B. O. D. If a sewage sample is fresh, it may contain some D. O.; to allow for this, a duplicate sample bottle was made up for determining the D. O., and this amount of D. O. was used along with the nitrate-oxygen in calculating the B. O. D. With the total available oxygen and the time required for decolorization of methylene blue dye both known, the B. O. D. can be calculated by using the relationship (described below) between the ultimate B. O. D. and the percentage B. O. D. completed within a given time. For convenience, a chart was drawn so that the B. O. D. could be read directly; this chart, shown in Figure 2, was based on the theoretical B. O. D. curve from the Relative Stability Table in Standard Methods (2) and the average of the volume of the glass-stoppered reagent bottles that were used.

All samples that were analyzed by the methylene blue test were also analyzed for their dilution B. O. D. and dichromate O. C. Most of the preliminary work on the M. B. test was carried out on samples from one of Atlanta's smaller sewage treatment plants because this plant receives its peak load earlier in the morning and samples were thus more easily obtained.
Figure 2. Relationship Between Time Required to Decolorize Methylene Blue and the Sample's B.O.D. of a Sewage Sample, Using 275 ml. Bottles and a Known Concentration of Oxygen.
In Figure 3, the well-known toxic effect of increasing the concentration of the dye is shown. The sample which shows the shortest time for the decolorization of the methylene blue represents the lowest concentration of the dye, while the sample with the highest concentration shows no decrease in color during the period of study.

Figure 4 shows a comparison of the time required for decolorization of a raw and blended sewage using dissolved oxygen instead of nitrate-oxygen, as well as two different dyes—methylene blue and resaurin. The blended sewage with more D. O. present decolorized both dyes more rapidly than did the raw sewage. This indicates that at full concentration blending does not retard the B. O. D. activity, but at very low concentrations does retard the B. O. D. reaction (as discussed in Appendix C). The onset of the decolorization for both dyes occurs at the same time. There is a slight rise of transmission of light initially within the first few minutes, which appears to be caused by a reduction in turbidity.

In Figure 5, three dyes and two different sources of oxygen with methylene blue are compared. The results, as shown in Line No. 1, show that after the D. O. is utilized the methylene blue is largely reduced, then re-oxidized again as the bacteria attack the nitrate oxygen. This is quite typical of the behavior of the sewage from this one plant. It was at one time thought that the problem of running D. O. might be eliminated by first bubbling nitrogen through the sample, but the color began to disappear immediately in spite of 36 or even 52 p.p.m. nitrate oxygen and did not reappear in many cases. Septic sewages behaved in the same manner as the deaerated sewage sample, and it became evident that the test as set up would not develop into a routine laboratory procedure, where sewages in all con-
Figure 3. Records of the Time Required to Decolorize a Sewage Containing Different Amounts of 0.5% Dye Solution (Resaurin).

Line No. 1 3.0 ml. dye
Line No. 2 2.0 ml. dye
Line No. 3 1.0 ml. dye
Line No. 4 0.5 ml. dye
Figure 4. A Comparison of the Times Required to Decolorize
Raw and Blended Sewages, with Two Dyes.

Line No. 1 Raw Sewage with 10.9 p.p.m. and MB
Line No. 2 Raw Sewage with 10.9 p.p.m. D. O. and Resaurin
Line No. 3 Blended Sewage with 14.9 p.p.m. D. O. and MB
Line No. 4 Blended Sewage with 14.9 p.p.m. D. O. and Resaurin
Figure 5. A Comparison of Different Dyes with Different Sources of Oxygen in the Same Sewage Sample.

Line No. 1  Sewage plus 18.2 p.p.m. Nitrate Oxygen with MB
Line No. 2  Sewage plus 16.6 p.p.m. D. O. with MB
Line No. 3  Sewage plus 16.6 p.p.m. D. O. with Resaurin
Line No. 4  Sewage plus 16.6 p.p.m. Nitrate Oxygen with MB
ditions must be handled. The results shown in Figure 5 indicate that methylene blue is the most suitable of the dyes tested.

After much of this preliminary, favorable work had been completed, using one source of sewage, sewage from another source was tried, and results obtained showed no agreement with the previous data, regardless of the condition of this sewage. In attempting to duplicate some of the earlier work the color persisted indefinitely, with only a slow, steady decline in the blue color. At times there was still color after five days, although the Sierp test for direct oxygen absorption as described by Falk and Rudolfs (3) gave a completely normal B. O. D. value. After several results like this were obtained, it was decided to obtain four samples on the same day from different sewage plants and to set up O. C. tests on each, diluting the stronger ones to have the same concentration of organic matter as the others. Dilution B. O. D.'s, Sierp tests, and relative stability tests were also set up on each. Settled sewage was used. The Sierp B. O. D. values ranged from 77-87 p.p.m., the dilution B. O. D. values were all approximately 70 p.p.m., and the relative stability values ranged from 35-68 p.p.m. The total O. C. value of 135 p.p.m. indicated that the five-day B. O. D. values should have been approximately 85 p.p.m. and, thus, should have agreed with the Sierp values. The lower dilution B. O. D. values could have been predicted on the basis of the use of settled sewage. However, a rational explanation of the difference in the type of curves obtained, as shown in Figure 6, and the different B. O. D. values which are indicated by different times at which the methylene blue decolorized, is not known. By mere chance, the 68 p.p.m. relative stability B. O. D. value was the closest to the 70
Figure 6. A Comparison of Four Sewages from Different Sources Which Have the Same O. C. and Dilution B. O. D. Values.
p.p.m. dilution B. O. D. value and was obtained on the sewage on which all of our early work was based.

The only possible explanation that might account for these striking differences in the relative stability B. O. D. values is the possible difference in the quantity of nitrifying flora which might be deficient in those with the low B. O. D. values. Experimental evidence does not substantiate this explanation, for all four sewages began to nitrify on the fourteenth day in an aerated sample, and the one which should have had the lowest B. O. D. was actually highest. With the high Sierp values, a toxic factor is probably not involved, and the pH is normal.

Many other results have been obtained during attempts to amalgamate the nitrate B. O. D. method and the relative stability test, but enough data have been included here to show that some interesting results have been obtained and that some fundamental differences in sewages from different sources render this method wholly unsuitable for the routine laboratory analysis of raw or settled sewages. No cause or explanation for the fundamental difference between sewages can be offered as yet, except the fact that some organisms have much longer lag periods than others.

An attempt to use this method to obtain the B. O. D. of pure substances ended in failure, indicating the wisdom at present of the five-day incubation period for the B. O. D. test.
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APPENDIX C
SAMPLING AND BLENDING RAW SEWAGE

Determining the B. O. D. of a raw sewage accurately is important for many reasons. One factor involved is the necessity of including the settleable suspended solids; Rudolfs and Gohm (1) for example, have shown that approximately one third of the total B. O. D. is furnished by the settleable solids fraction of the raw sewage. The measurement of the raw sewage also requires great care in order to get a proper sample. On the surface, therefore, it would seem simple and desirable to obtain a sewage with all of its settleable matter in suspension merely by processing a relatively large amount of raw sewage in a blender.

Recently, Setter, Price, Grossman, and De Grazia (2) gave a paper before the American Chemical Society describing a method in which the sewage was "blended" for a short period of time. The turbidity of the blended sewage was then determined photometrically, and the turbidity value was correlated with the suspended solids. Conceivable, of course, the chopping up of the material might increase its availability as food and thereby increase the B. O. D. Some blended samples might well include the chopped up particles from a large piece of solid matter, thus causing a very large increase in the O. C. and B. O. D. values. In our tests, this occurred once when the O. C. jumped from 450 to 800 p.p.m, and the B. O. D. from 230 to 345 p.p.m. However, in general the O. C. values have remained nearly constant, while the dilution B. O. D. values have been definitely lower than the values before blending.

There may be several explanations for this decrease in the B. O. D. values of the blended sewages. A direct chemical oxidation of the organic matter may occur as a result of both the oxygen dissolved by the blending
and the concurrent increase in the surface of the solid matter. The small increase which occurs in the O. C. values with blending does not favor this explanation, but we plan to set up an experiment to blend the sample in an atmosphere of nitrogen in order to check this point. Blending will destroy the plankton in the sewage, and this loss may contribute to the lower B. O. D. Blending also changes the particle size of the suspended matter, which change is accompanied by an increase in bacterial numbers as indicated by an increase in the number of colonies appearing in a standard plate count. The reduction in the particle size by blending may remove the discrete particles which the bacteria need to utilize the food at low food concentrations.

In 1940, Heukelekian and Heller (3) published data from which they drew the following conclusion: "Under the conditions of the experiment the growth of Escherichia coli did not take place in glucose and peptone concentrations of 0.5 and 2.5 p.p.m. The addition of glass beads to the medium at these concentrations permitted a considerable growth of these organisms. The effect of the glass beads was noticeable up to 25 p.p.m. concentration of glucose and peptone. Beyond this concentration the numbers of E. coli with and without beads were practically the same." The portion of their data substantiating this quotation is shown in Figure 1. This work was carried out with a pure culture, but several other articles have also been published indicating the importance of added or discrete surfaces during incubation of natural waters or mixed cultures. In 1943, Zobell (4) published a summary article in which he discusses the possible mechanisms by which solid surfaces increase the bacterial numbers and oxygen demand of dilute nutrient solutions. He indicates that in mixed cultures, 10 p.p.m. of organic matter rather than 25 p.p.m. is the critical value, at least for sea water.
**Fig. 1. Numbers of Escherichia coli (in Log_{10} per ml.) in Various Concentrations of Glucose and Peptone During 72 Hours' Incubation with and without Glass Beads**

(Initial number 170 per ml.)

Reproduced from Reference (3)
Figure 2 shows a plot of the theoretical relationship between oxygen depletion or demand in any B. O. D. sample bottle and the organic matter concentration. It is obvious that the organic matter concentration in the many individual B. O. D. bottles is well within the range of the 10 p.p.m. of organic matter in which the rate of reproduction of the bacteria and the oxygen demand are, in part, a function of the surface area available. If this is true, the question arises: will the addition of surface to the sample bottle raise the B. O. D. value of the blended sewage?

It has been found on experiment that either glass wool or asbestos added to the B. O. D. bottles will in general increase the B. O. D. value of the blended sewage; glass wool and asbestos were chosen because they are inert materials which possess a very small volume in relation to their surface areas. The results with glass wool for blended raw sewages and settled sewages are shown in Table I. The asbestos was added in 1.0 ml. of a suspension in dilution water; the glass wool was placed in the bottle before adding the dilution water and thoroughly wet before adding the sample. Less than 0.5 ml. of water was displaced by the amount of glass wool used.

If a discrete surface is needed for full biological activity at low concentrations, then a settled sewage may exhibit an increase in its B. O. D. value in the presence of some glass wool and a decrease in its B. O. D. with decreasing concentration. The concentration used and the presence of surface will affect the magnitude of the differences in the B. O. D. values of a settled sewage, as shown in Figure 3. Further, the blending of settled sewage causes a drop in the B. O. D. value, indicating that the settled sewage may have had some of its discrete surfaces broken down or that some other factor is changed and thus influences the rate of the biochemical oxidation.
TABLE I

EFFECT OF ADDED DISCRETE SURFACES UPON THE DILUTION B. O. D. VALUES OF BLENDED RAW OR SETTLED SEWAGES
(All values are the averages of not less than 8 bottles)

<table>
<thead>
<tr>
<th>Raw Sewage</th>
<th>Settled Sewage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blended</td>
<td>Blended</td>
</tr>
<tr>
<td></td>
<td>With Added Surface</td>
</tr>
<tr>
<td>P.P.m.</td>
<td>P.P.m.</td>
</tr>
<tr>
<td>150</td>
<td>127</td>
</tr>
<tr>
<td>82</td>
<td>60</td>
</tr>
<tr>
<td>270</td>
<td>225</td>
</tr>
<tr>
<td>244</td>
<td>186</td>
</tr>
<tr>
<td>210</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>With Added Surface</td>
</tr>
<tr>
<td>P.P.m.</td>
<td>P.P.m.</td>
</tr>
<tr>
<td>106</td>
<td>144</td>
</tr>
<tr>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>67</td>
<td>88</td>
</tr>
<tr>
<td>110</td>
<td>106</td>
</tr>
</tbody>
</table>
Figure 2.

Figure 3.

A Comparison of P. C. D. Values of a Settled Sewage at Three Different Dilutions with and without Surfaces, in Ten Bottles for Each Point.
The curves of Figure 4 indicate that over a period of more than five days the presence of added surface to a settled sewage not only increases the rate at which carbonaceous oxidation can proceed, but also favors the earlier onset of nitrification. These higher values with discrete surfaces agree with the long-time values of Zobell and Anderson (5) on the effect of the presence of a surface in the incubation of sea water.

Discussion

The B. O. D. value of raw sewages has been obtained both in the presence and absence of extra glass wool. The average value of several sewages shows no effect of the added surfaces, as might be predicted.

The results of the studies of Ruchhoft (6) appear to be in direct contradiction to the hypothesis of lower B. O. D. values in low concentrations of settled sewage in a B. O. D. dilution series. However, Ruchhoft uses cotton filtered raw sewage samples, while our laboratory has used settled sewage samples. The results of Ruchhoft indicate that the onset of nitrification occurs earlier in lower concentrations of sewage in a B. O. D. series, while our results indicate that the bacterial action, both carbonaceous and nitrogenous, is delayed by an increased dilution in the absence of discrete surfaces. If both results are correct, then it would seem that the B. O. D. samples in Ruchhoft's tests (6) at lower concentrations must contain sufficient discrete surfaces to give the proper carbonaceous oxidation and encourage nitrogenous oxidation. These results are in accord with those of ours shown in the upper curve of Figure 3, which shows practically no lag in the onset of nitrification.

Before coming to any definite conclusions on the basis of these data, it should be pointed out that, although the results have been quite con-
Figure 4.

The effect of the presence and absence of surface during incubation of a settled sludge.
consistent, more data are needed. It is true, however, that Moelmann (7) reported in 1925 that: "We have had difficulty in getting satisfactory results in dilutions in which the depletion is less than 2.0 p.p.m. ...", and this work or Ruchhoft's work would serve to explain those earlier results. It would seem that the original conclusions of Theriault and Hommon (8) are correct in that different dilutions of the samples for B. O. D. will give consistent B. O. D. values when there are discrete surfaces present in each B. O. D. bottle, and when the concentration is high enough to retard the early onset of nitrification. However, tentatively, it is suggested that the earlier conclusions may need to be modified for those sewage B. O. D. bottles which originally contain too low a concentration of sample with or without discrete surfaces; with discrete surfaces, the values may be too high, while without, the values may be too low. It is further concluded that blending of a raw sewage sample will increase the bacterial numbers, yet cause a reduction in the B. O. D. values; further, that the addition of glass wool may restore a large part of the B. O. D. loss in the blended sample.
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An Oxygen Consumed Test for Sewage

R. S. Ingols and P. E. Murray
State Engineering Experiment Station
Georgia School of Technology

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AN OXYGEN CONSUMED TEST FOR SEWAGE

R. S. INGOLS and P. E. MURRAY*
Georgia School of Technology
ATLANTA, GA.

As early as 1893, Wyatt
troduced a procedure for determining the amount of organic pollution in a water by noting the length of time required for the reduction in color of the permanganate ion by the organic matter present in the sample. Many authors* have published the findings of their attempts to improve the accuracy and reproducibility of the original oxygen consumed (O.C.) technique that uses permanganate. In America, the chemists have agreed to digest the sample with the permanganate ion by placing the flask with the sample and reagents in a boiling water bath, while in England† the standard practice is 4 hours digestion at 27°C.

In 1926, Adeney and Dawson published an acid dichromate method for analyzing water or sewage. In 1927, Abbott published a similar procedure using a lower temperature of digestion. Each of them added a small volume of sulfuric acid to the sample, took the sample to dryness on a water bath, and continued heating to drive off the chlorides. A small volume of a standard dichromate solution was added to the residue and the mixture again evaporated to a very small volume. More sulfuric acid was added and the mixture digested for two hours on a water bath (by Abbott) or at a higher temperature (by Adeney). The excess dichromate was then determined and the difference in titrations between this value and the blank titration value used as the O.C. method for the "Determination of Total Organic Carbon in Sewage and Industrial Wastes by Digesting the Sample with the Dichromate Ion in a Phosphoric-Sulfuric Acid Mixture. The amount of organic matter was determined by measuring the carbon dioxide evolved during the digestion. The O.C. values, calculated on the basis of the organic carbon, correlated fairly well with the 20 day biochemical oxygen demand (B.O.D.) values as in the case of Abbott. However, a technique for organic carbon which measures the carbon dioxide evolved is time consuming because of the apparatus that must be assembled and taken apart and the amount of attention that must be paid to the digestion. In 1941, Johnson and Halvorson reported an O.C. procedure using the iodate ion instead of the permanganate or dichromate ion for oxidizing the organic matter of polluted samples. Only abstracts of this method have been published, however.

The standard O.C. test may be used in analyzing certain toxic industrial wastes or in giving a rapid approximate sewage strength if the value is multiplied by some factor peculiar to the type of sample. It has been realized for some time that the results of the O.C. test are only slightly affected by the complex nitrogenous organic matter present in a sample. The English have realized this difficulty and have developed an O.C. test for nitrogenous material by using the hypochlorite ion as the oxidizing agent and have continued to use the permanganate ion for determining the carbonaceous matter in the samples. The use of the hypochlorite ion has not been adopted in this country.

According to Standard Methods for the Analysis of Water and Sewage, the results of the O.C. test are affected by the amount of carbonaceous matter in the sample. As shown in Table II, a 200 ppm. starch solution gives an O.C. value only one-half that of a 200 ppm. dextrose solution. It would appear that the conditions of the O.C. test do not favor the hydrolysis of complex organic substances, nitrogenous or carbonaceous. It would seem, therefore, that some adequate substitute for the O.C. test is highly desirable because there are times when a rapid simple chemical test is needed to indicate the total reducible matter as an alternate or adjunct to the B.O.D. test. Thus, an inadequate number of organisms in a sewage or industrial waste which contains toxic substances, or an unbalanced food content, may respond only partially to the food in the sample during the B.O.D. test while a good O.C. test could give a truer index of the amount of food present. The long incubation period of the B.O.D. test makes the results a matter of record only and prevents its use for direct plant control, while a satisfactory O.C. test will give results within a period of time that may be helpful for plant control.

Recently, Rhame published a method for the "Determination of B.O.D. Values by Chemical Oxidation." This test with modifications would seem to be a good substitute for the present permanganate O.C. test, because the new test is rapid, requires little attention, and yields values which give either reasonable correlation with

*Research Associate Professor and Research Assistant respectively of the State Engineering Experiment Station of Georgia School of Technology, Atlanta, Ga.

Table I

<table>
<thead>
<tr>
<th>Acid Res.</th>
<th>Water added</th>
<th>Time of digestion</th>
<th>Temp. of reflux</th>
<th>Total time</th>
<th>Total volume</th>
<th>Initial</th>
<th>終點</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml.</td>
<td>ml.</td>
<td>mins.</td>
<td>°C</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>155</td>
<td>23.5</td>
<td>23.5</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>145</td>
<td>24.7</td>
<td>24.7</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>135</td>
<td>24.2</td>
<td>24.2</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>125</td>
<td>23.3</td>
<td>23.3</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>
the 20 day B.O.D. values of the samples or theoretical oxidation values for some pure organic compounds.

When Rhame published his method he realized that it would require some refinement in technique. This paper describes a procedure which includes several modifications of Rhame's technic and presents data showing the correlation between the modified O.C. values and the standard B.O.D. and O.C. values.

**Method**

1. Dichromate oxidizing reagent:
   1 vol. of conc. sulfuric acid C.P. 95%
   1 vol. of orthophosphoric acid C.P. 85%
   1.25 g. potassium dichromate per liter
   (Dissolve the dichromate in 20-25 ml. distilled water before adding acid)

2. Sodium iodide solution 20% C.P.
3. Sodium thiosulfate solution 0.025N standard
4. Soluble starch solution
5. Silver nitrate and potassium dichromate solutions for determining chlorides according to Standard Methods®.

**Apparatus**

1. 250 ml. Erlenmeyer flasks with outer 24/40 Standard taper neck
2. Reflux Condenser with inner 24/40 Standard taper fitting

**Procedure**

Place 25 ml. of the oxidizing reagent in the 250 ml. flask with 10 ml. of sample. This mixture will boil at 145° C. under a reflux condenser. If raw sewage is used approximately 5 ml. of sample will be needed, then 5 ml. of water must be added to provide a volume of 10 ml. of sample plus the 25 ml. of reagent. If a larger volume is needed for dilute samples such as effluents and stream samples, then the excess water must be removed by evaporation until the temperature of the mixture reaches 145° C. (35.0 ml. total vol.)

Place the flask plus sample over a burner and reflux 60 minutes so that the fumes do not rise higher than one inch above the lowest cooling surface of the condenser. Cool; rinse down reflux condenser with approximately 100 ml. water; cool; add 3 ml. potassium iodide solution; wait 5 min.; then titrate the free iodine with the standard thiosulfate solution.

The oxidizing reagent can be made up accurately enough to be a primary standard or checked against the thiosulfate solution. The blank of the oxidizing reagent should be determined without heating the reagent. Determine the chlorides in the sample. The results to be calculated are based upon the difference in the titrations of the thiosulfate with the reagent alone and with the reagent after it has reacted with the sample, and corrected for the concentration of chlorides in the sample.

This formula will give the O.C. value which will approximate the 20 day B.O.D. because it is based on complete oxidation. If it is multiplied by the factor of 0.68, one will obtain a value that will approximate the 5 day B.O.D.

A mixture of phosphoric and sulfuric acids is used in the oxidizing reagent because the phosphoric acid alone does not have a high enough boiling point while the sulfuric acid water mixtures of the proportions to give the desired temperature bump violently. An all-glass apparatus is recommended to prevent contact of the hot vapors with organic matter not in the sample. The beakers recommended in the Rhame's article were discarded because of loss of the reagent and sample by splashing.

**Experimental Development Of Method**

The development of the procedure given above was carried out with solutions of 200 ppm. dextrose, starch, and peptone. This concentration was chosen because a 10 ml. aliquot of sample consumed about one-half of the dichromate of the oxidizing reagent in being completely oxidized. It soon became evident that the following factors must be studied:

1. The correct titration value for the blank.
2. The relative volume of water to be used with the oxidizing reagent during digestion and temperature of digestion.
3. The length of time for refluxing the mixture.
4. The use and choice of a catalyst for the oxidation of the organic matter in the sample by the dichromate.
5. The effect of the concentration of the chloride ion.

When using a good grade of dextrose or starch whose exact formula is known, it is possible to calculate precisely the theoretical amount of dichromate that should be reduced by the sugar or starch as well as the experimental amount of dichromate which is actually used by the solutions of these substances. The correct titration value for the blank is very important for checking the theoretical values of the amount of

### Table III

<table>
<thead>
<tr>
<th>Substance</th>
<th>Conc. (Ppm.)</th>
<th>Catalyst</th>
<th>Theoretical Oxygen Required</th>
<th>Average Experimental Value</th>
<th>Per cent of Theoret. Value</th>
<th>XO.68*</th>
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</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>200</td>
<td>None</td>
<td>214</td>
<td>210 98</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>200</td>
<td>selenium</td>
<td>214</td>
<td>210 98</td>
<td>145</td>
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<tr>
<td>Starch</td>
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<td>227</td>
<td>222 98</td>
<td>145</td>
<td></td>
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<tr>
<td>Starch</td>
<td>200</td>
<td>selenium</td>
<td>227</td>
<td>222 98</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Peptone</td>
<td>200</td>
<td>none</td>
<td>220</td>
<td>220 100</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Peptone</td>
<td>200</td>
<td>selenium</td>
<td>220</td>
<td>220 100</td>
<td>142</td>
<td></td>
</tr>
</tbody>
</table>

*Experimental value of O.C. multiplied by 0.68. To compare with the 5 day B.O.D. values.

**Developed by aerating sewage with starch for several days prior to setting up the dilutions.

### Table II

**Comparison of the Values Obtained By Oxidizing Three Organic Substances By Means of Three Methods of Oxidization**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Conc. (Ppm.)</th>
<th>Catalyst</th>
<th>Dichromate O.C.</th>
<th>Permanganate O.C.</th>
<th>Dilution B.O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average Exp.</td>
<td>Average Exp.</td>
<td>Average Exp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Value</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OF Theoret.</td>
<td>OF Theoret.</td>
<td>OF Theoret.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Value</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ppm.</td>
<td>Ppm.</td>
<td>Ppm.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>200</td>
<td>none</td>
<td>214</td>
<td>210 98</td>
<td>145</td>
</tr>
<tr>
<td>Dextrose</td>
<td>200</td>
<td>selenium</td>
<td>214</td>
<td>210 98</td>
<td>145</td>
</tr>
<tr>
<td>Starch</td>
<td>200</td>
<td>none</td>
<td>227</td>
<td>222 98</td>
<td>145</td>
</tr>
<tr>
<td>Starch</td>
<td>200</td>
<td>selenium</td>
<td>227</td>
<td>222 98</td>
<td>145</td>
</tr>
<tr>
<td>Peptone</td>
<td>200</td>
<td>none</td>
<td>220</td>
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<tr>
<td>Peptone</td>
<td>200</td>
<td>selenium</td>
<td>220</td>
<td>220 100</td>
<td>142</td>
</tr>
</tbody>
</table>

*Experimental value of O.C. multiplied by 0.68. To compare with the 5 day B.O.D. values.

**Developed by aerating sewage with starch for several days prior to setting up the dilutions.
dichromate reduced by the sugar and starch. According to the present concepts, the blank value will be different for various conditions, so that several conditions were studied. When all of the water of the sample is evaporated off from a sample acid dichromate mixture, as indicated by the volume remaining in the flask, the temperature rises to approximately 155°C. The temperature continues to rise rapidly to 170°C unless a reflux condenser is used to prevent the further loss of some volatile constituent.

As shown in Table I, the oxidizing capacity of the dichromate falls off rapidly when a blank is heated at 155°C or higher. It is also true that the organic oxidation proceeds rapidly at these high temperatures, but it is difficult to choose the proper length of time of heating or the correct blank value when the time factor is so critical. The results shown in Table I indicate that 10 ml. of sample plus 25 ml. of acid dichromate reagent gives the blank value nearest to the value obtained without any heating, even though the blank containing 25 ml. reagent and 10 ml. water had a higher temperature during refluxing than those mixtures with more water. This would give the shortest digestion period with the least error from an excessive period of digestion.

The data of Table I further shows that the dichromate remaining decreases as the amount of water boiled with it increases. It is concluded from these data that the dichromate was reacting with the water to form hydrogen peroxide which decomposed to give free oxygen and water. An experiment was set up with a constant volume of a reagent of pure phosphoric acid with dichromate and with varying amounts of water. The phosphoric acid water mixtures had much lower temperatures of refluxing (105-115°C), but the results again indicated that the water reacted with the dichromate. It is important to know whether hydrogen peroxide under these conditions will oxidize organic matter first or break down first. The problem was attacked experimentally by making up a small volume of 1:1 sulfuric acid-phosphoric acid mixture containing 0.5 per cent by volume of 30 per cent hydrogen peroxide. Ten ml. of 200 ppm. dextrose solution was refluxed with 25 ml. of the acid peroxide reagent for one hour. Then one ml. containing 31 mg. potassium dichromate (the amount in 25 ml. of the acid dichromate reagent) was added and the heating continued for one hour. There was no reduction in the dichromate after the hour's heating. This indicated that the hydrogen peroxide had oxidized all of the sugar before the dichromate was added. It was concluded that the hydrogen peroxide will oxidize the organic matter before breaking down if any is formed during the digestion period and that no correction should be made in the dichromate titration run on the reagent directly in the cold.

Because of the simple procedure that has been outlined above in the absence of chlorides, the determination of dextrose was repeated in the presence of several different concentrations of chlorides. The average of six determinations at each of four different chloride concentrations showed that the dichromate was reduced by the amount theoretically required to oxidize the chloride to chlorine in concentrations up to 240 ppm. chloride in the presence of dextrose.

From the data presented above, it was decided to use the mixture of 10 ml. of sample with 25 ml. of acid dichromate reagent in order to attain the highest possible digestion temperature with the smallest breakdown in the dichromate if an excessive time of refluxing was used.
other catalysts were tried without any success. The increase of the peptone O.C. value when using selenium makes it appear that for many complex substances one should use the selenium catalyst, but the small increase indicates either that the oxidation with the selenium was complete or that a better catalyst must be found. It was concluded that heating, refluxing, for one hour with selenium will give complete oxidation.

The permanganate O.C. values shown in Table II are in very poor agreement with the theoretical values for the oxidation of starch and sugar. The dilution B.O.D. values shown in Table II for dextrose and peptone are in good agreement with previously published values for these compounds. The basic chemical identity between starch and dextrose means that starch should have the same or slightly higher B.O.D. than dextrose. The need for a special seed to attain this B.O.D. value for starch indicates the need for special precautions for all B.O.D. determinations on industrial wastes and the poor seeding characteristics of ordinary sewage under certain conditions. The high concentration of the acids in the dichromate reagent makes the reagent an excellent solvent for paper. The dispersed paper gives an O.C. value that compares with starch. Since the dilution method for B.O.D. gives no oxygen demand for paper, this will be a source of discrepancy in the analysis of raw sewage.

A comparison of the dilution B.O.D. values with the new dichromate O.C. values multiplied by 0.68, for a number of sewage samples, is presented in Fig. I and Table III. The results indicate that the O.C. values x 0.68 run somewhat higher than the dilution B.O.D. values. This might be expected from the presence of substances known to be oxidizable chemically but not biologically, such as paper and humus. The differences in the B.O.D. and dichromate O.C. values are increased by the selenium with the raw sewage, but the O.C. values are not increased by the selenium with the settled sewage. This indicates that the settled sewage does not contain as much material which resists oxidation as does the raw sewage.

Discussion

There are several interesting differences between this dichromate O.C. procedure and the one suggested by Adeney and Dawsonson or Abbott. Both of these older procedures removed the chlorides before digesting the sample with the dichromate, while this proposed procedure recommends a separate determination of the chlorides and an arithmetic correction during the calculation. It is obvious that the accuracy of any of the three methods will decrease as the ratio of the chlorides to the organic matter increases.

The two earlier procedures controlled their temperatures externally by using steam (1) or a thermostatic hot plate (2), while the digestion temperature of this procedure is controlled by the boiling point of the mixture of 25 ml. of the sulfuric-phosphoric acid reagent, with 10 ml. of the sample. Each of the three procedures are alike in determining the O.C. value by calculating the dichromate reduced from the difference in the amount of dichromate remaining after digestion and the amount present originally or in a blank. By using some (but not an excessive amount of) water, the need for running a blank with each set of samples in this procedure has been eliminated; whereas the earlier procedures removed all water from the digesting mixture to permit a direct breakdown of the dichromate.

Because many organic molecules contain some oxygen in their structure, this O.C. test, which measures the reducing equivalents in an organic mixture, would seem to be a better method for measuring the strength of sewage or industrial wastes than the complete organic carbon methods which measure the carbon dioxide evolved. Further, this proposed O.C. test is easy to conduct, uses simple apparatus, and is rapid.

In Rhame's article, the median values for the dichromate O.C. test using the 0.68 factor and B.O.D. values were roughly equivalent, while with the present procedure for the O.C. test, the O.C. values are all higher than the dilution B.O.D. values. On a purely theoretical basis, the O.C. value which is based on complete oxidation should run higher than the B.O.D. value unless there has been either (1) poor sampling, (2) a loss of sample or reagent during the O.C. test, or (3) an apparent failure of the reagent to oxidize the sample due to the use of a low blank titration value in the O.C. calculation. Where pure food substances are used, the O.C. and B.O.D. values can and do approach each other very well.

Summary

This paper presents a rapid analytical procedure for determining chemically the amount of oxidizable material present in sewage. A small aliquot of sewage is added directly to a small volume of a 1-1 mixture of phosphoric and sulfuric acids plus some potassium dichromate and the mixture refluxed for sixty minutes in the presence of selenium. The amount of dichromate remaining after oxidizing the sewage is determined chemically and the oxygen consumed value is calculated by the difference in the dichromate present originally and finally, and is corrected for the chloride concentration of the sample.

Acknowledgement

The work of this paper is part of a project supported by a grant-in-aid from the National Institute of Health of the United States Public Health Service for research upon rapid analytical procedures for water and sewage. The samples of sewage were provided by Mr. Ray, the sewage plant chemist for the City of Atlanta, who delivered them daily while collecting his samples from the small city plants.

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FINAL REPORT

PROJECT NO. 117

GRANT-IN-AID RG264C

RAPID CHEMICAL AND PHYSICAL ANALYTICAL TECHNIQUES
IN WATER AND SEWAGE ANALYSIS

Prepared for

NATIONAL INSTITUTES OF HEALTH
UNITED STATES PUBLIC HEALTH SERVICE

By

GEORGE W. REID
and
ROBERT S. INGOLS

OCTOBER 1, 1946--JANUARY 1, 1950
FINAL REPORT
PROJECT NO. 117
GRANT-IN-AID RG264C

RAPID CHEMICAL AND PHYSICAL ANALYTICAL TECHNIQUES
IN WATER AND SEWAGE ANALYSIS

Prepared for
NATIONAL INSTITUTES OF HEALTH
UNITED STATES PUBLIC HEALTH SERVICE

By
GEORGE W. REID
and
ROBERT S. INGOLS

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This Report Contains 16 Pages
I. SUMMARY

The research on rapid methods of water and sewage analysis supported by the National Institutes of Health has included investigations of individual techniques, comparison of time and equipment required for various techniques of analysis, and methods in general. Emphasis has been placed on micromethods, rapid semiquantitative procedures, and other time-saving modifications of existing techniques. Accomplishments include publications, six unpublished manuscripts, a 215-page literature search, and three specific recommendations to the Standard Methods Committees on modifications of techniques. Specific studies have been made of oxygen consumed, fluoride, magnesium, nitrogen, relative stability, hardness, chlorine, chromium, nitrate, and sulfate. Comparative studies have been made of B.O.D., O.C. and direct oxygen-demand measurements, dissolved oxygen, and cost of water and sewage analytical procedures.

II. INTRODUCTION

This project, supported by a grant-in-aid from the Surgeon General, U. S. Public Health Service, was initiated to study "Rapid Chemical and Physical Analytical Techniques in Water and Sewage Analysis."

The primary objective was the determination of the possibilities of using micromethods, rapid semiquantitative procedures, and physical methods of analysis in accomplishing the desired result.

In the solution of problems concerned with water and sewage analysis, attempts have been made to devise new and suitable analytical techniques and to modify such existing ones as are pertinent.
III. PERSONNEL

The project staff was as follows:

George W. Reid, Project Director, S.M. and Associate Professor of Sanitary Engineering.

R. S. Ingols, Chief Chemist, Ph.D. and Research Associate Professor of Sanitary Chemistry.

E. H. Shaw, Chemical Engineer, M.S. (resigned July 1, 1948).

P. E. Murray, Chemist, B.S. (resigned April 1, 1948).

J. C. Hildebrand, Chemist, B.S. (resigned September 1, 1949).

H. L. Lawless, Chemist, B.S. (resigned June 1, 1949).

R. W. Nippler, B.S., Chemist.

J. R. Munger, Laboratory Assistant.

C. E. Renn, Consultant.¹

L. C. MacMurray, Consultant.¹

IV. LABORATORY FACILITIES

The Georgia Institute of Technology has made available a special laboratory in which much of the developmental work on new procedures has been carried out. The laboratory contained practically all of the standard equipment found in analytical and physical laboratories. Among the special items which the school furnished the project is a Brown Recording Potentiometer and a 6 x 12 foot B. O. D. incubator room.

Other facilities of the Georgia Institute of Technology, including research instruments and machine shops, have been used regularly by the project. The machine shop fabricated a shaking machine for the Sierp apparatus, an electrode assembly for the physical-chemical measurement

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(1) Connected with the coordinate NIH project at Johns Hopkins University.
V. EXPERIMENTAL WORK

A. Calcium Determination

Calcium ion has been determined by a technique involving the use of urea to raise the pH of the solution containing both the calcium and oxalate ions, permitting the formation of large, readily filterable crystals of calcium oxalate. These are less contaminated by magnesium or aluminum oxalate, or excess oxalate due to the presence of the sulfate ion, than are the small crystals formed by the standard method. In addition, the overall time for a set of duplicate determinations for calcium is reduced by the use of urea from 90 minutes to 40-50 minutes.

B. Oxygen Consumed Test

The oxygen-consumed (O. C.) test was developed early and has been used regularly for other analytical studies, but no new developmental work on the procedure has been conducted recently. However, considerable data have been collected for the Standard Methods Committee of the Sewage Works Federation in order to permit evaluation of the technique for possible inclusion in the next issue of Standard Methods. The results obtained by the Cincinnati group of the Public Health Service indicate that the accuracy of the technique compares favorably with that of similar techniques developed at about the same time, but, because of the use of a smaller sample, it lacks the precision of the technique developed in Cincinnati.

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(2) Either published or to be published. See Tables I and II.
In this rapid analytical procedure for determining chemically the amount of oxidizable material present in wastes, a small aliquot of sewage is added directly to a small volume of a one-to-one mixture of phosphoric and sulfuric acids containing some potassium dichromate, and the mixture is refluxed for 60 minutes in the presence of selenium.

**TABLE I**

PUBLISHED WORK

<table>
<thead>
<tr>
<th>Subject</th>
<th>Publication</th>
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<tbody>
<tr>
<td>Calcium</td>
<td>&quot;Urea Hydrolysis for Precipitating Calcium Oxalate.&quot; Analytical Chemistry 21, 524 (1949).</td>
</tr>
<tr>
<td>General</td>
<td>&quot;Rapid Analytical Techniques for Water and Sewage Analysis.&quot; Public Works 80, No. 1, 27 (1949).</td>
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</table>

After oxidizing the sewage, the dichromate remaining is determined chemically. The oxygen-consumed value is calculated by the difference in the dichromate present before and after oxidation, and is corrected for the chloride concentration of the sample.
TABLE II
UNPUBLISHED WORK

<table>
<thead>
<tr>
<th>Subject</th>
<th>Publication</th>
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</thead>
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| Sulfate | "Sulfate Ion Determination with Bezidine Dihydrochloride" (Water & Sewage Works)*  
|         | "A Volumetric Sulfate Ion Determination Using the Barium Ion and Versenate" (Analytical Chemistry)* |
| Nitrogen & Phosphorous | "Microkjeldahl Procedure in Water and Sewage Analysis" |
| Fluoride | "Determination of Fluoride Ion With Ferric Thiocyanate" (Analytical Chemistry)** |
| Chromium | "Notes on the Determination of Chromium with Diphenyl Carbozide" (Analytical Chemistry)* |
| Procedures | "Cost Study of Water and Sewage Analytical Techniques" |

*Submitted for Publication.  
**Accepted for Publication.

C. Fluoride Ion Determination

The new ferric thiocyanate procedure, which has been accepted for publication by the editors of Analytical Chemistry, is rapid, and the color is stable. However, the technique is complicated by its sensitivity to the sulfate and hydroxyl ions. The ferric thiocyanate complex is decolorized by both the fluoride ion and the sulfate ion, with approximately 0.1 p.p.m. fluoride ion being equivalent to 20 p.p.m. of sulfate ions. In order to determine the extent of decolorization due to the fluoride ions, zirconyl ions are added and the ferric thiocyanate color is restored by an amount equivalent to the fluoride ion concentration. The zirconyl ion does not react with the sulfate ion.
From a re-examination of the original paper presenting the determination of fluoride with ferric thiocyanate it appeared that some study must be made of the effect of the sulfate ion on the ferric thiocyanate color complex. This work indicated that sulfate ions do change the color intensity of the ferric thiocyanate in the same manner as does the fluoride ion, but a concentration approximately 100 times that of sulfate ions is required for a similar change. However, this is the normal ratio of concentrations of sulfate ion to fluoride ion in natural water. Foster recommends that a small amount of ferric or thiocyanate ions be added to compensate for the color loss due to the sulfate ions, but, although the original color intensity is restored, the original sensitivity is lost. Thus, some other approach is needed. Because it is realized that the intensity of the ferric thiocyanate color is already known to be a function of pH, ferric ion concentration, thiocyanate ion concentration, and total ion concentration, it does not seem possible to compensate for a variable sulfate ion concentration. It has been found, however, that by the addition of a small amount of zirconyl ion to a second color tube containing the sample the ferric thiocyanate color intensity whose loss was caused by the fluoride ion, could be restored without changing the ferric sulfate complex or, conversely, the resultant ferric thiocyanate color complex.

The technique as presented requires two tubes containing samples, one with and one without zirconyl ions, and a third containing the reagents in distilled water. Stable colors are developed rapidly in

the presence and absence of the fluoride ion, permitting immediate readings, but it is then necessary to use a nomograph. From this discussion, it is evident that the procedure lacks the simplicity of the technique of Standard Methods. However, because no sulfate ion interference will be encountered, the ferric thiocyanate complex should be very useful for plants, adding the fluoride ion for control of dental caries. A small fluctuation in coagulant dosage would not produce an appreciable change in sensitivity of the ferric thiocyanate toward the fluoride ion. A manuscript is under preparation on the ratio of ferric to thiocyanate in the color complex.

D. Magnesium Ion Determination

Two rapid volumetric tests for total hardness have been published recently. One procedure, published in Russian literature, using trisodium phosphate has not been successful but the method of Schwarzenbach has proved very desirable. The Schwarzenbach technique employs a standard solution of the disodium salt of ethylenediaminetetraacetic acid (versenate) and a dye Erochromezwartz T (Nr241). Calcium and magnesium ions form soluble complexes with the versenate radical, and the dye changes color as the magnesium ion concentration is reduced below $10^{-5}$ M. The titration is rapid, accurate, and reproducible to one part in 100 above concentrations of $0.2 \times 10^{-4}$ M. In order to determine the magnesium ion in a mixture containing the calcium ion, one ion must be removed or its concentration determined. The sample must be buffered to pH 9.8-10.0 during titration but this can be carried out with an ammonium hydroxide–ammonium chloride mixture. The titration is rapid and the end point is

sharp. In the presence of the magnesium ion the dye is wine red and in its absence, the dye is blue.\(^5\)

E. Microkjeldahl Procedure\(^2\)

A detailed procedure for use of a modified microtechnique for nitrogen analysis on sewage, water, slime, and sludge is being written, the accuracy of the technique being limited only by sampling. The procedure is simply the Hengar technique adapted to the needs of water and wastes.

F. Relative Stability Determination\(^2\)

The possibilities of developing a short-time B.O.D. test in which time would be the measurable variable in the technique has been investigated. A combination of photoelectric cell and a recording potentiometer is used to measure the changes in color intensity of a sewage sample to which a redox potential indicator (methylene blue) has been added. The results obtained are unsatisfactory, apparently because of the excessive time consumed (lag period) during which the bacteria make the necessary changes in the redox potential of the sample and to the low initial redox potential of some of the samples which immediately decolorize the indicator.

At the request of the Standard Methods Committee of the Sewage Works Federation some further work on the relative stability test has been performed. The results indicate that the values obtained by the relative stability test are highly reproducible, but not accurate; this was indicated in the published manuscript. It is felt that, in spite of its

\(^5\) Since the technique is published, it has been recommended to the Standard Methods Committee of the American Water Works Association, who were already considering the method.
inaccuracy as far as raw sewage is concerned, the technique should be left in Standard Methods as a means of analyzing the effluent of biologically treated sewage at small plants. Also, it has value in comparison to the B.O.D. so far as nitrification is concerned.

G. Amperometric Titration for Hardness and Chlorine

No further work has been done on hardness since the second annual report, but a careful comparison has been made of the amperometric technique of Haller and Listek\(^6\) against each of the volumetric techniques of Palin\(^7\) and Connell.\(^8\) The present O.T.A. test differentiates quite well between free and combined chlorine residuals at low chlorine values of 0.5 p.p.m. or less. However, it is frequently necessary to determine the nature of chlorine residuals in excess of 0.5 p.p.m. This can be done either in the amperometer titration procedure or with the volumetric methods of Palin and of Connell. It was found, however, that the amperometric procedure is time-consuming and that dichloramine is determined along with chlorine dioxide in the alkaline-treated sample. The necessity for four large portions for this procedure can also present difficulties in some research work.

The Connell technique serves reasonably well in differentiating between free chlorine and chloramine, but it does not indicate the type of chloramine that is present. The Palin technique differentiates between chlorine, trichloramine, dichloramine, and monochloramine, but it does not differentiate between chlorine and chlorine dioxide and is not

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The Palin technique has been found by comparison to be so clear-cut in its differentiation of the types of chlorine compounds that further research has been initiated to determine the meaning of the titration values with organic amine compounds and to study the residual values required for cysticidal concentrations of *Endamoeba hystolytica*. Research reported at Harvard indicates that the differentiation into the various chloramine compounds by the Palin technique agrees with the spectrophotometric determination of the various compounds. On the basis of the study, a recommendation has been made to the Standard Methods Committee of the American Water Works Association that the Palin technique be considered for the next issue of *Standard Methods*.

It is believed that a minor variation in the present Palin technique will permit the procedure to differentiate between either chlorine or trichloramine and chlorine dioxide. At present no attempt is made to account for chlorine dioxide, but, by use of the technique of Haller and Listek, a second aliquot can be treated with alkali to pH 12 while the first aliquot is run through the Palin procedure. After the second aliquot has been neutralized, the Palin procedure can be repeated; the alkaline treatment will leave the chlorine unchanged while the chlorine dioxide will be destroyed, and trichloramine will be changed to monochloramine. Thus, it is possible to differentiate between chlorine and chlorine dioxide or chlorine dioxide and trichloramine, but this suggested modification is not advised for differentiation between chlorine and trichloramine. The extraction of the trichloramine with carbon tetrachloride is still desirable.
H. Chromium Determination

A hypothesis is advanced that chromate is adsorbed on the glass of the oxidation or digestion flask. The amount adsorbed becomes appreciable in samples containing less than 0.1 mg. chromium per 50 ml. sample (2.0 p.p.m. chromium). This adsorption is reduced by the use of new flasks. Adsorption is more pronounced from an acid medium; therefore, the alkaline digestion gives better recovery of small amounts of chromium.

The addition of cobalt ion is suggested for the more rapid breakdown of the excess hydrogen or sodium peroxide in an alkaline digestion procedure.

I. Nitrate Determination with Brucine

At present, nitrates may be determined by phenoldisulphonic acid or by reduction followed by Nesslerization. Either test is slow, but the phenoldisulfonic acid test color is extremely stable and the ammonia of the reduced test may be run in connection with other ammonia analyses.

However, the brucine sulfate test is rapid and simple except that its color is not stable indefinitely and the brucine sulfate reagent must be renewed at regular intervals. Although generally the size of sample is no problem, in water analyses the brucine test operates on a very small aliquot (5 ml.). For samples having more than 10-12 p.p.m. nitrate, a smaller aliquot is recommended for better accuracy and reproducibility. Checks on the interference of various ions and organic material indicate that these should not interfere with the accuracy of the results at the concentrations normally found in sewage.
J. Sulfate Ion Determination with Benzidine

The standard methods technique for sulfate ion has been improved by refinements in precipitation by controlled cooling. Two discs of filter paper (Whatman 542, 2, 1 cm. dia.) in a Gooch crucible are used for filtration and ice is used for cooling.

K. Sulfate Ion Determination with Versenate and Barium Chloride

The "Versenate test" for total hardness, as described by Schwarzenbach, consists of titrating the calcium and magnesium ions with the disodium salt of ethylenediaminetetraacetic acid (Versenate). The sample is buffered to a pH of 10 and the indicator used is Eriochromeschwartz T (F-241). The end point consists of a change from wine red to blue. The calculation of the total hardness is made by multiplying the number of ml. of the versenate by a factor dependent upon the strength of the standard and the size of the sample.

This technique incorporates the above method with additions in order to determine the sulfate ion concentrations generally found in water. It can be modified to determine them in any concentration. The value of this technique lies in its speed and accuracy.

The principle of the technique is the removal of the sulfate ion with a known amount of standard barium chloride solution and the titration of the excess barium chloride with versenate, using Eriochromeschwartz T indicator. From this, the amount of sulfate can be computed with a high degree of accuracy.

L. B.O.D. Studies

A comparison of the B.O.D.'s of raw and blended sewage to the theoretical B.O.D. and the C.O.D. values indicates the advisability of ob-
taining C.O.D. values along with the B.O.D. to provide a better understanding of the strength of sewage. No greater degree of definite formulation can be offered at this time.

M. Direct B.O.D. Studies

A modified Seirp apparatus for studying the rate of the B.O.D. reaction by following the dissolved oxygen directly with the dropping mercury electrode has not been successful. Some time has been spent in the development of the apparatus, but the test results were good only up to 48 hours. Dr. Morris, Harvard, according to personal correspondence, experienced a similar toxicity of mercury to sewage after an incubation period of 48 hours.

N. Electrometric Techniques for Dissolved Oxygen

A technique has been developed by Olson and co-workers for determining dissolved oxygen by the use of an electrode system consisting of a small platinum wire and calomel half-cell, with an electrical device that alters the polarity and measures the current from the depolarizing materials present. The technique was developed to study the B.O.D. of sewage, but calibration curves with sewage indicate that the electrode system does not function in sewage. It was found that sewage interferes with the decrease in current which is obtained with decreasing oxygen tension. Thus, the zero dissolved-oxygen curve is superimposed on the air-saturated oxygen curve in sewage. Some material in sewage is capable of depolarizing the platinum electrode (in the absence of oxygen) to the same extent as does oxygen in pure water.

If the material which depolarizes the platinum consists of a large molecule, then it is possible that the use of a film over the platinum electrode might help the situation. For this, it must be assumed that the film is permeable to oxygen and impermeable to the other depolarizing material. Two films were studied: agar and collodion. The agar film provides a satisfactory electrical covering, but it is very poor mechanically and seldom works adequately. As might be expected, the use of a film gives a slow rate of reaction to the change in dissolved oxygen. The collodion gives better mechanical properties but poorer electrical responses to changes in the dissolved oxygen level. Neither protective film gives values which indicate that extended B.O.D. work with sewage would be possible.

O. Cost Study of Water and Sewage Analytical Procedures and Resulting Equipment

An attempt has been made to group various analytical procedures according to the principal method of analysis used and to evaluate the relative times required by the two groups of procedures. One of the NIH projects has indicated a very rapid system of analysis using expensive instruments. The work of this project has shown that volumetric and colorimetric procedures can give most of the essential chemical information for water in a short time without expensive instruments. A brief review of the essential analyses that must be performed by small water or sewage treatment plants will be developed, the purpose being to give a general outline of the laboratory facilities that should be maintained for proper control of water and sewage. Proper control will, of necessity, be a balance between the control desired by the regulatory authorities and the most economical control which the plants can give
while maintaining a reasonable picture of operations. Obviously, control will be scaled to the size and location of plants.

The scheme of tests to be recommended will include the simplest techniques, so that they are economical from the standpoints of both equipment and personnel required. Recommendations will be developed for the size, location, and facilities required for the laboratory.

P. Literature Search

The Bibliography on Water and Sewage Analysis constitutes an indexed guide to the pertinent literature on water and sewage analysis, prepared so that future work might benefit through use of and acquaintance with existing data. References have been classified according to subject under "tests" for various materials and properties. Access to references on "methods of analysis" and related subjects has been made possible by lists of pertinent reference numbers. References are numbered consecutively throughout the book, and all items include bibliographic reference to abstracts where these are known to exist. An author index is included.

VI. FUTURE WORK

Much of the work on the project is in the final stage. This work on specific techniques and on a general system will be completed at the expense of the Georgia Institute of Technology. At the completion of

Final Report, Project No. 117

the program, a covering manuscript will be forwarded to the National Institutes of Health.

Respectfully submitted:

George W. Reid,
Project Director

Robert S. Ingols,
Chief Chemist

Approved:

Gerald A. Rosselot, Director
State Engineering Experiment Station