LBL-15816
UC-69

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

ENERGY & ENVIRONMENT DIVISION

RECEIVED
LAWRENCE
BERKELEY LABORATORY
MAY 17 1983
LIBRARY AND
DOCUMENTS SECTION

QUANTITATION OF OIL SHALE WASTEWATER QUALITY: A Manual of Analytical Methods

Christian G. Daughton

December 1982

TWO-WEEK LOAN COPY
This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 6782.

ENERGY
AND ENVIRONMENT
DIVISION

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

LBL-15816

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

QUANTITATION OF OIL SHALE WASTEWATER QUALITY

A Manual of Analytical Methods

Edited by

Christian G. Daughton
Sanitary Engineering and
Environmental Health Research Laboratory
College of Engineering
School of Public Health
University of California
Berkeley

DOE Contract No.
DE-AC03-76SF00098

Prepared for

U.S. Department of Energy

DOE Project Manager: Arthur Hartstein
Office of Fossil Energy, Washington, DC 20545

DOE Technical Project Officer: David Sheesley
Laramie Energy Technology Center
Laramie, Wyoming 82071

December 1982

LBL-15816

Energy and Environment Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

CONTENTS

Preface iii

Acknowledgments iv

Authors v

Abbreviations vi

I. RAPID FRACTIONATION OF OIL SHALE WASTEWATERS BY REVERSE-PHASE SEPARATION ..... I-1 to 18

C.G. Daughton, B.M. Jones, and R.H. Sakaji

II. COULOMETRIC QUANTITATION OF CARBON IN OIL SHALE PROCESS WASTEWATERS: VIA UV-PEROXYDISULFATE OR HIGH-TEMPERATURE OXIDATION ..... II-1 to 54

G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

III. COLORIMETRIC AND TITRIMETRIC QUANTITATION OF AMMONIA IN OIL SHALE WASTEWATERS ..... III-1 to 21

C.G. Daughton, J. Cantor, B.M. Jones, and R.H. Sakaji

IV. COLORIMETRIC AND TITRIMETRIC QUANTITATION OF CHEMICAL OXYGEN DEMAND IN OIL SHALE WASTEWATERS ..... IV-1 to 35

B.M. Jones, R.H. Sakaji, and C.G. Daughton

V. MICROBIAL BIOMASS IN OIL SHALE WASTEWATERS: QUANTITATION AS PROTEIN ..... V-1 to 14

C.G. Daughton, B.M. Jones, and R.H. Sakaji

APPENDIX

Table Summarizing Data Obtained for Oil Shale Wastewaters A-1

PREFACE

This manual is a compendium of analytical methods for the quantitation of various water quality parameters. Each method is specifically adapted for application to the highly complex sample matrices of the aqueous wastes that are generated by the pyrolytic production of shale oil. These methods have evolved from specific needs of the LBL-SEEHRL Oil Shale Waste Treatment Program. Although the methods have been developed specifically for oil shale wastewaters, the stringent requirements imposed by these sample matrices would probably allow for the successful direct application of these methods to other aqueous waste samples; the major limitation would be that of insufficient lower detection limits because oil shale wastewaters commonly require methods with wide linear dynamic ranges. Although these methods appear to give excellent results for the waters analyzed during this study, it cannot be overemphasized that these methods must be validated for each new water under investigation.

Discussions of theory, literature review, methods comparisons, validation and precision data, and detailed operator protocols are presented for each of the methods. These include a simple and rapid fractionation scheme for organic carbon, and quantitation of oil and grease, organic and inorganic carbon, ammonia, chemical oxygen demand, and microbial biomass. Some of the protocols are routine standard methodologies that have been validated for oil shale process wastewaters, while others are modified standard methods or totally new approaches. The question of accuracy has not been fully addressed in these Chapters, because it is a tremendously complex issue. The origins of the wastewaters that are mentioned in each Chapter are fully described in Chapter II, Table I (p. II - 23), and the data obtained from each of the methods is summarized for all of the waters in the Appendix.

Several of these Chapters are expanded or updated versions of previously available LBL-SEEHRL Oil Shale Wastewater Treatment Program in-house reports: Chapter I (LBID-485, February 1982), Chapter II (LBID-561, June 1982), and Chapter III (LBID-465, December 1981). The development of other methodologies will be described in future supplements or subsequent editions to this manual.

The mention of trade names or commercial products in this manual is for illustrative purposes and does not constitute endorsement or recommendation for use by the U.S. Department of Energy.

ACKNOWLEDGMENTS

This work was supported by the Assistant Secretary for Environment, Office of Environmental Compliance and Overview, Environmental Control Technology Division, and the Assistant Secretary for Fossil Energy, Office of Oil Shale, Division of Oil, Gas, and Shale Technology of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

The authors thank S. Alikani, G. Harris, and K. Yu, laboratory technicians of the LBL-SEEHRL Oil Shale Program, for their capable assistance, and we thank J.P. Fox for helpful comments on Chapter II. The statistical analyses were performed by G.W. Langlois, and all illustrations were drafted by R.H. Sakaji.

AUTHORS

Christain G. Daughton, Ph.D.
Deputy Leader, Lawrence Berkeley Laboratory Oil Shale Program
Assistant Research Toxicologist

Sanitary Engineering and
Environmental Health Research Laboratory
University of California, Berkeley
Richmond, CA 94804
415/231-9597; FTS 451-7117

Bonnie M. Jones, M.S.
Staff Scientist Chemical Engineer
Richard H. Sakaji, M.S.
Staff Scientist Chemical Engineer
Gregg W. Langlois, M.S.
Senior Research Associate
Jeremy Cantor, B.S.
Principal Research Technician

Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720
415/486-4150; FTS 451-4150

ABBREVIATIONS

BOD	biochemical oxygen demand; usually refers to 5-day test
COD	chemical oxygen demand
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon; synonymous with soluble organic carbon (SOC)
HpF	hydrophilic fraction from RPF
IC	inorganic carbon (nonspecific)
LpF	lipophilic fraction from RPF
OC	organic carbon (nonspecific)
RPF	reverse-phase fractionation
SCOD	soluble chemical oxygen demand
TC	total carbon; includes TIC and TOC
TDC	total dissolved carbon; includes DIC and DOC
TIC	total inorganic carbon; includes particulate and dissolved forms
TOC	total organic carbon; includes particulate and dissolved forms

Chapter I

RAPID FRACTIONATION OF OIL SHALE WASTEWATERS BY REVERSE-PHASE SEPARATION

C.G. Daughton, B.M. Jones, and R.H. Sakaji

Introduction 2
Theory 3
Chemical Class Fractionation by Reverse-Phase Separation 3
Protocol Summary 4
Chemical Class Fractionation 4
General Procedure 4
Oil and Grease Procedure 5
Methods Comparison 6
References 8
Tables
I. Reproducibility of RPF Method for Oil Shale Process Waters 10
II. Applicability of RPF Oil and Grease Method to Oil Shale Process Waters 11
III. Ranking of Retort Waters by LpF Content as Measured by Both DOC and "Oil and Grease" 12
Figures
1. Reverse-Phase Fractionation (RPF) of Aqueous Samples: Separation of Hydrophobic Solutes (HpF) and Aliphatic Oil (LpF) .. 13
2. Quantitation of Oil by Tangent-Baseline Method and Infrared Spectroscopy 14
Protocol: Reverse-Phase Fractionation 15-18

Chapter I: RAPID FRACTIONATION BY REVERSE-PHASE SEPARATION

INTRODUCTION

Two basic approaches are available to the analyst for the routine quantitation of particular chemical classes or individual compounds in aqueous heterogeneous mixtures. The first approach quantitates solutes by methods of detection that are specific for individual chemical classes or particular compounds; many of these methods involve functional group derivatizations or colorimetric reactions that enable the compounds of interest ("analytes") to be distinguished from myriads of other solutes. Each analyte, however, requires a specific method of reaction and detection. The alternative approach employs an initial step that effects a sufficient degree of separation of the analyte from the mixture, and thereby enables the use of routine, nonspecific, bulk-property methods as means of detection.

Tremendous qualitative differences can exist between quantitatively identical values obtained by any bulk- or colligative-property method such as chemical oxygen demand (COD), dissolved organic carbon (DOC), or organic nitrogen. In oil shale wastewater treatment, for example, the removal of a large percentage of DOC may be inadequate if the toxicity, color, and odor of the wastewater are strictly associated with the remaining percentage. Identical values for any colligative property obviously can result from solutions of different compounds. The information from nonspecific quantitative data yielded by colligative properties can be greatly amplified, however, by the physical separation or "fractionation" of the sample matrix prior to the detection step.

The physical separation of chemical classes is usually accomplished by "isolation" processes such as liquid-liquid partitioning (solvent extraction), ion exchange, or sorption (e.g., onto charcoal or macroreticular resins), and by "concentration" methods such as lyophilization and ultrafiltration (Jolley, 1981; Leenheer and Farrier, 1979). The isolation schemes usually depend on various sequences of pH adjustment in the acidic, neutral, and basic ranges followed by extraction with water-immiscible solvents and back-extraction into aqueous phases, or passage of the sample through series of exchange resins or sorbents. The fractionated classes are combinations of acidic, neutral, or basic organic compounds. The analytes are then recovered from the various fractions by selectively removing the water or organic solvent. These procedures risk the chemical or physical alteration or contamination of the sample by introduction of solvents, acids, caustics, and heat. Although these methods are generally capable of effecting extensive separations of many different chemical classes, they are too time consuming for use in routine experiments or for monitoring waste treatment performance.

We have developed a new fractionation procedure which effects a crude separation of solutes from complex oil shale wastewaters, but which is also simple, rapid, and applicable to other types of aqueous wastes (Daughton et al., 1981a,b, 1982). This method separates a wastewater into polar (hydrophilic) and nonpolar (hydrophobic, lipophilic) fractions by passing the sample through a disposable reverse-phase chromatographic cartridge. We call this approach reverse-phase fractionation (RPF). The method is rapid, and alteration or contamination of the sample is minimized. This crude fractionation step greatly increases the information that can be derived from the subsequent application of colligative detection methods. Moreover, these fractions (especially the hydrophilic fraction) themselves can be directly subjected to experimental treatments (such as biooxidation or physicochemical treatment) for comparison with parallel treatments of the raw unfractionated water. It is crucial to

recognize that the fractions generated by a separation scheme are arbitrarily defined, being strictly dependent on the idiosyncrasies of the scheme; operationally, "polarity" is a relative characteristic.

THEORY

Chemical Class Fractionation by Reverse-Phase Separation

Reverse-phase chromatography utilizes a stationary phase that is less polar than the mobile phase, which is commonly water modified with organic solvents. After solutes in the mobile phase have been retained by the stationary phase, they can be eluted by further modification of the mobile phase with organic solvent. Solvent "strength" (i.e., capacity to elute sorbates or retained compounds) increases with increasing hydrophobicity of the solvent. A typical reverse-phase stationary material is composed of silica particles whose inherently polar surface silanol groups are covalently bonded to aliphatic moieties (e.g., octadecylsilyl, C-18 groups), which create an immobilized hydrophobic layer around each silica particle.

If an aqueous sample containing organic solutes were passed through a bed of unmodified reverse-phase silica, the composition of the effluent generally would be unchanged. If the stationary phase were pretreated, however, with a water-miscible organic solvent such as methanol, the less polar solutes would partition into the hydrophobic stationary phase; the more polar solutes would remain in the aqueous effluent. The methanol serves to "wet" or "activate" the reverse-phase material. Although the mechanism of retention of organic solutes by the stationary phase has not been delineated, several mechanisms have been proposed. The most straightforward model assumes that the aliphatic chains simply serve as a stationary liquid phase (Snyder and Kirkland, 1979). Activation allows the unlike stationary and mobile phases to contact one another; this contact promotes the partitioning process. The solutes that partition into the activated stationary phase from the mobile aqueous phase can be eluted with an organic solvent of sufficient strength.

The general principle of RPF can be demonstrated by the following experiment. An aqueous mixture of bromophenol blue and p-nitrophenoxide ion is prepared. Bromophenol blue is a relatively hydrophobic, intensely purple sulfonphthalein dye substituted with two dibromophenyl groups. p-Nitrophenoxide, in contrast, is a relatively polar, bright yellow ionized phenol. The mixture of these two compounds is deep violet, and when it is applied to unactivated C-18 material, the effluent is also deep violet; no color is retained by the stationary phase. In contrast, when the cartridge is activated, the lipophilic fraction (LpF) is retained and the hydrophilic fraction (HpF) appears in the aqueous effluent; the effluent is bright yellow and the stationary phase becomes deep purple. A water-miscible organic solvent can easily elute the bromophenol blue from the stationary phase. Complete physical separation of the two solutes is thereby effected.

Similarly, the application of retort water to a cartridge containing unactivated C-18 material produces an effluent that is unchanged. Activation of the cartridge with methanol permits retention of a large percentage of the organic solutes. Raw retort water generally is dark brown and has a pungent tarry smell; these organoleptic characteristics are common to nitrogenous heterocycles. The aqueous effluent from an activated C-18 cartridge, however, smells strongly of ammonia, whose odor had been totally obscured by the intense odor of the heterocycles. If the water is removed from this HpF fraction by drying, ammonia is concomitantly removed, and its absence enables the intense odor of fatty acids to be recognized. The aqueous effluent is colorless or pale

yellow; the reverse-phase packing material retains nearly all of the color. A large percentage of the retained compounds can be eluted with organic solvent (e.g., methanol). If the organic solvent is removed from the organic eluate, the residuum (i.e., LpF) possesses the characteristic odor and color of raw retort water. A portion of the LpF is irreversibly retained. This is probably a result of sorption of solutes with basic functionalities by surface silanol groups that remained unreacted during the bonding of the silica; the particular cartridges used in the protocol reported here are packed with material that is not endcapped.

It must be emphasized that RPF is not analogous to liquid chromatography (LC). In LC, the sample is applied as a plug, and the mobile phase carries the components through a highly efficient analytical column. In RPF, the sample and mobile phase are synonymous, and the column has been miniaturized and packed with less efficient chromatographic material. It is therefore essential to ascertain the volume of sample that can be applied before the stationary phase is saturated and the solutes that normally would be retained begin to breakthrough. The analyst should also be aware that the polarity of the stationary phase can be altered during fractionation by the partitioning of solutes, i.e., the "mutual zone of solubility effect" (Saner, Jadamec, and Sager, 1979). With mixtures of solutes comprising a wide range of capacity factors (K'), the initial sorption of many solutes with high K' values shifts the selectively away from solutes with lower values. The permanent alteration of the stationary phase may limit the reuse of the cartridges for RPF.

PROTOCOL SUMMARY

Chemical Class Fractionation

General Procedure.

Miniature reverse-phase cartridges are available from several manufacturers (e.g., C-18 Sep Paks from Waters Assoc., Inc., Milford, MA; Chrom-Prep PRP-1 cartridges from Hamilton Co., Reno, NV; Disposable Extraction Columns from J.T. Baker Chemical Co., Phillipsburg, NJ; Bond Elut extraction columns from Analytichem International, Harbor City, CA) We have extensively investigated C-18 Sep Paks because of their ease of use; other cartridges should give comparable results, although modifications in the protocol would be required. The C-18 Sep Pak cartridges are made from virgin polyethylene tubes and contain about 350 mg of packing material (80-µm diameter particle size) which is held in place by fritted polypropylene discs. The cartridges have interchangeable influent and effluent female Luer-slip ends.

Activation is achieved by applying 5 mL of methanol to the cartridge followed by rinsing with 20 mL of water; sufficient rinsing is required for removal of "free" residual methanol and minimization of background interferences. The sample is then passed through the cartridge at a sufficiently slow rate (e.g., 5 to 10 mL/min for retort waters; 100 mL/min can be used successfully on dilute waters). The total quantity applied must be predetermined from breakthrough experiments; these volumes are commonly 2.5 to 10 mL for retort waters, and up to several liters for cleaner waters. With larger sample sizes, however, the octadecyl surface may become deactivated; addition of methanol to the sample (e.g., up to several percent) may prevent deactivation (Andrews and Good, 1982). The cartridge should be held vertically to prevent channeling during sample application and elution. The initial milliliter of aqueous effluent (HpF) is discarded because of its dilution by the 400 µL of water that remain from activation of the cartridge. Subsequent HpF can be collected for analysis or for subjection to waste treatment schemes.

The retained organic compounds (LpF) can be eluted after the residual, unpartitioned 400-µL aqueous sample is rinsed from the cartridge with water. We have observed that some sorbate can be stripped from the stationary phase during the rinse. This could be caused by sample overloading or because the pH and osmolality of the wash water differ from that of the sample. The solvent used for initial elution must be mutually miscible in water and in whatever strong solvent may be subsequently used. Methanol, however, will elute nearly all retort water compounds that have not been irreversibly bound to the stationary phase; less polar solvents will not elute the more polar compounds. Compounds of different polarities can be eluted depending on the strength of the organic solvent. For other types of sample, tetrahydrofuran or dichloromethane may be needed for elution.

The particle size of the C-18 packing material and porosity of the frits give the cartridges the ability to act as depth filters. The particulates in retort water are effectively retained by both the inlet fritted polypropylene disc and by the silica particles. Because organic elutrients will literally dissolve these retained, tarry and oily residues, it is possible to calculate the concentration of suspended hydrophobic solutes by determining the difference in LpF concentrations yielded by raw and filtered samples.

Oil and Grease Procedure.

Oil and grease is a broad classification of organic compounds that is arbitrarily defined by the procedure applied. As specified in Standard Methods (1980), it is the group of substances that can be determined quantitatively on the basis of their common solubility in Freon 113. Oil and grease includes aliphatic hydrocarbons (e.g., paraffins, waxes, and oils), lipids, fatty acids, and soaps. This class of compounds is analogous to the group of lipophilic solutes that compose the LpF of the RPF procedure.

Liquid-liquid partitioning followed by gravimetric quantitation is the standard method for determining the dissolved or suspended oil and grease concentrations of a wastewater sample. This method is fraught with difficulties when applied to oil shale process waters. A stable emulsion forms between the aqueous and organic extraction phases requiring the addition of acid and large quantities of salt. The high total alkalinity of retort water requires the addition of large amounts of acid, leading to CO_2 offgassing, excessive foam production, protonation of aliphatic carboxylic acids, and precipitation of elemental sulfur. The large quantity of additional salts will tend to "salt-out" some of the less hydrophilic solutes that are not oils (i.e., decrease their solubilities in the aqueous phase). In addition, the partitioning process itself is not selective. Polar compounds can be coextracted as organic ligands or ion-pairs, and both polar and nonpolar species can be concentrated at the organic-aqueous interface by surface-active agents, such as carboxylic acids. During the drying step, compounds are lost continuously via volatilization; standardization of losses from volatilization is impossible because the rate of volatilization is substantial and not constant. Finally, quantitation by gravimetric detection is notoriously inaccurate and totally nonselective.

Consequently, we have adapted our general fractionation procedure to the determination of dissolved oil and grease (Fig. 1). The hydrophobic analytes, LpF, are retained by the stationary phase; the HpF is discarded. At this point in the general procedure, the protocol is modified because the method of detection precludes use of a "switchover" solvent of mutual miscibility. The aqueous residuum in the cartridge must therefore be removed by mechanical means, best achieved by lyophilization. The nonpolar compounds (oil and grease) can then be eluted with Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) and

quantitated via infrared spectroscopy (IR) by measuring absorbance of the asymmetric methylene C-H stretch at 2930 cm^{-1} (Fig. 2). True aliphatic oil can be determined by passing the Freon eluent through an activated (i.e., dried) normal-phase silica cartridge. This step removes LpF solutes that contain functional groups and allows aliphatic hydrocarbons to pass through in the effluent for quantitation. A major difficulty with development of the LpF procedure was a high IR background absorbance that was sporadically encountered. This problem has several possible origins which include trace quantities of phthalate plasticizers and residual alkylsilyl bonding reagent from the cartridges. We also found substantial lot-to-lot variation in both background absorbance and apparent partitioning efficiency; this same variability was noted by Saner et al. (1979).

METHODS COMPARISON

The RPF procedure, when used for determining oil and grease of a wastewater sample, has distinct advantages over the partition-gravimetric procedure. As mentioned above, the RPF procedure obviates the need for liquid-liquid partitioning and gravimetric detection along with the attendant problems of emulsion formation and loss of volatile hydrocarbons during sample concentration. The procedure is simple to perform, minimizes the use of glassware (generally one volumetric flask is the only glassware required per sample), and minimizes solvent consumption (usually about 20 mL of solvent is required per sample, regardless of sample size, compared with 100 mL of solvent for the partition-gravimetric method). Dilute samples are concentrated directly (i.e., trace enrichment) by the cartridges. This means that the detection limit is restricted only by the sample matrix; large sample sizes can be applied to the cartridges if the solute concentrations are low. The detection limit that we usually observe is 20 mg/L oil (as mineral oil) in the Freon eluent.

The major advantage of the RPF procedure is throughput. An analyst can easily determine LpF oil and grease by the RPF procedure for 25 to 50 samples in an eight-hour working period. The major disadvantage of the method is that it is not known if the compounds in the LpF are the same as the compounds that would be quantitated by the partition-gravimetric method. This is also a limitation of the partition gravimetric method because true fortification/validation experiments cannot be performed.

The reproducibility of the LpF method for determining oil, and oil and grease, was determined for Oxy-6 and 150-Ton retort waters (Table I). The relative standard deviations for both parameters were usually less than 10 percent. The oil and grease concentrations for Oxy-6 retort water (and presumably for other waters as well) varied from sample to sample because of changes in composition over time. Some of these values were obtained from samples withdrawn from different drums over a period of a year.

The oil values of unfiltered samples represented less than a fifth of the total oil and grease concentration (Table I). In contrast, the oil values for filtered samples of both Oxy-6 and 150-Ton retort waters were not detectable. Apparently, the "oil" in these waters, in contrast to the total oil and grease, is associated exclusively with the particulate fraction.

The LpF method was used to determine oil and grease in seven retort process waters (Table II). The values were determined for both unfiltered raw sample (i.e., total oil and grease) and for filtered sample (i.e., dissolved oil and grease). The difference between these values represents particulate oil and grease. The samples are arranged in order of decreasing total oil and grease concentrations. This trend is maintained for dissolved oil and grease with the

exception of Oxy-6 retort water. Omega-9 and 150-Ton contained the lowest and highest LpF concentrations, respectively, differing by over an order of magnitude. The three highest LpF concentrations were found in 150-Ton, S-55, and TV. Although the former two waters were from simulated in-situ projects, all three waters were produced from above-ground retorts. The lower concentrations of oil and grease in the remaining four samples can be explained by noting that these four wastewaters may have been diluted by groundwater during in-situ retorting. It is apparent from these values that the quantity of oil is much lower than originally believed, although the effect of long-term storage on the separation of the oil from the water is not known; compare with 580 mg/L for Omega-9 (Farrier, Poulson, and Fox, 1979), and 3836 mg/L for 150-Ton (Harding et al., 1979). The above-ground retorts also had lower percentages of total oil and grease that was dissolved (i.e., 53 to 70 percent versus 83 to 101 percent dissolved oil and grease) even though their absolute dissolved values were generally higher.

The RPF method for determining oil and grease has a further distinct advantage over the partition-gravimetric method in that routine methods such as DOC or COD can be used to quantitate the compounds in the retained fraction. This can be done indirectly by calculating the difference in values for the unfractionated sample and for the HpF (aqueous effluent). The analyst should be aware, however, that the results can be altered by redefining various parts of the protocol. For example, the volume, pH, and osmolality (e.g., degree of dilution) of the sample and rinse water can drastically alter the results.

The quantitation of LpF oil and grease by DOC was done for the seven retort process waters (Table III). The relative rankings of the waters for Tables II and III were the same. By dividing the LpF oil and grease values (i.e., those obtained by the IR procedure) by the LpF DOC values, qualitative information can be obtained about the composition of the LpF. This value, which has arbitrary units, reflects the degree of saturation of the carbon. All of the waters appeared to contain about the same types of LpF constituents (i.e., degrees of saturation ranged from 0.10 to 0.14) except for 150-Ton, whose degree of saturation was 0.22.

The percentage of the total DOC that resided in the HpF for Oxy-6 retort water and gas condensate (Table III) was 40 and 11 percent, respectively. Of the organic solutes that have been quantitated for these two waters, the major polar chemical classes (i.e., those that would be expected in the HpF) are aliphatic mono- and di-carboxylic acids and low-molecular-weight nitriles, alcohols, and ketones. These classes comprise up to a maximum of about 45 and 10 percent of the DOC for Oxy-6 retort water and gas condensate, respectively (Leenheer, Noyes, and Stuber, 1982).

We have used the RPF procedure routinely for monitoring the performance of our biological and physicochemical treatment research and for generating fractions for further experimental treatment work. Some major conclusions from this work have been summarized (Jones et al. 1982a, b; Jones, Sakaji, and Daughton, 1982; Sakaji et al. 1982).

REFERENCES

Andrews, J.S.; Good, T.J.
"Trace Enrichment of Pesticides Using Bonded-Phase Sorbents", Am. Lab. 1982, 14(4), 70-5.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.
"Analytical Problems in Monitoring Retort Water Treatment", in Energy and Environment Division 1980 Annual Report, Lawrence Berkeley Laboratory Report LBL-11989, 1981a.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.
"Oil Shale Waste Treatment: Analytical Methods Development", 53rd Annual California Water Pollution Control Association Conference, Long Beach, California, April 29-May 1, 1981b.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.; Thomas, J.F.
"Analytical Methods for Quantitating Performance of Oil Shale Wastewater Treatment", in Energy and Environment Division 1981 Annual Report, Lawrence Berkeley Laboratory Report LBL-13500, 1982.

Farrier, D.S.; Poulson, R.E.; Fox, J.P.
"Interlaboratory, Multimethod Study of an In Situ Produced Oil Shale Process Water", in Oil Shale Symposium: Sampling, Analysis and Quality Assurance; Gale, C. (Ed.); U.S. EPA: Cincinnati, Ohio, 1980, p 182-210; EPA-600/9-80-022.

Harding, B.L.; Linstedt, K.D.; Bennett, E.R.; Poulson, R.E.
"Removal of Ammonia and Alkalinity from Oil Shale Retort Waters by the Use of Weak Acid Cation Exchange Resins", in Proceedings of the Second Pacific Chemical Engineering Congress, Denver, CO, August 28-31, 1977, Vol. 1, p 442-9.

Jolley, R.L.
"Concentrating Organics in Water for Biological Testing", Environ. Sci. Technol. 1981, 15, 874-80.

Jones, B.M.; Langlois, G.W.; Sakaji, R.H.; Daughton, C.G.
"Ozonation and UV Irradiation: Effect on Oil Shale Wastewater Biorefractory Organic Solutes", LBL-SEEHRL Oil Shale Waste Treatment Program, May 1982a, Lawrence Berkeley Laboratory, LBID-549 (27 pp.).

Jones, B.M.; Sakaji, R.H.; Daughton, C.G.
"Physicochemical Treatment Methods for Oil Shale Wastewater: Evaluation as Aids to Biooxidation", Fifteenth Oil Shale Symposium Proceedings; Gary, J.H. (Ed.); Colorado School of Mines: Golden, CO, 1982, p 581-97 (LBL-14666).

Jones, B.M.; Sakaji, R.H.; Thomas, J.F.; Daughton, C.G.
"Microbial Aspects of Oil Shale Wastewater Treatment", in Energy and Environment Division 1981 Annual Report, Lawrence Berkeley Laboratory Report LBL-13500, 1982b.

Leenheer, J.A.; Farrier, D.S.
"Applications of Dissolved Organic Carbon Fractionation Analysis to the Characterization of Oil Shale Processing Water", in Oil Shale Symposium: Sampling, Analysis and Quality Assurance; Gale, C. (Ed.); U.S. EPA: Cincinnati, Ohio, 1980, p 273-85; EPA-600/9-80-022.

Leenheer, J.A.; Noyes, T.I.; Stuber, H.A.
"Determination of Polar Organic Solutes in Oil-Shale Retort Water", Environ. Sci. Technol. 1982, 16, 714-23.

Sakaji, R.H.; Jones, B.M.; Thomas, J.F.; Daughton, C.G.
"Processes for the Physico-Chemical Treatment of Oil Shale Wastewaters", in Energy and Environment Division 1981 Annual Report, Lawrence Berkeley Laboratory Report LBL-13500, 1982.

Saner, W.A.; Jadamec, J.R.; Sager, R.W.
"Trace Enrichment with Hand-Packed CO:PELL ODS Guard Columns and Sep-Pak C18 Cartridges", Anal. Chem. 1979, 51, 2180-8.

"Standard Methods for the Examination of Water and Wastewater"
15th ed.; American Public Health Association: Washington, D.C., 1980 p 460-5.

Snyder, L.R.; Kirkland, J.J.
"Introduction to Modern Liquid Chromatography", 2nd ed.; John Wiley & Sons, Inc.: New York, NY, 1979; Chapter 7.

Table 1. Reproducibility of RPF Method for Oil Shale Process Waters

test	sample	mean (mg/L) oil and grease as mineral oil	n	rsd (%)
oil and grease	Oxy-6 RW[a]	204	8	13.2
oil and grease	Oxy-6 RW	262	8	5.8
oil and grease	Oxy-6 RW (filtered)[b]	273	15	5.2
oil	Oxy-6 RW	47.1	9	4.1
oil	Oxy-6 RW	32.9	5	9.8
oil	Oxy-6 RW	27.2	9	9.5
oil	Oxy-6 RW (filtered)	nil	15	-
oil	150-Ton[c]	121	10	6.0
oil	150-Ton (filtered)	nil	10	-

[a] Oxy-6 retort water; Occidental Oil Shale, Inc., Logan Wash, CO, MIS Retort #6

[b] filtered through 0.8-μm pore diameter polycarbonate membrane

[c] retort water from run 13, LETC 150-ton above-ground simulated in-situ retort

Table II. Applicability of RPF Oil and Grease Method to Oil Shale Process Waters

	oil and grease as mineral oil (mg/L)				
	unfiltered		filtered[a]		% dissolved[b]
sample[c]	mean	range	mean	range	oil and grease
150-Ton[d]	641	58	448	37	70
S-55[e]	334	78	178	20	53
TV[f]	276	20	175	18	63
Oxy-6 RW[g]	242	40	219	39	90
Geokinetics[h]	160	24	162	8	101
Oxy-6 GC[i]	86	48	71	35	83
Omega-9[j]	58	33	56	44	97

[a] filtered through 0.8-µm pore diameter polycarbonate membrane

[b] (filtered ÷ unfiltered) X 100 (i.e., percent of total oil and grease that is dissolved)

[c] sample volumes applied to cartridges were 2.5 or 5.0 mL; n = 4 for each sample type

[d] retort water from run 13, LETC 150-ton above-ground simulated in-situ retort

[e] retort water from run 55, LETC 10-ton above-ground simulated in-situ retort

[f] sour water from a near-term commercial surface retorting process

[g] retort water from Occidental Oil Shale, Inc., Logan Wash, CO, MIS Retort #6

[h] retort water from retort #9, Geokinetics, Inc., Book Cliff, UT; a true in-situ process

[i] gas condensate from Occidental Oil Shale, Inc., Logan Wash, CO, MIS Retort #6

[j] retort water from LETC 1976 Rock Springs, WY, site 9 true in-situ experiment

Table III. Ranking of Retort Waters by LpF Content as Measured by Both DOC and "Oil and Grease"

sample[a]	raw DOC[b]	HpF DOC (mg/L)[c]	LpF DOC (mg/L)[d]	LpF oil & grease[e]	degree of saturation[f]
150-Ton	3054	974	2080	448	0.22
Oxy-6 RW	3194	1271	1923	219	0.11
TV	2550	727	1823	175	0.10
S-55	2263	1009	1254	178	0.14
Geokinetics	1646	498	1148	162	0.14
Oxy-6 GC	671	74	597	71	0.12
Omega-9	803	254	549	56	0.10

[a] sample volumes applied to C-18 cartridges were 4.0 mL

[b] filtered through 0.8-μm pore diameter polycarbonate membrane; DOC of unfractionated water

[c] C-18 effluent DOC

[d] calculated indirectly; difference between DOC of raw water and HpF

[e] values of filtered samples from Table II

[f] (LpF oil & grease) ÷ (LpF DOC); i.e., extent of hydrogenation

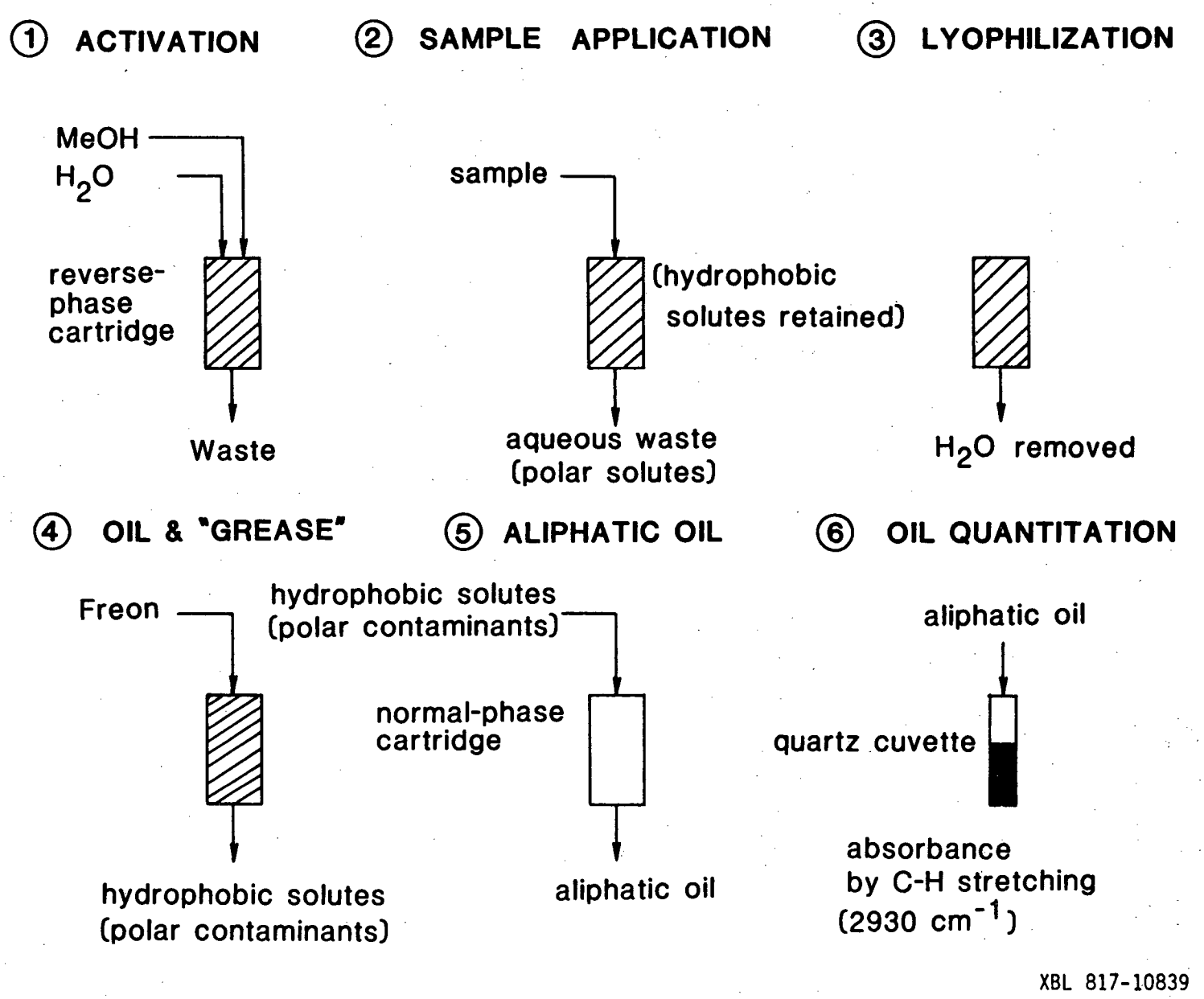


Figure 1. Reverse-Phase Fractionation (RPF) of Aqueous Samples: Separation of Hydrophobic Solutes (HpF) and Aliphatic Oil (LpF).

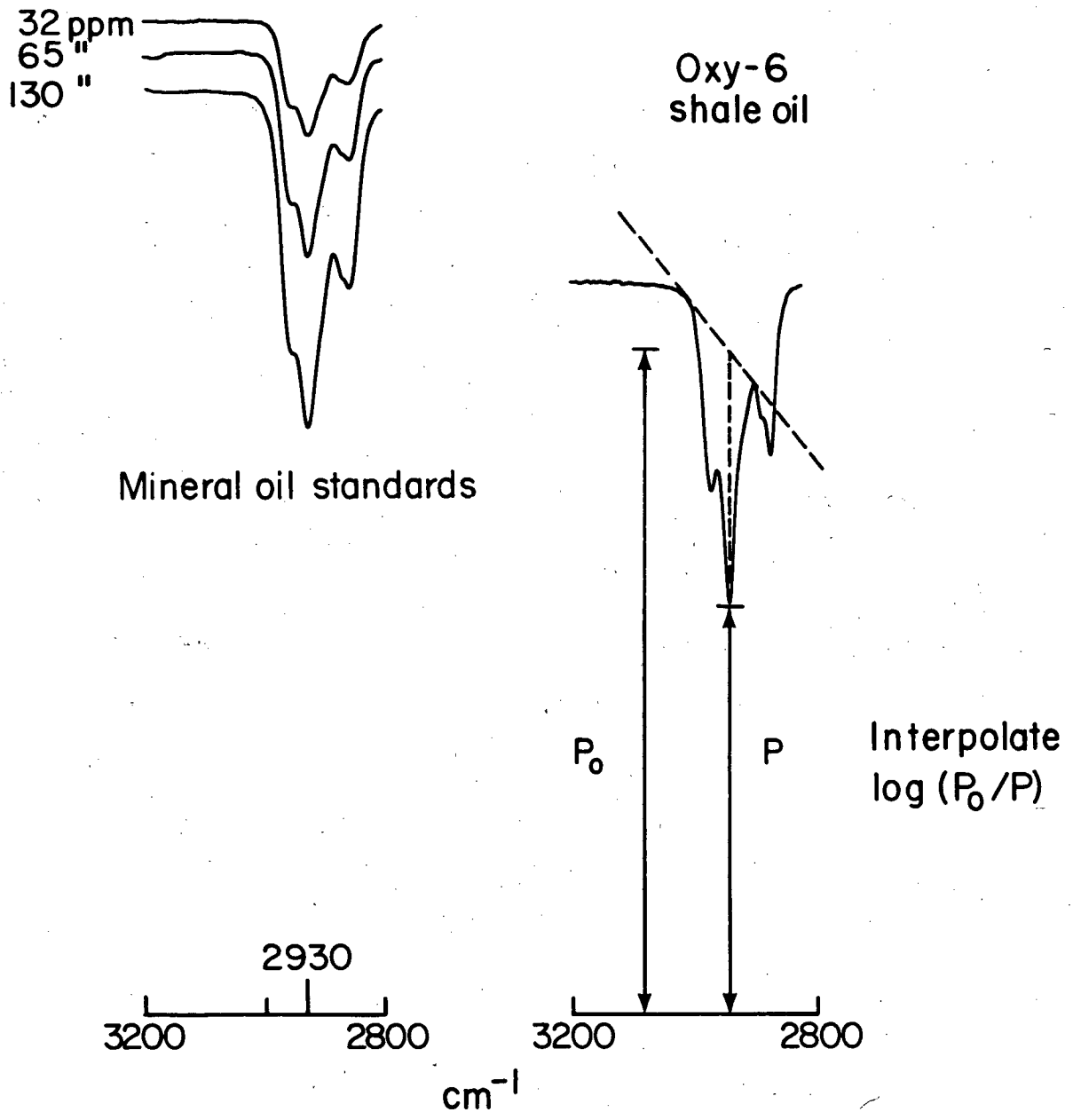


Figure 2. Quantitation of Oil by Tangent-Baseline Method and Infrared Spectroscopy.

PROTOCOL: REVERSE-PHASE FRACTIONATION

Glassware & Equipment

Reverse-phase C-18 cartridges (C-18 Sep-Paks, Waters and Associates, Milford, MA; Part No. 51915)
Normal-phase Si cartridges (Si Sep-Paks, Waters and Associates; Part No. 51905)
Positive-displacement pipettes (e.g., 1.0-mL and 2.5-mL from SMI, Inc., Emeryville, CA)
10-mL gastight glass syringe with Teflon-tipped plunger and male Luer tip (two)
Numbered aluminum tags (e.g., for gas chromatography columns; one per cartridge)
Lyophilization connectors. No. -0- Vikem or silicone solid stopper, 13-gauge stainless-steel tubing with female Luer hub, Teflon male Luer union (Hamilton Co., Reno, NV), and polypropylene male Luer plug (Value Plastics, Inc., Fort Collins, CO) (1 set per cartridge).
To construct lyophilizer connection apparatus, insert 4-cm length of 13-gauge stainless steel tubing with a female Luer hub through the narrow end of a No. -0- stopper. Connect the Luer hub to the cartridge end-sleeve with a male Luer union. Seal the open end of the cartridge with a male Luer plug.
Lyophilization apparatus (manifold, vacuum pump, etc.)
Volumetric flasks (size and number will depend on organic solute concentration in each sample; generally one per sample)
Teflon male Luer unions (for coupling C-18 and Si cartridges)
Infrared scanning spectrophotometer (repetitive scan recommended, e.g., Perkin-Elmer 298; matched quartz cuvettes, 1-cm path length)

Glassware Preparation

Acid-wash all glassware (soak in 25 percent nitric acid overnight and rinse with ASTM Type I water)
Rinse volumetric flasks with Freon 113

Reagents

(Note: all reagents are made from Analytical Reagent Grade chemicals.)

ASTM Type I water
Tetrahydrofuran (THF)
Methanol (MeOH)
1,1,2-Trichloro-1,2,2-trifluoroethane (Freon 113)

Procedure

N.B. The maximum number of samples and blanks is limited by the capacity of the lyophilizer.

I. Hydrophilic Fraction (HpF)

A. **C-18 Cartridge preparation**
(Note: Use cartridges from the same lot for any one experiment; record lot #)
1. To introduce solvents, connect male Luer tip of gastight syringe to the longer end-sleeve of the C-18 cartridge.
2. Pre-wash each C-18 cartridge with 10 mL of THF.
(Note: Always disconnect the syringe or pipette from the cartridge prior to withdrawing the plunger)
3. To activate each cartridge, apply 5 mL of MeOH, followed by 20 mL of ASTM Type I water, using gastight syringes. Partially remove residual water by forcing approximately 10 cc of air through the cartridge using a gastight syringe.

B. **Sample application**
1. Withdraw subsample from aqueous sample with an SMI pipette. To introduce sample, connect male Luer tip of SMI pipette to the longer end-sleeve of the C-18 cartridge.
2. Apply sample to a cartridge immediately after activation, at a flow rate of 5 to 10 mL/min. The SMI pipette tips fit directly inside the cartridge end-sleeve. The cartridge should be held vertically during sample application.

C. **Collection of HpF**
(Note: This fraction is discarded if only "oil and grease" values are desired; refer to Part II)
1. Discard the initial milliliter of effluent; this fraction is diluted by the residual water occluded by the cartridge.
2. Collect effluent in appropriate container for subsequent analysis or treatment.

II. Lipophilic fraction (LpF): —"Oil & Grease" and "Aliphatic Oil"—

A. **Blank preparation**
1. Blanks are prepared by following step **I. A.**
2. Blanks should be prepared in duplicate for each elution volume for oil & grease and aliphatic oil

B. **Sample application**
1. Follow steps **I. A.** and **I. B.**
2. Remove residual sample from cartridge by forcing approximately 10 cc of air through the cartridge using a gastight syringe.
3. Rinse residual occluded sample from cartridge with 1 to 2 mL of ASTM Type I water (positive displacement pipette). Partially remove residual rinse-water by forcing approximately 10 cc of air through the cartridge using a gastight syringe.

C. **Sample lyophilization**
1. DO NOT MARK CARTRIDGES WITH MARKING PEN! Identify each cartridge with a numbered aluminum tag. Seal both ends of the cartridge with male Luer plugs. Store cartridges at -20 ^{o}C until lyophilization.

2. Prepare lyophilizer.
3. Start the vacuum pump; operate lyophilizer at less than 0.10 torr.
4. Remove male Luer plug from long end-sleeve of cartridge. Attach each cartridge to lyophilizer manifold (e.g., VirTis 6205-1650, with 0.5-in OD Quickseal valves).
5. Submerge each cartridge in a MeOH-dry ice bath for 30 seconds.
6. Immediately after this freezing step, apply the vacuum; if any loss in vacuum occurs, check for leaks.
7. Lyophilize for about two hours. Lyophilization is complete when the cartridges are at room temperature and no further condensation forms when they are wiped dry.
8. When lyophilization is complete, release the vacuum and turn off the pump.
9. Remove samples from lyophilizer.

D. **Elution of LpF**

1. For the determination of oil & grease, elute the lyophilized cartridges with 5 mL of Freon; force residual Freon through with 10 cc of air. Elute in the same direction as sample application to avoid washing out nonsoluble particulates. Collect eluent and residual in an appropriately-sized volumetric flask. Bring to volume with Freon.
2. For the determination of aliphatic oil, prewash the Si cartridges with 10 mL of Freon; force residual Freon through with 10 cc of air and discard. Elute the retained solutes from the C-18 cartridges directly through the Si cartridges (cartridges coupled with male Luer unions) with 5 mL of Freon; force residual Freon through with 10 cc of air. Collect eluent and residual in an appropriately-sized volumetric flask. Bring to volume with Freon.
3. The stoppered volumetric flasks containing the eluates can be stored at 4 ^{o}C.

E. **Sample quantitation**

1. Prepare a set of standards using mineral oil or appropriate reference material (e.g., shale oil).
2. For the stock solution, place appropriate volume of oil in a tared 50-mL volumetric flask (if using mineral oil, place 75 µL in flask). Determine the mass of the oil and bring to volume with Freon.
3. For working standards, place 100, 250, 500, or 1000 µL of stock solution (positive displacement pipettes) in 5-mL volumetric flasks, and bring to volume with Freon.
4. Turn on IR spectrophotometer; allow a 20-minute warm-up period.
5. For a Perkin-Elmer model 298 IR spectrophotometer, set the repetitive-scan feature for the range 3200 to 2800 cm^{-1}, and set for medium slit width.
6. Fill one matched quartz cuvette with Freon 113; place in the reference slot.
7. Place sample in second matched cuvette.
8. Scan between 3200 and 2800 cm^{-1} at 4 min/full-range scan.
9. Quantitate the absorbance by tangent base-line measurement of peak height (Fig. 2). Keep peak height below 80 per cent of full-scale by making appropriate dilutions.

10. Interpolate the oil & grease or oil values from the oil standard curve:

    mg/L oil = m x (log P_o/P sample - log P_o/P blank) + b

    (P_o=incident energy; P=transmitted energy)

    a. Determine slope (m) and intercept (b) of the regression equation.
    b. Calculate the values of the samples from their log P_o/P values.
11. If absorbance peaks are beyond the range of the standard curve, dilute sample and bring to volume with Freon. Repeat analysis.
12. Report data in the following column format:

A[1]	B	C	D[2]	E[3]
log P_o/P	log P_o/P x dilution factor[4]	B - log P_o/P blank[5]	oil in volumetric flask (mg/L)	oil in sample (mg/L)

[1] absorbance determined by tangent base-line measurement of peak height

[2] obtained by interpolation from standard curve for value in column C

[3] transform column D by degree of enrichment or dilution that occurred during elution of samples (i.e., if 2.5 mL of sample was applied to the cartridge and the cartridge was eluted into a 10-mL volumetric flask, multiply the resultant value in D by 4 to determine the true sample concentration, i.e., the value for column E)

[4] if a dilution was required to keep peak within 80 per cent of full-scale, multiply by dilution factor (e.g., if sample was diluted one volume in four volumes total, multiply the values in column A by 4 to obtain value for column B)

[5] blank should be consistent with sample treatment, i.e., if sample was eluted into 10-mL volumetric flask, blank should be eluted into 10-mL volumetric flask.
Typical blank absorbance values for different final volumes:

oil and grease (10 mL)	0.010
oil and grease (5 mL)	0.015
oil (5 mL)	0.023

Protocol prepared by: B.M. Jones, R.H. Sakaji, and C.G. Daughton

Chapter II

COULOMETRIC QUANTITATION OF CARBON IN OIL SHALE WASTEWATERS VIA UV-PEROXYDISULFATE OR HIGH-TEMPERATURE OXIDATION

G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

Abstract 2
Introduction 3
Carbon Analysis 4
Classes of Carbon 4
Quantitation: Direct Versus Indirect 5
Commercial Instrumentation 5
Methods of Oxidation 6
Methods of Detection 7
Comparison Study 8
High-Temperature Combustion 9
Low-Temperature UV-Persulfate Oxidation 10
Coulometric Titrimetry 12
Inorganic Carbon Determination 13
Methods and Materials 14
Results and Discussion 15
Pure Compounds: Recovery and Reproducibility Comparison Study 15
Process Wastewaters: TDC and DOC Reproducibility Comparison Study 16
References 20
Tables
I. Origins of Oil Shale Process Wastewaters Used in the Comparison Study 23
II. Recovery Study: High-Temperature Combustion Carbon Analyzer 24
III. Recovery Study: UV-Persulfate Carbon Analyzer 25
IV. Precision of the Purge Operation for Determining Indirect Organic Carbon in a Composite Sample of Oil Shale Process Waters 26
V. Comparison of Analyzers: Quantitation of Direct/Indirect Organic Carbon in Oil Shale Process Waters 27
Figures
1. Terminology for Carbon Classifications Used in Carbon Analysis 28
2. Schematic of Coulometrics High-Temperature Combustion Apparatus 29
3. Schematic of UV-Peroxydisulfate Low-Temperature Oxidation Apparatus 30
4. Proposed Scheme for Coulometric Titration of Carbon Dioxide 31
5. Schematic of Coulometrics Acidification/Purge Apparatus for Inorganic Carbon 32
6. Comparison of Percent Recovery Means (X) and Ranges (---) for Standard Solutions 33
7. Time-Course Purge Study for Direct Organic Carbon Determination 34
Protocol: Dissolved Organic Carbon 35
High-Temperature Combustion/Coulometric Titration 35
UV-Enhanced Persulfate Oxidation/Coulometric Titration 40
Protocol: Inorganic Carbon 46
Acidification-Purge/Coulometric Titration 46
Appendix A
Carbon Analyzer Background Counts Program 50
Appendix B
Water Analysis Program: TDC, DOC, and DIC 52-54

ABSTRACT

The routine quantitation of organic or total carbon (OC and TC, respectively) in aqueous samples is generally achieved after stoichiometric conversion of each carbon atom into identical unit-carbon molecules by chemical/photochemical oxidation or high-temperature combustion followed by detection with nondispersive infrared spectroscopy (IR), flame ionization (FID), or coulometric titrimetry. The quantitation of OC and TC in synfuel process wastewaters presents several major problems to various conformations of instruments designed for the conversion and detection steps.

A carbon analyzer was fabricated from commercially available units and parts. This new design obviates the problems of (i) instrument downtime caused by fouling of high-temperature combustion catalysts and corrosion of furnace combustion tubes, (ii) limited linear dynamic range and upper detection limit (viz., IR), and (iii) frequent detector calibration (viz., IR and FID). This new approach to carbon analysis couples an ultraviolet photochemical reactor with an automatic coulometric titrator. Aqueous acidic peroxydisulfate serves as a source of free-radical oxidant and carries the sample from a sample injection loop to the photoreactor, where oxygen gas assists mixing and serves as a carrier gas for the evolved CO_2. This carbon analyzer was compared statistically with an ASTM-approved high-temperature combustion system. The CO_2 that was generated from each oxidation unit was quantitated by coulometric titrimetry. Low-temperature UV-enhanced persulfate oxidation of pure compound standards compared favorably with the recoveries from the ASTM-approved combustion unit. Results of a two-way analysis of variance indicated there was no significant difference between analyzers for recovery of pure compounds ($\alpha = 0.05$). Neither was there a significant difference in total or dissolved organic carbon values from each of the two types of analyzers ($\alpha = 0.05$) for nine oil shale process waters.

INTRODUCTION

Wastes that contain a complex mixture of organic and inorganic compounds present numerous problems when attempts are made to quantify the "total" amount of solutes or to quantitate the degree of contaminant removal by a waste treatment process. Methods that are specific for given compounds or even entire chemical classes may contribute information relevant only to a small portion of the solutes present in complex wastes; these methods can also be inaccurate because of positive and negative interferences by other compounds in the sample matrix. Complex solutions and heterogeneous wastes often necessitate the measurement of "bulk" or colligative properties that are shared by as many solutes in the matrix as possible.

Bulk properties that are conducive to analytical measurements include total dissolved solids, electrical conductivity, and the oxidative states of the solutes. A method that is commonly but incorrectly employed to estimate the total concentration of organic solutes is biochemical oxygen demand (BOD). Although BOD is partially a function of the quantity of carbon and its average oxidative state, it is merely an estimator of the material that can be oxidized by acclimated, aerobic bacteria. The overall oxidative state of solutes in a solution is more closely reflected by chemical oxygen demand (COD), which also is often misused as an estimator of organic carbon. Neither of these methods can distinguish organic from certain inorganic compounds (ammonia and thiosulfate will yield BOD and COD values, respectively), and both are unable to detect compounds that are refractory to the particular means of oxidation.

Methods that determine specific elemental concentrations (e.g., C, N, S, or P) can give more direct information. One of the most widely employed element-specific methods is carbon analysis. Inorganic and organic carbon species can be quantitated separately or together. Further qualitative information can be obtained by determining other parameters, such as COD, and relating them to carbon concentration, or by fractionating solutes into chemical classes prior to carbon analysis. For example, by relating the COD of an organic waste to the organic carbon concentration (i.e., "specific COD", see: Daughton, Jones, and Sakaji, 1981), the overall oxidative state of organic solutes can be estimated. Fractionation of organic compounds can be achieved, for example, by a rapid method that uses reverse-phase separation of polarity classes (see: Chapter I).

Problems associated with the quantitation of treatment performance for synfuel wastewaters such as oil shale process waters have been discussed (Daughton, Jones, Sakaji, and Thomas, 1982). Retort waters often contain large concentrations of organic and inorganic carbon. A large portion of the organic carbon is refractory to extensive mineralization by biooxidation. The inorganic carbon is partially responsible for the extreme buffering capacity and high alkalinity of these waters, which makes pH adjustment economically infeasible as a step in waste treatment. Of the numerous classes of organic solutes present in oil shale wastewaters, nitrogen heterocycles and nitriles are among the most difficult to oxidize biologically or chemically (e.g., by BOD and COD determinations) (Naik et al., 1972; Standard Methods ..., 1981) and have proved resistant to certain methods of oxidation used for organic carbon measurements (Armstrong, Williams, and Strickland, 1966; Gershey et al., 1979). These compounds also appear to be the major factor that limits the success of biotreatment of these waters (Jones, Sakaji, and Daughton, 1982).

This report discusses the advantages and disadvantages of various instrumental techniques for determining organic and inorganic carbon in highly contaminated waters. A new approach to organic carbon analysis is presented,

and the performance of this instrument in quantitating dissolved carbon in oil shale process wastewaters and in standard solutions of pure compounds is compared with that of an ASTM-approved carbon analyzer (ASTM, in press).

CARBON ANALYSIS

Classes of Carbon

There are eight major groups of carbon that can be determined by "carbon analysis". These classes are defined by organic and inorganic carbonaceous content and by whether suspended matter (e.g., particulates and colloids) is included (Fig. 1). Total carbon (TC) includes all forms of carbon in an aqueous sample; this in turn is composed of total organic carbon (TOC) and total inorganic carbon (TIC). "Inorganic carbon" in this report is synonymous with "oxides of carbon", "mineral carbon", and "carbonate carbon"; the predominant species in retort waters are carbonate and bicarbonate salts. If the particulate and colloidal materials are removed from liquid samples (e.g., via centrifugation or filtration), the carbon that remains is called total dissolved carbon (TDC). Total dissolved carbon includes both dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC); usually "dissolved" is arbitrarily defined as material that passes through a membrane filter of specified pore diameter (e.g., 0.20 to 0.45 μm).

An operational definition of "dissolved" or "soluble" is exceedingly complex. Filtration is generally assumed to separate the particulates from the dissolved species, but problems attendant with this approach are numerous. Filtration methods other than molecular weight ultrafilters can allow the passage of colloidal material into the filtrate, while at the other extreme, filtration can actually remove dissolved compounds (Fox, 1980) by any of several mechanisms. For oil shale process wastewaters, several variables influence the eventual separation of filtrate from retentate. The type of filter is the most important feature. "Depth" (e.g., glass fiber) versus "screen" (e.g., membrane) characteristics distinguish the two major groups of filters. The membrane filters include mixed cellulose esters and nylon, which themselves have depth filter characteristics, and polycarbonate. The screen type filters are affected by the loading of particles on their surfaces. During filtration, as the pores become partially blocked by particulates, the nominal pore size is reduced, thereby promoting the retention of particles that would normally not be retained (Laxen and Chandler, 1982); this problem can be partially solved by the use of tangential flow filtration apparatus. The chemical sorption or precipitation of solutes by electrostatic (Zierdt, 1979) or chemical interactions of the solution with the membrane surface also can effect removal of dissolved solutes. In addition, the partitioning of solutes into the immobilized, retained particulate phase has been hypothesized (Daughton et al., 1981). The composition of the filtrate can also be influenced by the type of filtration device. Vacuum filtration will remove portions of dissolved gases such as CO_2 and volatile organic species; pressure filtration is recommended in these instances.

Contamination of the filtrate by the filter is a final consideration for samples with low solute concentrations. Water extractable materials (e.g., wetting agents), humectants, and particulate debris, all of which remain after the manufacture of membranes, can significantly contaminate the filtrate (Cooney, 1980). From our experience, polycarbonate membranes offer the best compromise of features for the filtration of oil shale wastewaters; they are hydrophilic and yet have fewer extractables and smaller dispersion in nominal pore diameter, and they permit larger volumes of filtrate from oil shale process waters.

The rationales for distinguishing between dissolved and total carbon include: (i) dissolved carbon is the major form of carbon available to microorganisms and (ii) sampling error is minimized (e.g., size-exclusion dictated by the bore of the syringe needle) for liquids that contain large quantities of both particulate and colloidal forms of carbon. For these reasons, our laboratory has restricted itself to the determination of dissolved organic and inorganic carbon. This report will only address the determination of dissolved species. The investigation of particulate materials should entail another major study.

Quantitation: Direct Versus Indirect

Approaches to the quantitation of the carbon content of organic compounds generally require two steps: (i) the liberation of each carbon atom as an identical C-1 molecule which is not influenced by the bonding in the parent compounds and (ii) the detection and quantitation of these units.

The first step usually involves conversion of bound carbon to gaseous species (i.e., CO_2 or CH_4) by chemically or thermally mediated oxidation or reduction. The quantitation of organic carbon can be accomplished by either the direct or indirect method (Fox et al., 1980). The indirect method involves the determination of both TDC and DIC; DOC is then calculated by difference. The direct method requires the removal of inorganic carbon prior to the determination of dissolved carbon (TDC then becomes equivalent to DOC). The removal of DIC can be accomplished by precipitation with barium hydroxide or by boiling or purging with an inert gas after acidification (Van Hall, Barth, and Stenger, 1965). Acidification converts inorganic carbon to carbonic acid which subsequently hydrolyzes into H_2O and CO_2. The latter approach (i.e., purging) is the most widely accepted (ASTM, 1977; Standard Methods ..., 1981).

The direct method can result in the precipitation of organic compounds such as higher-molecular-weight saturated fatty acids during acidification and occlusion or partitioning of other organic solutes by these precipitates; subsequent purging of CO_2 can volatilize lower-molecular-weight organic solutes, especially carboxylic acids (Fox, Farrier, and Poulson, 1978). The indirect method requires the least sample manipulation, but lengthens the sample-throughput time because two analyses are required for each sample.

Inorganic carbon is determined directly by the conversion of each carbon atom to a single detectable species (i.e., CO_2). This can be accomplished at low temperature (60 ^{o}C) by conversion of mineral carbon species to CO_2 via acidification. The unambiguous determination of inorganic carbon is dependent on the specific conversion of only mineral carbon species to CO_2 and the resistance of all organic compounds to both oxidation and detection by this type of determination.

Commercial Instrumentation

The conversion of carbonaceous species to CO_2 is generally accomplished by one of four methods: high-temperature combustion, chemical oxidation, UV oxidation, or UV-enhanced chemical oxidation. The evolved CO_2 can be detected in batch or continuous mode. Detection methods can be physical, chemical, or electrical and span the range of low-sensitivity gravimetric methods to high-specificity infrared spectroscopy (IR). The advantages and disadvantages of each conversion and detection method are described below.

Methods of Oxidation.

(1) High-temperature combustion (950 °C).

Both organic and inorganic carbon compounds can be oxidized at high temperatures to yield CO_2. Oxidation generally occurs within a ceramic, stainless steel, or quartz combustion tube which is heated in a temperature-controlled furnace. Combustion tubes are packed with an oxidation catalyst which also serves to lengthen the sample residence time. A wide variety of sample introduction methods exist. These include syringe injection of aqueous samples and "boat" (ladle) introduction for solid or heterogeneous mixtures. Combustion products are swept from the tube to the detector by a carrier gas, usually oxygen or nitrogen; sufficient oxygen for carbon oxidation originates from combustion catalysts and water.

One problem associated with the combustion method is the production of large quantities of water vapor and other gases, which can result in excessive pressures in the combustion tube. This procedure is therefore restricted to sample volumes less than several hundred microliters. In addition, samples with high salt concentrations, especially alkaline metals, may cause the rapid degradation of the catalyst and attack the tube material itself; this is a major problem with quartz. High-temperature combustion characteristically contributes high background values as a result of trace carbon contamination of the catalyst and carrier gas. These systems therefore lack precision and accuracy below 2 mg-C/L (Takahashi, 1979), and Baker et al. (1974) found them unsuitable for the analysis of natural waters containing less than 15 mg-C/L. High-temperature combustion, however, does provide the most complete oxidation (Collins and Williams, 1977; Gershey et al., 1979) within a relatively short analysis time (3-5 minutes; Takahashi, 1979); this is an advantage not shared by alternative oxidation procedures.

(2) Chemical oxidation (persulfate; chromic acid in H_2SO_4).

Wet chemical methods can be adapted for the oxidation of organic compounds for TDC, DOC, or DIC analyses. These methods typically have lower system background values than high-temperature combustion units, and therefore, they are capable of lower limits of detection. They are not, however, applicable to all waters; certain organic compounds are resistant to chemical oxidation (Menzel and Vaccaro, 1964). Other classes of organic compounds may require significantly longer contact times with the oxidant, thereby increasing the analysis time beyond practical limits (Van Hall, Safranko, and Stenger, 1963). The persulfate oxidation procedure described by Menzel and Vaccaro (1964) requires less time than other chemical oxidation methods, but yields incomplete recoveries of polycyclic aromatic and long-chain hydrocarbons. Persulfate oxidation, in general, gives significantly lower recoveries (by 10 percent) than either combustion or photooxidation methods (Gershey et al., 1979; Williams, 1969). In addition, the presence of a high concentration of chloride ion in a sample can consume oxidant, contributing either a positive or negative interference, depending on the detection method.

(3) Ultraviolet oxidation.

Ultraviolet (UV) energy alone is incapable of mineralizing inorganic carbon oxides for the determination of total or inorganic carbon, or of oxidizing particulates for determination of TOC. Therefore, this technique is applicable only to dissolved organic carbon analyses. For this method to be effective, samples must be irradiated at wavelengths less than 210 nm for long periods (0.5 to 3.0 hours), a significant disadvantage when large numbers of samples must be analyzed. Although UV oxidation compares favorably with combustion for the recovery of organic carbon from natural waters (Gershey et al., 1979; Goulden and Brooksbank, 1975) it is incapable of complete mineralization of many of the

nitrogen- and sulfur-containing organic compounds (Armstrong et al., 1966; Gershey et al., 1979) that typify oil shale process waters. The effects of extensive UV irradiation of these waters have been reviewed by Jones et al. (May, 1982).

The energy output of the UV lamp is critical for this method of oxidation. The output will decrease during the life of the lamp, and variability in output also exists between individual UV lamps (Collins and Williams, 1977). Ultraviolet oxidation methods are compatible with detectors designed for quantitating low concentrations of carbon (e.g., 0 to 25 mg/L). Baker et al. (1974) report UV oxidation to be as efficient as chemical oxidation for the determination of organic carbon in freshwater. Gershey et al. (1979) found, in fact, that the recovery of DOC from seawater is higher with UV photooxidation than with chemical oxidation.

(4) UV-enhanced chemical oxidation.

This method is applicable to TDC and DOC analyses, but not to direct DIC analyses. Most methods coupling UV and wet chemical oxidation incorporate the Technicon Auto-Analyzer system; the sample is introduced into the oxidant stream, usually potassium persulfate (Collins and Williams, 1977; Goulden and Brooksbank, 1975), and is pumped through a silica coil surrounding a UV lamp. Residence time in the coil varies from 8 to 45 minutes. This method results in higher precision than obtained by high-temperature combustion (Goulden and Brooksbank, 1975). Leachable organic material from the pump tubing, however, can contribute a significant background signal; preconditioning of the pump tubing is often required for at least 24 hours to minimize this problem.

Another design for UV-enhanced chemical oxidation uses a UV lamp submerged in a reactor vessel containing the chemical oxidant (Wölfel and Sontheimer, 1974). The sample is introduced directly to the solution and the evolved CO_2 is swept by the carrier gas to the detector. A commercial system that incorporates a UV lamp submerged in an acidic potassium peroxydisulfate solution and nondispersive IR detection of CO_2 was recently introduced (model #DC-80, Dohrmann Division, Xertex, Inc., Santa Clara, CA). The manufacturer reports an analysis time of 3 to 4 minutes, and complete recoveries of several nitrogen heterocycles (e.g., pyridine, proline, and nicotinic acid, each at approximately 100 mg/L concentration) (Takahashi, Martin, and Harper, 1981).

Methods of Detection.

Evolved CO_2 can be quantitated by several physical methods, including manometric, gravimetric, and volumetric determinations. These are limited to batch analysis, are extremely time consuming, suffer from low sensitivity and high lower-detection limits, cannot be automated, and are subject to interferences from co-produced gases. Gravimetric determinations, for example, depend on the absorption of CO_2 on soda-asbestos, soda lime, or into an alkaline solution (Blom and Edelhausen, 1955). The increase in weight as a result of gas absorption is measured, and the carbon content is interpolated from the recovery of standard solutions analyzed in parallel.

Thermal conductivity, electrical conductivity, and flame ionization detection (FID) are three methods for directly or indirectly detecting CO_2 in a gas stream on a continuous basis. These methods vary from moderate sensitivity with a narrow linear range (thermal conductivity) to high sensitivity with a wide linear dynamic range (FID). Thermal conductivity detectors determine the change in conductivity within a heated cavity as a result of changes in the gas composition. This method of detection is nonspecific and subject to interferences from co-produced gases. In addition, the sample throughput is limited (25 min/sample). Thermal conductivity is only

moderately sensitive compared with other methods of detection (Willard, Merritt, and Dean, 1974). In comparison, flame ionization detection is extremely sensitive. The introduced gases burn in a hydrogen flame (Jeffery and Kipping, 1972) and a proportion of the molecules acquire sufficient energy to ionize. This ionization gives the flame an electrical conductivity which can be detected and amplified (Littlewood, 1962). Since an FID responds only to oxidizable carbon atoms, CO_2 from oxidized or combusted organic material must be reduced to CH_4 over a nickel catalyst prior to detection (Willard et al., 1974). Any hydrocarbon gas that survives the combustion/oxidation step would also be quantitated. The precision of an FID for organic carbon determinations will depend on the efficiency of conversion of CO_2 to CH_4. Flame ionization detectors are reported to have wide linear dynamic ranges (Willard et al., 1974), but require frequent daily calibration.

Non-dispersive IR detection has the advantage of being highly specific for CO_2 with excellent sensitivity. The CO_2 content of the carrier gas is compared with a nonabsorbing reference gas (Delahay, 1962) and the difference in absorbance at 2380 cm^{-1} (4.2 μm) is quantitated (Beckman, 1980). The range of the detector depends on the cell path length and detector configuration. Manufacturers claim a range for aqueous samples of 0 to 2000 mg-C/L; the standard curve from an IR detector over this range, however, is notoriously nonlinear. The precision of the instrument relies on a constant gas flow rate. Carbon dioxide is quantitated by peak height interpolated from a standard curve. This type of detector requires frequent calibration and, for high precision, the samples should fall within the linear portion of the standard curve.

Detection of CO_2 by coulometry, as in any titrimetric technique, requires the addition of reagent until a predetermined endpoint has been attained. For coulometry, the reagent (i.e., electrons) is generated electrolytically, and the quantity of titrant required for the stoichiometric indirect titration of the CO_2 is equivalent to the number of coulombs generated (Ewing, 1981). Stoichiometric titration obviates the need for frequent calibration because the electron itself becomes a primary standard (Willard et al., 1974).

We have found this detection method to possess an excellent linear dynamic range (at least three orders of magnitude) and, with appropriate gas scrubbers, to be accurate and precise for the quantitation of CO_2. For oil shale wastewaters, we have decided that coulometric detection is the method of choice.

COMPARISON STUDY

Of the four oxidation/combustion methods discussed previously, only high-temperature combustion and UV-enhanced persulfate oxidation appeared to be suitable for the routine determination of organic carbon in oil shale process waters. The alternative methods, chemical and UV oxidation, were not applicable to oil shale process waters due to reported incomplete oxidation of certain organic compounds and lengthy analysis times. We have fabricated a hybrid carbon analyzer that combines the strengths of two commercial carbon analyzers while avoiding their weaknesses. A study was initiated to statistically compare this newly configured instrument with an ASTM-approved carbon analyzer which this laboratory has used routinely for analysis of oil shale wastewaters.

Nearly all commercial instruments for carbon analysis employ one of two designs: (1) high-temperature combustion coupled with coulometric titrimetry (e.g., Coulometrics, Inc.) or IR detection (e.g., Ionics; O.I. Corp.; Beckman)

or (2) low-temperature oxidation coupled with IR detection (e.g., Dohrmann; Astro; O.I. Corp.; Ionics). While both high-temperature combustion and IR detection are applicable to the analysis of oil shale process waters, we have experienced significant problems with each. High-temperature combustion units have been subject to frequent and unpredictable downtimes because of damaged combustion tubes and fouled catalysts. Infrared detectors have exhibited substantial drift, requiring frequent standard curve determinations.

The ASTM-approved analyzer used in this study was obtained from Coulometrics, Inc. (Wheat Ridge, CO). This system couples high-temperature combustion (quartz combustion tube) with an automatic coulometric titrator. The newly configured analyzer, subject of this comparison study, combines a commercially available photochemical reactor with the same automatic coulometric titrator. The major anticipated advantages of this new approach were reduced maintenance and downtime, lower capital and maintenance costs, and ease of automation.

High-Temperature Combustion

The Coulometrics high-temperature combustion system (model #5020, Coulometrics, Inc., Wheat Ridge, CO) (Fig. 2) oxidizes both organic and inorganic carbonaceous compounds. Samples are introduced to a quartz combustion tube by direct injection with a Hamilton (Reno, NV) CR-700 "constant rate" carbon analyzer syringe. This syringe can be set for any volume up to 200 µL, and the contents are forcibly expelled by a spring-driven piston through a 90° bevel needle to ensure reproducible emplacement of the sample within the heated portion of the combustion tube. The syringe and injection port form a gas-tight Luer-slip union. The combustion tube, successively packed with a WO_3-coated quartz wool plug, barium chromate catalyst, and a sintered plug of reduced silver for removal of HI and HBr, is heated to 950 °C in a digitally controlled furnace. Oxygen (99.6 percent purity) is used as a carrier gas and as an additional oxidant source. The oxygen is pretreated by passage though a heated (950 °C) "precombustion" tube packed with barium chromate; contaminative combustion products (e.g., acidic gases) and CO_2 are removed by a gas scrubber containing 45 percent potassium hydroxide before the oxygen passes into the injection port. The scrubbed oxygen stream sweeps the volatilized injected sample through the combustion tube. Combustion of the liquid sample results in conversion of organic and inorganic carbon to CO_2, production of acidic gases (e.g., SO_x and NO_x), and steam. Much of the water vapor condenses and is collected in an ambient temperature burette trap. The gaseous phase then passes through a drying tube (magnesium perchlorate) followed by a scrubber packed with acid potassium dichromate-manganese dioxide for removal of contaminative acidic gases. The gas stream, theoretically containing only CO_2 and O_2, then enters the coulometric titration cell where the CO_2 is absorbed and quantitated.

High-temperature combustion of organic compounds provides complete oxidation within a short period of time and is thus well suited to the analysis of DOC in retort waters. These wastewaters characteristically contain large numbers of nitrogen and oxygen heterocycles that may be resistant to wet chemical or UV oxidation. Problems have been encountered, however, with combustion tube deterioration and sample introduction methods. The high salt concentration in retort water causes rapid deterioration of the combustion catalyst and the alkaline metals attack the quartz combustion tube. This results in frequent downtime for replacement and conditioning of new combustion tubes; these tubes can rarely be reused because of stress fractures that almost always develop during cooling. The life of the combustion tube and packing

material can be prolonged with the use of tungsten trioxide at the influent end of the combustion tube packing; tungsten trioxide aids in the rapid oxidation of carbonates and prevents the formation of sodium carbonates, which are more thermally stable (ASTM, in press). In addition, the sample introduction method is somewhat unsatisfactory. The constant-rate syringe lacks precision and accuracy for reproducibly measuring repetitive sample volumes; this necessitates volume corrections for each data point. The volume set-point for this syringe is also easily disrupted during operation. Sample analysis time is increased because the syringe must remain in the injection port throughout the analysis period; this prevents rapid preparation of the subsequent sample for injection. The restricted internal diameter of the needle severely limits the utility of this approach for particulate sampling.

Low-Temperature UV-Persulfate Oxidation

To circumvent the disadvantages associated with high-temperature combustion, the alternative approach of low-temperature oxidation was evaluated. The high-temperature system with syringe injection was replaced with a modified Dohrmann UV-persulfate reactor for sample oxidation and a low-pressure injection loop for sample introduction (Fig. 3).

The design of the Dohrmann photochemical reactor obviates many of the disadvantages of conventional UV-persulfate reactors. Direct immersion of a low-pressure mercury vapor lamp in the persulfate solution (85 mL) eliminates the need for a silica coil around a UV lamp; this significantly reduces the sample residence time for complete oxidation. In addition, attenuation of the UV output by the lamp quartz envelope, dead air space, and coil wall is minimized. Therefore, more UV energy is available, and the time required for complete sample oxidation is minimized. A carrier gas/sparging system (O_2, N_2, He, Ar, or purified air) provides complete mixing of the reactor contents. The system described in this report uses O_2 (99.6 percent).

The photoreactor unit (Fig. 3) was assembled from parts that were purchased from Dohrmann (Xertex Inc., Santa Clara, CA). The following major parts were required: reactor body assembly (#512-090), reactor cap assembly (#512-091), silicone connectors (#517-798) for joining 1/16-inch o.d. Teflon tubing to the reactor, silicone plugs for unused reactor ports (#577-803), UV lamp (#512-092), Teflon sleeve for tapered joint of reactor cap (#050-409), and transformer for UV lamp (#010-454). A power supply for the UV lamp was fabricated from the Dohrmann transformer, using readily available electrical supplies which included an aluminum instrument housing, instrument fan, ready light and on-off toggle switch, and an electrical outlet for auxiliary power supply to other equipment. In-line fuses were installed for the transformer and auxiliary electrical outlet. A Teflon gas delivery line was connected to the fritted glass impinger in the reactor bottom with a 1/4-inch to 1/8-inch silicone reducing connector; the effluent gas line was similarly connected to the reactor cap. It is very important to ensure that the contact of all silicone rubber connectors and plugs with the carrier gas stream is minimized. Teflon lines and glass and metal fittings should be abutted to or slipped inside one another. This is necessitated by the high permeability of silicone rubber to carbon dioxide (Brookes, 1969). An alternative connector material is shrink-fit Teflon tubing.

The low-pressure injection valve (model #50-20, Rheodyne, Berkeley, CA) incorporates a calibrated 200-µL sample loop, which minimizes error in sample volume measurement and reproducibility. The system is designed so that the samples and reagents only contact Teflon, glass, and stainless steel. The sample is loaded into the 200-µL loop with a Glenco (#19925, Houston, TX)

1.0-mL gas-tight syringe (rotary valve in "load" position) via a Valco zero-volume fill port assembly (#VISF-1, Houston, TX); excess sample is expelled through the waste line. Five to ten loop volumes are loaded to ensure complete flushing of the previous sample from the loop (Rheodyne, 1979). When the valve is switched to the "inject" position, peroxydisulfate solution sweeps through the loop and carries the sample to the reactor.

The sample enters the bottom of the reactor through a sidearm (Fig. 3). The sample fluid and persulfate solution immediately enter a region of high turbulence created by impinged oxygen that is introduced through the bottom of the reactor. A portion of the reactor fluid is withdrawn for recycle from a sidearm at the mid-portion of the reactor; this fluid is combined at a tee fitting with the flow of fresh persulfate reagent from a reservoir and recycled through the injection valve and back into the reactor via the lower sidearm. A glass loop connects the top and bottom of the reactor contents. Reactor fluid is drawn off to waste from the top horizontal section of the loop. The upward flow of the impinged oxygen creates a downward flow of reactor fluid through the loop; this ensures that nonoxidized sample is not isolated from the main reactor and promotes further mixing. By ensuring that the pumping rate for the waste is equal to or greater than the influent rate for fresh persulfate, the volume within the reactor is maintained at a constant level; identical pumping rates can be ensured by setting the wastage rate higher than the influent and removing the waste from a set surface level. The wastage rate is limited, however, by careful consideration of the amount of reactor headspace that is removed; since the carrier gas flow rate is 200 cm^3/min, a wastage rate of up to 2 mL/min would result in loss of nearly 1 percent of the evolved CO_2, depending upon the volume of gas that enters the waste line. The gaseous oxidation products are swept through the effluent line connected to the reactor cap by the oxygen carrier gas.

The influent, waste, and recycle lines were plumbed through a four-channel peristaltic pump (model 375-A, Sage Instruments Division, Orion Research Inc., Cambridge, MA). Organic contaminants were found to leach from both silicone and Tygon pump tubes, which resulted in high background carbon counts (10 mg-C/L-min); this was most likely a result of plasticizers and unreacted oligomers. Overnight preconditioning of tubing in a hypochlorite solution could only temporarily reduce the background (3.3 mg-C/L-min). Collins and Williams (1977) reported the need for tubing preconditioning and observed decreased background contributions during operation because of a reduction in leachable materials. To avoid these problems, the influent and recirculation tubes were replaced with Viton tubing (a copolymer of vinylidene fluoride and hexafluoropropylene; Cole-Parmer Instrument Co., Chicago, IL) which gave an acceptable background carbon concentration (2.0 to 3.2 mg-C/L-min) without preconditioning. The disadvantage of Viton tubing is its reduced elasticity which necessitates more frequent replacement (lifetime = 50 to 80 hours of operation) and its higher cost. The recirculation pump tube (0.063-inch i.d.) was manifolded to yield the desired flow rate of 3.0 mL/min, then recombined after the pump and joined, via a stainless steel tee with the influent persulfate line (0.031-inch i.d.) (0.6 mL/min) to yield a 3.6-mL/min flow rate through the injection valve into the reactor. The flow rate of the waste (silicone pump tube, 1.0 mm i.d.) was 0.6 mL/min, balancing the flow of fresh reagent into the reactor.

Sample material entering the reactor is exposed to the individual and combined effects of persulfate- and UV-oxidation. Ultraviolet radiation

enhances the disproportionation of persulfate into sulfate free radicals and hydroxyl free radicals, two powerful oxidants (House, 1962; Takahashi et al., 1981):

$$S_2O_8^{-2} + h\nu = 2SO_4^{\bullet-}$$
$$H_2O + h\nu = H^\bullet + {}^\bullet OH$$
$$SO_4^{\bullet-} + H_2O = SO_4^{-2} + {}^\bullet OH + H^+$$

Ultraviolet energy also can cause excitation of organic compounds, facilitating their oxidation to CO_2 by sulfate and hydroxyl radicals:

$$R + h\nu = R^*$$
$$R^* + SO_4^{\bullet-} + H_2O = nCO_2 + \ldots$$
$$R^* + {}^\bullet OH + H_2O = nCO_2 + \ldots$$

The oxidation of retort water organic solutes by hydroxyl radical has been discussed by Jones et al. (May, 1982).

High chloride ion concentration in a sample can interfere with the mineralizaton of organic analytes by competing for oxidant (House, 1962).

$$SO_4^{\bullet-} + Cl^- = SO_4^{-2} + Cl^\bullet$$

This interference could possibly be minimized by complexing the excess chloride ions with mercuric ion (Takahashi et al., 1981).

The oxygen carrier gas is passed through a potassium hydroxide scrubber for removal of contaminative acidic gases prior to entering the UV-persulfate reactor. The CO_2 produced in the reactor is swept through two magnesium perchlorate drying tubes, an acid potassium dichromate/manganese dioxide scrubber, and into the coulometer for quantitation.

Coulometric Titrimetry

The automatic CO_2 coulometric titrator was obtained from Coulometrics, Inc. (model #5010). The titration cell consists of a 200-mL Berzelius tall-form Pyrex beaker and a rubber stopper which holds the cathode, influent gas line, and anode cell (Fig. 4). For absorption/titration of evolved CO_2, the coulometer cell is filled with approximately 75 mL of a proprietary ethanolamine solution which contains thymolphthalein blue as an indicator (pK_a 9.4-10.0); the solution changes from blue to colorless upon acidification. The anode cell is a fritted glass tube that contains potassium iodide pellets, a proprietary anode solution, and a silver electrode which is connected to the coulometer circuitry. The anode solution is most likely a saturated potassium iodide solution that acts as a salt bridge; use of potassium chloride in place of potassium iodide would result in the precipitation of silver chloride within the anode cell. The platinum wire cathode surrounds the outside of the fritted-end of the anode cell. The major components of the coulometer are a colorimeter for detection of the titration endpoint (displayed as percent transmittance) and anticipator circuitry, which switches the titration current from high (100 milliamps) to low (5 milliamps) and from low to off as the colorimetric endpoint (i.e., transmittance of 30 percent at 612 nm) is approached. The rate of current generation for titration is determined by comparing the colorimeter output with preset voltages to determine the distance from the endpoint. A logic block receives the comparator signal and sets the current source at the

determined rate (Huffman, 1977). The current passing through the cell is converted to a digital readout which can be manipulated to display carbon concentration as milligrams per liter.

Carbon dioxide in the gas stream is quantitatively absorbed by monoethanolamine (MEA), forming hydroxyethylcarbamic acid (Fig. 4). Dissociation of the acid yields one hydrogen ion per molecule of CO_2 absorbed. The transient carbamate is hydrolyzed by water, producing bicarbonate and regenerating MEA. The equilibrium reactions occurring in the bulk solution are (Danckwerts and McNeil, 1967; Danckwerts and Sharma, 1966):

$$RNH_2 + CO_2 = RNHCOO^- + H^+$$
$$RNHCOO^- + H_2O = HCO_3^- + RNH_2,$$

where R is the 2-hydroxyethyl moiety of MEA and the carbamic acid.

Absorption of CO_2 with the concomitant production of hydrogen ion decreases the pH of the coulometer solution; the hydrogen ion protonates the thymolphthalein blue indicator, yielding the colorless form (Fig. 4). The increased transmittance of the solution is detected by the photometer which initiates the generation of electrons at the silver anode. Two possible fates for the electrons have been postulated. Hydrogen ions, produced stoichiometrically with CO_2 absorption, could be reduced by electrons leaving the platinum cathode, yielding hydrogen gas. Alternatively, the electrons leaving the platinum cathode could cause the hydrolysis of water, producing hydroxide ion and hydrogen gas. The hydroxide ion would then reduce the hydrogen ion (produced from CO_2 absorption), regenerating water. As the CO_2 concentration decreases during titration, the increase in pH causes dissociation of the indicator to the colored form. When all the CO_2 has been titrated, the photodetector determines that the endpoint has been reached. The generation of current is then suspended, and the integrated measurement of the number of coulombs used is converted to display mg-C/L. Although the dried carrier gas continually evaporates water from the coulometer solution, thereby decreasing the transmittance possibly beyond the endpoint, small quantities of CO_2 serve to continuously readjust the transmittance; we have not, however, encountered reproducibility problems after extended operation.

The major advantage of coulometric titration is that titrant is generated stoichiometrically with 100 percent efficiency. The linear dynamic range and upper limit of the coulometer exceed those of detection by nondispersive infrared spectroscopy, flame ionization, and thermal conductivity. This often eliminates the need for dilution of samples. The coulometer calibration, performed electronically, is extremely stable and obviates the need for frequent empirical calibration with standards, as is required for other detectors. Coulometric titrimetry for detection of CO_2 seems particularly well suited for analysis of carbon in oil shale process waters because of the wide range of concentrations of inorganic and organic carbon present. An occasional problem of sample over-titration, however, has been observed; this problem appears to be related to the rate at which CO_2 enters the coulometer cell and the response lag-time for the high-to-low titration trip-point.

Inorganic Carbon Determination.

The Coulometrics carbonate-carbon apparatus (model #5030) uses the acidification/purge technique (Fig. 5). The sample is injected into the reaction tube with a 200-µL gas-tight syringe fitted with a septum-piercing needle (e.g., Unimetrics TP 4250S with repetitive volume adjustment). A

repipette (e.g., 5-mL Dispensette, Brinkmann Instruments Co., Westbury, NY), connected to the top of the reactor tube with Teflon tubing and unions, is used to dispense 2.0 mL of 2N perchloric acid. Ambient air, scrubbed through an aqueous solution of potassium hydroxide, sweeps the acid and sample into the bottom of the reactor tube where the mixture is heated to 60 °C. The CO_2 that evolves from the carbon oxides is swept through a silver sulfate scrubber for removal of interfering acidic gases (e.g., SO_x and NO_x) and into the coulometer. This method of inorganic carbon determination is only accurate if organic compounds are not oxidized by the acid treatment.

METHODS AND MATERIALS

The two carbon analyzers were evaluated and compared for the quantitation of TDC and direct and indirect DOC in nine oil shale process wastewaters. The recovery of 17 pure compounds in standard solutions was also investigated. Of the organic solutes present in oil shale process wastewaters, nitrogen heterocycles were of primary interest because they are proposed to be responsible for much of the difficulty in treatment processes (Jones et al., 1982) and also because they resist many oxidation schemes. A series of water-soluble methyl-substituted pyridines was selected for recovery studies based on their reported occurrence in synfuel wastewaters (Raphaelian and Harrison, 1981; Torpy, Raphaelian, and Luthy, 1981) and because of their adequate solubilities in water. Acetonitrile and cyanuric acid were selected because they are resistant to complete and rapid oxidation by photochemical methods (Dohrmann-Envirotech, 1981; Takahashi et al., 1981). Several other water-soluble aromatic and nitrogen-heterocyclic organic compounds also were included in this study because of their possible resistance to complete mineralization. A compound known to be quantitatively mineralized by less rigorous oxidative methods, potassium acid phthalate, was quantitated at several concentrations to determine the linear response of each unit.

The nitrogen heterocycle standards were of the highest grade commercially available (Noah Chemical, Farmingdale, NY; Jewel Nero Consulting, Sun Valley, CA). The acetonitrile was of HPLC grade; all other standards were of analytical reagent grade. A solution of each compound was prepared with acidified, CO_2-free ASTM Type I water. The mass of compound added to a Class A 50-mL volumetric flask was determined with a semi-micro Mettler analytical balance (model HL52). From the resulting concentration, the theoretical carbon concentration of each standard was calculated. The CO_2-free water was prepared by boiling ASTM Type I water for one hour; upon cooling, the boiling vessel was connected to a series of three drying tubes containing calcium chloride, Ascarite, and soda lime. The cooled water was acidified to pH 3 to minimize the uptake of atmospheric CO_2. This procedure precluded the need to purge the standards prior to analysis for DOC and therefore minimized the possibility of loss of carbon from volatilization; the TOC and DOC of these standards were therefore equivalent. Standard solutions and diluted samples were stored at 4 °C in 25-mL glass scintillation vials with Teflon-lined screw caps. Ten single-operator replicate injections of CO_2-free water (blanks) were analyzed on each instrument to determine the background contribution during a 5-minute analysis time. Ten single-operator replicates of each standard were then analyzed for TDC (in this instance DOC) concentration on each carbon analyzer.

Samples of nine oil shale process wastewaters (Table 1) were filtered (0.4-μm pore-diameter polycarbonate membranes, Bio-Rad Laboratories, Richmond, CA) under pressure and diluted to yield concentrations of

approximately 500 mg-C/L for TDC and DOC analyses. These samples were stored in a manner identical to the standards. Samples for direct DOC analysis were acidified with concentrated sulfuric acid (100 µL acid per 10.00 mL of sample) and purged for 10 minutes with high purity helium (120 cm^3/min). This represents less than a 1-percent dilution error, and the final DOC values were not corrected. It may be important to note that the procedural order (i.e., filtration, dilution, and acidification) and the rate of acidification may affect the carbon concentration of a sample. The appropriate blank value for each system was determined by the method previously described. Ten single-operator replicates of each process water sample were analyzed on each system for both TDC and direct DOC.

For the determination of direct DOC, the extent and precision of the purging operation were also determined. High purity helium (99.995 percent) was delivered through Teflon tubing to a six-place aluminum manifold purge station at 720 cm^3/min, giving a flow rate through each glass capillary purge station of about 120 cm^3/min. The time-course removal of volatile carbon (presumably DIC) was followed for a wastewater that was composed of equal volumes of nine process waters (Table I); this composite water was chosen to moderate possible idiosyncrasies of individual waters. Duplicate samples of the composite water (filtered, diluted, and acidified) were purged for each of nine time intervals up to 15 minutes and then analyzed for TDC (i.e., what normally would be DOC if purging of DIC were complete) with three replicate determinations for each duplicate; the duplicates were run at separate times using the same manifold to eliminate variability from differences in flow between manifolds. The imprecision of purging was determined by purging duplicate samples at five stations for 10 minutes each with five replicate determinations for each duplicate.

Each analyzer was interfaced with a programmable printing calculator (Hewlett-Packard, model HP 97S) which monitored the coulometer output at 15-second intervals. Values for the system blank and sample dilution were stored in the calculator memory. The DOC value recorded after the 5-minute analysis time was subjected to a stability test prior to print-out. This test compared the final value with the value that was recorded 15 seconds earlier. If the difference in values was greater than 1 percent, the data-acquisition loop was re-entered and a subsequent value obtained and tested for stability. When the stability test was satisfied, the final value (minus the system blank and multiplied by the sample dilution factor) was automatically printed.

Samples of each process water also were analyzed for DIC concentration (10 replicates). The analysis time for DIC determinations was 3 minutes, and the data were manipulated as described above. The mean DIC values were subtracted from the respective mean TDC values for each retort water; this yielded an indirect DOC value for comparison with the direct DOC determination. All statistical analyses were based on the appropriate sections in Sokal and Rohlf (1969) and Rohlf and Sokal (1969).

Detailed operating protocols for all instruments used in the comparison study are appended.

RESULTS AND DISCUSSION

Pure Compounds: Recovery and Reproducibility Comparison Study

The theoretical concentrations and the observed recoveries for the high-temperature combustion of pure compound solutions are presented in Table II. Complete recoveries were obtained for all compounds except pyridine (95 percent) and acetonitrile (97 percent). The degree and position of alkyl

substitution for the N-heterocycles did not affect the recoveries. The relative standard deviations (rsd) were less than 1 percent for most compounds, and they did not exceed 3 percent for any compound. Recovery of the potassium acid phthalate standards deviated slightly from linearity at the lowest concentration (100 mg-C/L); a similar deviation was also noted for the UV-persulfate system. With increasing concentrations of acid phthalate standards, the precision of recovery increased.

Ultraviolet-enhanced peroxydisulfate oxidation resulted in complete recoveries for the majority of pure compounds tested; acetonitrile and cyanuric acid, however, were resistant to oxidation (Table III) as reported by others (Takahashi et al., 1981; Dohrmann-Envirotech, 1981). The recovery of pyridine (95 percent) was identical to that for the high-temperature system; this may indicate that the pyridine contained impurities that reduced its actual concentration. The quantitation of acetonitrile was incomplete after the 5-minute analysis period; higher recoveries (i.e., 100 percent) were obtained by increasing the analysis time to 10 minutes. Cyanuric acid, an s-triazine, was completely resistant to UV-persulfate oxidation, regardless of the analysis time. Similar findings were reported by the manufacturer of the UV reactor for cyanuric acid and melamine (Dohrmann-Envirotech, 1981). It is not known whether other triazines present a similar problem, but these compounds have not been reported in oil shale process waters. With the exception of cyanuric acid, the relative standard deviations for sample recoveries were less than 2 percent for most samples, and did not exceed 4 percent.

The close agreement between analyzers for the recovery of all the pure compounds, except cyanuric acid, is illustrated in Figure 6. The pattern of small deviations above and below 100 percent recovery is similar for both analyzers; this is probably the result of impurities in the stock reference compounds and errors in sample preparation. The ranges of percent recoveries suggest that the high-temperature analyzer was slightly more precise.

To determine if the observed differences in sample recoveries were significant, a two-way analysis of variance (anova) was conducted. The calculated F-value (F_s) for the variability between analyzers was 1.23, which was less than the critical F-value (F_α) of 4.41 at $\alpha = 0.05$. Therefore, there was no significant difference ($P>0.05$) between carbon analyzers for the recovery of carbon from solutions of pure compounds.

Because of the anticipated problems with the ability of the UV-persulfate oxidation system to completely oxidize acetonitrile and cyanuric acid, the mean recoveries of these compounds were tested by a priori comparisons between analyzers. There was no significant difference ($P>0.05$) between analyzers for the recovery of acetonitrile: $F_s(<1)<F_{.05}(4.41)$. There was a highly significant difference ($P<0.001$) between analyzers for the recovery of cyanuric acid despite the nonsignificant overall anova: $F_s(19.24)>F_{.001}15.4$.

Process Wastewaters: TDC and DOC Reproducibility Comparison Study

Results from the purge time-course study are presented in Figure 7. The rsd values for the three replicate determinations for each duplicate were less than 1.0, except for one sample. A one-way anova conducted on these data indicated a highly significant variance component ($P<<0.001$) due to purge time: $F_s(46.5)>>F_{.001}(8.96)$. Results of the Student-Newman-Keuls step-wise multiple comparison test indicated that there was a significant difference ($P<0.05$) between means for purge times of 0.0, 0.5, and 1.0 minutes; there was no significant difference between purge times of 1.0 and 15 minutes ($P>0.05$). Since oil shale wastewaters differ greatly in DIC concentrations,

they may require more or less purging than the composite water used in this study. From the results for this composite water, however, 10 minutes was probably a more than sufficient purge time for DIC removal from the nine waters used in this study. This study, however, does not address the question of whether volatile organic compounds also are lost during purging.

Results of the purge precision study (Table IV) indicated that the variability between purge stations was small. A one-way anova conducted on these data showed no significant difference ($P>0.05$) among stations: $F_s(3.91)<F_{.05}(5.19)$.

The values obtained from each carbon analyzer for TDC, DIC, and direct and indirect DOC concentrations in nine oil shale process wastewaters are presented in Table V. There was close agreement between the two analyzers (each labeled "a" or "b" in Table V) for TDC and DOC determinations for each water. Since high-temperature combustion techniques are generally assumed to give complete recovery of carbon, even though there is no definitive means of proving the completeness of mineralization (Gershey et al., 1979), it therefore can be concluded from these results that UV-enhanced persulfate oxidation of oil shale process waters yields complete oxidation of dissolved organic material. If compounds resistant to UV-persulfate oxidation were present in retort wastewaters, their concentrations were too low to significantly affect the overall recovery of carbon.

The rsd values for the recovery of TDC and DOC by either analyzer were less than 3 percent and generally less than 2 percent. To determine if a significant difference existed between carbon analyzers for the recovery of TDC, a two-way anova was conducted on the square root-transformed data. There was no significant difference ($P>0.05$) between analyzers for TDC recovery: $F_s<F_{.05}$ $(4.88<5.32)$. There was a significant interaction effect between analyzers and wastewaters, $F_s>F_{.05}$ $(3.99>1.94)$, but the results of Tukey's test indicated that an insignificant portion was due to nonadditive effects, $F_s<F_{.05}$ $(0.21<5.59)$, and therefore did not violate the assumptions of the anova model. The additive interaction between treatments (i.e., between wastewaters and analyzers) obviously resulted from the wide range in TDC values between wastewaters.

A two-way anova was conducted on the square root-transformed DOC data with similar results. There was no significant difference ($P>>0.05$) between carbon analyzers for the quantitation of DOC: $F_s<F_{.05}$ $(0.98<5.32)$. The interaction term was significant but additive, and it therefore did not violate the assumptions of the statistical model.

For each carbon analyzer, the direct and indirect DOC data for the nine process waters (Table V) were compared by a two-way anova on the log-transformed values. There was no significant difference ($P>>0.05$) between direct DOC and indirect DOC measurements for either high-temperature combustion or UV-persulfate oxidation: $F_s<1<F_{.05}$ (5.59) for both anova's. The purging of samples for direct DOC analysis therefore did not appear to remove measurable quantities of volatile organic carbon compounds nor did the acidification step result in noticeable loss of organic species by precipitation. This is in agreement with the results from indirect versus direct carbon determinations on oil shale wastewaters reported by Fox et al. (1980); it should be noted, however, that extensive volatilization may have already occurred in all of these waters because they had been stored for long periods. The imprecision of the indirect DOC method was greater than that of the direct method, as shown by its larger rsd's (Table V). Since indirect DOC is the difference between TDC and DIC, its associated rsd contains the propagated error from both the TDC and DIC

analyses. The standard deviation for indirect DOC was calculated as:

$$s_{TDC-DIC} = (s^2_{TDC} + s^2_{DIC})^{\frac{1}{2}}$$

where s^2 is the variance for the TDC and DIC determinations.

The discrepancies between some of the paired direct and indirect DOC values possibly resulted from problems with determining DIC values required for calculation of indirect DOC. TDC values for S-55, Omega-9, 150-Ton, and Oxy-6 gas condensate (Table V) were 14, 26, 31, and 40 percent lower, respectively, than values from earlier analyses. There was agreement, however, for DOC values between data sets from different days, indicating that the TDC discrepancies resulted from variability in DIC concentrations. Although the rsd's for DIC were less than 2 percent (Table V), several of the process waters exhibited TDC values which were lower than values obtained in previous analyses. The following are offered as possible origins of this problem: (i) Samples containing more DIC than 1000 mg-C/L must be diluted prior to determination of DIC. Sodium carbonate standards of 1000 mg-C/L routinely gave 95 percent recovery, whereas standards diluted from the same stock gave 100 percent recovery. It is unknown whether this was a problem with inadequate acidification/purging or with inefficient absorption of the CO_2 by the coulometer solution. The latter was not a problem, however, when the same amount of CO_2 was generated by the high-temperature or UV-persulfate units. (ii) Certain samples (e.g., Oxy-6 gas condensate, S-55, Omega-9, and 150-Ton) yielded significantly lower TDC values when diluted and stored (4 ^{o}C) for more than one week. It is not known whether storage of these diluted samples under headspace would result in uptake or loss of CO_2, but the former would seem more likely for these alkaline waters. (iii) Certain samples (e.g., Paraho) would not yield stable DIC values on particular days. This problem seemed to be related to gross interference by other gases that evolved during acidification/purging.

The number of problems that have been encountered with the DIC determinations on oil shale wastewaters is surprising, and this method requires further validation. For this reason, we recommend that DOC be determined directly. An alternative route to DIC quantitation that deserves investigation is by the use of the photochemical reactor with the UV-lamp turned off. This would preclude the need for the Coulometrics DIC unit, although it may be necessary to replace the acidic persulfate reagent with a nonoxidizing acid (e.g., dilute perchloric or sulfuric acid).

The statistical analyses of data from the comparison study indicated that no significant difference existed between the two carbon analyzers for the precision and accuracy of DOC recoveries from pure compounds, or for the quantitation of TDC and DOC in retort wastewaters. Since the UV-persulfate system gave incomplete recoveries for two of the 17 pure compounds analyzed, use of this oxidation procedure for the analysis of waters other than those reported should be preceded by a similar validation study. The routine determination of direct DOC should always be validated by indirect DOC measurements. Incomplete recovery of cyanuric acid may indicate an inability to completely oxidize other symmetrical triazines containing electron-donating substituents (e.g., melamine).

Recalcitrant compounds could probably be more effectively oxidized by using ozone/oxygen for the carrier gas through the photooxidation unit and replacing the acidic persulfate solution with an alkaline medium. This would promote formation of hydroxyl radical (Jones et al., May 1982). Two problems, however, would need to be overcome: (i) the difficulty in purging the resultant carbon

dioxide from the alkaline reaction medium and (ii) the difficulty in selectively removing residual ozone from the carrier gas stream prior to its entry to the coulometer.

Some important qualitative differences did exist in the performance and operation of the analyzers. The syringe injection method and the downtime from exhausted packing material and deteriorated combustion tubes severely hampered the routine use of the high-temperature unit. Following the DOC analyses of the pure compounds and retort wastewaters in the study reported here (approximately 320 sample injections), replacement of the combustion tube was necessary. Symptoms of the malfunctioning tube were an increased system blank and incomplete recoveries of acid phthalate standards. The calibration of the constant rate syringe was easily disturbed during use and required frequent checking. When calibrated according to the manufacturer's instructions, the actual volume delivered was never within several microliters of 200 µL; this necessitated different volume-correction terms for all the reported data.

The design of the UV-persulfate oxidation/coulometric titration carbon analyzer circumvented these problems. There was a minimum of downtime associated with the UV-persulfate reactor, and maintenance was limited to replacement of worn pump tubing and replenishment of persulfate reagent. The 200-µL sample loop required flushing with at least 10 loop-volumes of sample to eliminate the dilution effect of the persulfate reagent which had flushed the previous sample from the loop. If large sample volumes (e.g., 5 mL) are not available, a septum injection system (Dohrmann P/N 880-034), used in conjunction with a gas-tight syringe, could easily be installed for sample introduction. The loop injector has the main advantages of ease of use, increased precision, and reduction of intersample preparation time; it also can be easily automated.

A cost comparison of the UV-persulfate system and the Coulometric high-temperature total carbon analyzer shows that the UV-persulfate system ($8,526) was slightly less expensive that the Coulometrics analyzer ($9,200). The parts for the photochemical system include: UV-lamp ($212), reactor body and cap ($366), transformer ($147), injection value ($90), 4-channel peristaltic pump ($975), syringe ($28), miscellaneous electrical parts and plumbing ($408), and coulometric titrator ($6300). The photochemical system is significantly less expensive with regard to downtime, supplies, and maintenance costs. Routine annual supplies and maintenance costs include potassium persulfate ($70), Viton pump tubes ($200), and UV lamp ($212; assuming at most one per year) compared with combustion tubes ($2,000; assuming about one per month) and precombustion tubes ($250) for the high-temperature system; combustion tube and catalyst lifetimes are a function of the concentration and number of process water sample injections.

Based on recoveries of the potassium acid phthalate standards (Tables II and III), it appeared that the operation of both analyzers was best at higher carbon concentrations (>500 mg/L); this affords an advantage to either system for the analysis of oil shale process waters. We have concluded, however, that the UV-persulfate oxidation/coulometric titration carbon analyzer provided improved performance over the high-temperature combustion/coulometric detection system for analysis of oil shale wastewaters on the basis of ease of operation, downtime, and maintenance costs, while maintaining accuracy and precision of sample recovery.

REFERENCES

Armstrong, F.A.J.; Williams, P.M.; Strickland, J.D.H.
"Photo-oxidation of Organic Matter in Sea Water by Ultra-Violet Radiation, Analytical and other Applications", Nature (London) 1966, 211, 481-3.

ASTM
Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1977, (1110 pp.).

ASTM
"Standard Method of Test for Total and Organic Carbon in Water: Oxidation-Coulometric Detection", D-4129 (in press).

Baker, C.D.; Bartlett, P.D.; Farr, I.S.; Williams, G.I.
"Improved Methods for the Measurement of Dissolved and Particulate Organic Carbon in Fresh Water and Their Application to Chalk Streams", Freshwater Biol. 1974, 4, 467-81.

Beckman Instruments, Inc.
"Models 864/865 Non-Dispersive Infrared Analyzers", Beckman Instruments, Inc., Process Instuments, Bulletin 4201A: Fullerton, CA, 1980 (11 pp.).

Blom, L.; Edelhausen, L.
"Direct Titration of Carbon Dioxide", Anal. Chim. Acta 1955, 13, 120-8.

Brookes, R.
"Properties of Materials Suitable for the Cultivation and Handling of Micro-organisms", in Methods in Microbiology; Norris, J.R. and Ribbons, D.W., Eds.; Academic Press: New York, NY, 1969; pp 21-75.

Collins, K.J.; Williams, P.J. le B.
"An Automated Photochemical Method for the Determination of Dissolved Organic Carbon in Sea and Estuarine Waters", Mar. Chem. 1977, 5, 123-41.

Cooney, D.O.
"Interference of Contaminants from Membrane Filters in Ultraviolet Spectrophotometry", Anal. Chem. 1980, 52, 1068-71.

Danckwerts, P.V.; McNeil, K.M.
"The Absorption of Carbon Dioxide into Aqueous Amine Solutions and the Effects of Catalysis. Part I: Theoretical Treatment of Equilibria and Absorption Kinetics", Trans. Inst. Chem. Eng. 1967, 45, 32-49.

Danckwerts, P.V.; Sharma, M.M.
"The Absorption of Carbon Dioxide into Solutions of Alkalis and Amines (with some Notes on Hydrogen Sulphide and Carbonyl Sulphide)", Chem. Eng. (London) 1966, Rev. Ser. 2, 244-80.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.
"Analytical Problems in Monitoring Retort Water Treatment", in Energy and Environment Division 1980 Annual Report, Lawrence Berkeley Laboratory, 1981, LBL-11989.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.; Thomas, J.F.
"Analytical Methods for Quantitating Performance of Oil Shale Wastewater Treatment", in Energy and Environment Division 1981 Annual Report, Lawrence Berkeley Laboratory, 1982, LBL-13500.

Delahay, P.
Instrumental Analysis; The Macmillan Company: New York, NY, 1962; p 225.

Dohrmann-Envirotech
Equipment Manual, DC-80 Total Organic Carbon Analyzer System, 3rd ed. Dohrmann Divison, Envirotech Corp.: Santa Clara, CA, July 1981, P/N 915-121.

Ewing, G.W.
"Titrate with Electrons", Am. Lab. 1981, 13(6), 16-22.
Fox, J.P.
"The Partitioning of Major, Minor, and Trace Elements During Simulated In-Situ Oil Shale Retorting", Ph.D. Dissertation, University of California, Berkeley, 1980 (Lawrence Berkeley Laboratory Report LBL-9062, p 197-241).
Fox, J.P.; Farrier, D.S.; Poulson, R.E.
"Chemical Characterization and Analytical Considerations for an In Situ Oil Shale Process Water", Laramie Energy Technology Center, 1978, LETC/RI-78/7.
Fox, J.P.; Jackson, D.E.; Sakaji, R.H.; Daughton, C.G.; Selleck, R.E.
"Spent Shale as a Control Technology for Oil Shale Retort Water", in Energy and Environment Division Annual Report, Lawrence Berkeley Laboratory, 1980, LBL-10746.
Gershey, R.M.; Mackinnon, M.D.; Williams, P.J. le B.; Moore, R.M.
"Comparison of Three Oxidation Methods Used for the Analysis of the Dissolved Organic Carbon in Seawater", Mar. Chem. 1979, 7, 289-306.
Goulden, P.D.; Brooksbank, P.
"Automated Determinations of Dissolved Organic Carbon in Lake Water", Anal. Chem. 1975, 47, 1943-6.
House, D.A.
"Kinetics and Mechanism of Oxidations by Peroxydisulfate", Chem. Rev. 1962, 62, 185-203.
Huffman, E.W.D., Jr.
"Performance of a New Automatic Carbon Dioxide Coulometer", Microchem. J. 1977, 22, 567-3.
Jeffery, P.G.; Kipping, P.J.
Gas Analysis by Gas Chromatography; Pergamon Press: New York, NY, 1972; pp 65-6.
Jones, B.M.; Langlois, G.W.; Sakaji, R.H.; Daughton, C.G.
"Ozonation and UV Irradiation: Effect on Oil Shale Wastewater Biorefractory Organic Solutes", LBL-SEEHRL Oil Shale Waste Treatment Program, May 1982, Lawrence Berkeley Laboratory, LBID-549 (27 pp.).
Jones, B.M.; Sakaji, R.H.; Daughton, C.G.
"Physicochemical Treatment Methods for Oil Shale Wastewater: Evaluation as Aids to Biooxidation", Fifteenth Oil Shale Symposium Proceedings; Gary, J.H., Ed.; Colorado School of Mines: Golden, CO, 1982, p 581-97 (Lawrence Berkeley Laboratory Report LBL-14666).
Laxen, D.P.H.; Chandler, I.M.
"Comparison of Filtration Techniques for Size Distribution in Freshwaters", Anal. Chem. 1982, 54, 1350-5.
Littlewood, A.B.
Gas Chromatography; Academic Press: New York, NY, 1962; pp 288-94.
Menzel, D.W.; Vaccaro, R.F.
"The Measurement of Dissolved Organic and Particulate Carbon in Seawater", Limnol. Oceanog. 1964, 9, 138-42.
Naik, M.N.; Jackson, R.B.; Stokes, J.; Swaby, R.J.
"Microbial Degradation and Phytotoxicity of Picloram and other Substituted Pyridines", Soil Biol. Biochem. 1972, 4, 313-23.
Raphaelian, L.A.; Harrison, W.
"Organic Constituents in Process Water from the In-Situ Retorting of Oil from Oil-Shale Kerogen", Argonne National Laboratory Report, February 1981, ANL/PAG-5, (33 pp.).

Rheodyne Inc.
"HPLC Sample Injectors: Design Characteristics and Accuracy/Precision Capabilities", Rheodyne, Inc.: Berkeley, CA, Technical Notes, Sept. 1979, 1 (8 pp.).

Rohlf, J.F.; Sokal, R.R.
Statistical Tables; W.H. Freeman and Co.: San Francisco, CA, 1969; 253 pp.

Sokal, R.R.; Rohlf, J.F.
Biometry; W.H. Freeman and Co.: San Francisco, CA, 1969; 776 pp.

"Standard Methods for the Examination of Water and Wastewater", 15th ed.; American Public Health Association; American Water Works Association; Water Pollution Control Federation: Washington, D.C., 1981, pp 471-5.

Takahashi, Y.
"A Review of Analysis Techniques for Organic Carbon and Organic Halide in Drinking Water", Proceedings of the Conference on Practical Application of Adsorption Techniques in Drinking Water, 1979 (preprint).

Takahashi, Y.; Martin, J.T.; Harper, F.C.
Advances in Total Organic Carbon Analysis for High Chloride Water Samples, Dohrmann Division, Xertex Corp.: Santa Clara, CA, Oct. 1981.

Torpy, M.F.; Raphaelian, L.A.; Luthy, R.G.
"Wastewater and Sludge Control-Technology Options for Synfuels Industries, Vol. 2: Tar-Sand-Combustion Process Water -- Removal of Organic Constituents by Activated-Sludge Treatment", Argonne National Laboratory Report, November 1981, ANL/ES-115, Vol. 2 (53 pp.).

Van Hall, C.E.; Barth, D.; Stenger, V.A.
"Elimination of Carbonates from Aqueous Solutions prior to Organic Carbon Determination", Anal. Chem. 1965, 37, 769-71.

Van Hall, C.E.; Safranko, J.; Stenger, V.A.
"Rapid Combustion Method for the Determination of Organic Substances in Aqueous Solutions", Anal. Chem. 1963, 35, 315-9.

Willard, H.H.; Merritt, L.L., Jr.; Dean, J.A.
Instrumental Methods of Analysis, 5th ed.; D. Van Nostrand Company: New York, NY, 1974; pp 528-9, 702.

Williams, P.M.
"The Determination of Dissolved Organic Carbon in Seawater: A Comparison of Two Methods", Limnol. Oceanog. 1969, 14, 297-8.

Wölfel, P.; Sontheimer, H.
"Ein neues Verfahren zur Bestimmung von organisch gebundenem Kohlenstoff im Wasser durch photochemische Oxidation" Vom Wasser 1974, 43, 315-25 (Ger.).

Zierdt, C.H.
"Adherence of Bacteria, Yeast, Blood Cells, and Latex Spheres to Large-Porosity Membrane Filters" Appl. Environ. Microbiol. 1979, 38, 1166-72.

Table I. Origins of Oil Shale Process Wastewaters Used in the Comparison Study

process water	water type	retort/process	shale source	retorting atmosphere	max. retorting temp. (°C)	operator/ collection date
		Field in-situ Retorts				
Oxy-6 RW	RW[1]	retort #6/MIS[2]	Logan Wash, CO	air/steam	unknown[3]	Occidental Oil Shale Inc., 1979
Oxy-6 GC	GC[4]	retort #6/MIS	Logan Wash, CO	air/steam	unknown	Occidental Oil Shale Inc., 1979
Geokinetics	RW	retort #9/hor. TIS[5]	Book Cliff, UT	air	unknown	Geokinetics, Inc., 1978
Omega-9	RW	site #9/hor. TIS	Rock Springs, WY	air	unknown	LETC, 1976
Rio Blanco Sour	RW	retort #0/MIS	Tract C-a, CO	air/steam	unknown	Rio Blanco Oil Shale Co., 1980
		Field Surface Retorts				
Paraho	RW	Paraho direct mode	Anvil Points, CO	air/ recycle gas	750	Development Engineering, Inc., 1977-1978
TV	GC & RW	near-term commercial	confidential	unknown	unknown	confidential
		Simulated in-situ Retorts				
150-Ton	GC & RW	LETC 150-ton, run 13	Anvil Points, CO	air	800	LETC, 1976
S-55	GC & RW	LETC 10-ton, run 55	Anvil Points, CO	air/steam	650	LETC, 1978

[1] retort water. [2] modified in situ. [3] retorting temperatures for MIS field retorts are not accurately known; localized temperatures may reach 1000 °C. [4] gas condensate. [5] horizontal true in situ.

Table II. Recovery Study: High-Temperature Combustion Carbon Analyzer

Compound	DOC Concentration (mg-C/L) theoretical (a)	observed[1] (b)	(b/a) X 100	rsd[1]
potassium acid phthalate	100.0	104.3	104.3	1.39
potassium acid phthalate	500.0	506.5	101.3	0.54
potassium acid phthalate	1000.0	1002.6	100.3	0.50
phenol	606.8	606.0	99.9	0.61
acetonitrile	479.0	465.6	97.2	2.79
3,5-dimethylpyrazole	501.5	502.3	100.2	0.45
pyridine	476.3	454.1	95.3	0.64
2-methylpyridine	435.2	434.1	99.7	0.81
4-methylpyridine	455.3	453.5	99.6	0.69
2,4-dimethylpyridine	437.5	435.7	99.6	0.66
2,6-dimethylpyridine	431.6	430.4	99.7	1.09
2,4,6-trimethylpyridine	420.7	424.3	100.8	0.73
2,3,6-trimethylpyridine	416.7	430.4	103.3	0.58
2-ethylpyridine	443.4	441.7	99.6	0.92
3-ethylpyridine	447.8	449.3	100.3	0.69
3-ethyl-4-methylpyridine	472.1	503.8	106.7	0.21
2-n-propylpyridine	435.4	441.1	101.3	0.55
2-methylpyrazine	502.5	503.6	100.2	1.77
cyanuric acid	252.2	253.5	100.5	1.05

[1] n=10 for each standard solution

Table III. Recovery Study: UV-Persulfate Carbon Analyzer

Compound	DOC Concentration (mg-C/L) theoretical (a)	observed[1] (b)	(b/a) X 100	rsd[1]
potassium acid phthalate	100.0	102.8	102.8	3.72
potassium acid phthalate	500.0	503.3	100.7	0.70
potassium acid phthalate	1000.0	999.4	99.9	0.44
phenol	606.8	602.9	99.4	0.90
acetonitrile	479.0	426.3	89.1	3.44
3,5-dimethylpyrazole	501.5	502.5	100.2	0.70
pyridine	476.3	454.5	95.4	0.65
2-methylpyridine	435.2	438.9	100.8	1.01
4-methylpyridine	455.3	447.5	98.3	1.29
2,4-dimethylpyridine	437.5	434.2	99.3	1.16
2,6-dimethylpyridine	431.6	429.9	99.6	0.88
2,4,6-trimethylpyridine	420.7	421.7	100.2	0.66
2,3,6-trimethylpyridine	416.7	430.6	103.3	0.80
2-ethylpyridine	443.4	436.3	98.4	1.66
3-ethylpyridine	447.8	453.1	101.2	1.38
3-ethyl-4-methylpyridine	472.1	495.7	104.9	0.71
2-n-propylpyridine	435.3	435.2	100.0	0.87
2-methylpyrazine	502.5	500.0	99.5	0.68
cyanuric acid	252.2	5.4	2.2	123

[1] n=10 for each standard solution

Table IV. Precision of the Purge Operation for Determining Indirect Organic Carbon in a Composite Sample of Oil Shale Process Waters

Purge Station	DOC[1] (mg/L)	rsd
1	6244	0.70
	6291	0.50
2	6369	0.69
	6361	0.54
3	6364	0.51
	6328	0.35
4	6369	0.32
	6394	0.19
5	6310	0.45
	6385	0.16

[1] each duplicate is the mean of 5 single-operator replicates

Table V. Comparison of Analyzers: Direct/Indirect Organic Carbon (mg/L)[1] in Oil Shale Process Waters

Wastewater	DOC (direct)	rsd	DOC (indirect) (TDC-DIC)	rsd	TDC	rsd	DIC	rsd
Paraho							209.8	1.3
a (high temperature)	41809	1.4	43205	0.55	43415	0.55		
b (UV-persulfate)	42066	1.1	42470	0.66	42680	0.66		
150-Ton							1932	1.8
a	3147	0.58	2925	1.4	4857	0.44		
b	3259	0.46	3128	1.4	5060	0.53		
Oxy-6 retort water							984.9	1.0
a	2829	0.80	2832	1.6	3817	1.2		
b	2942	0.40	2967	0.79	3952	0.54		
Geokinetics							1994	0.67
a	1627	1.1	1680	3.0	3674	1.3		
b	1656	0.55	1688	1.3	3682	0.45		
TV							824.8	0.54
a	2651	0.23	2545	0.81	3370	0.59		
b	2726	0.61	2661	1.3	3486	0.97		
Oxy-6 gas condensate							2213	0.38
a	651.7	2.6	522.0	3.7	2735	0.63		
b	641.0	0.51	653.0	2.4	2866	0.47		
S-55							339.5	1.6
a	2213	0.40	2256	2.1	2595	1.8		
b	2285	0.34	2294	0.63	2633	0.51		
Omega-9							1387	1.3
a	694.7	0.34	732.0	6.5	2119	2.1		
b	718.4	0.44	787.0	2.5	2174	0.29		
Rio Blanco sour							364.3	1.4
a	206.3	1.4	191.3	2.7	555.6	0.25		
b	207.0	1.3	183.8	3.0	548.1	0.47		

[1] mean of 10 single-operator replicates.

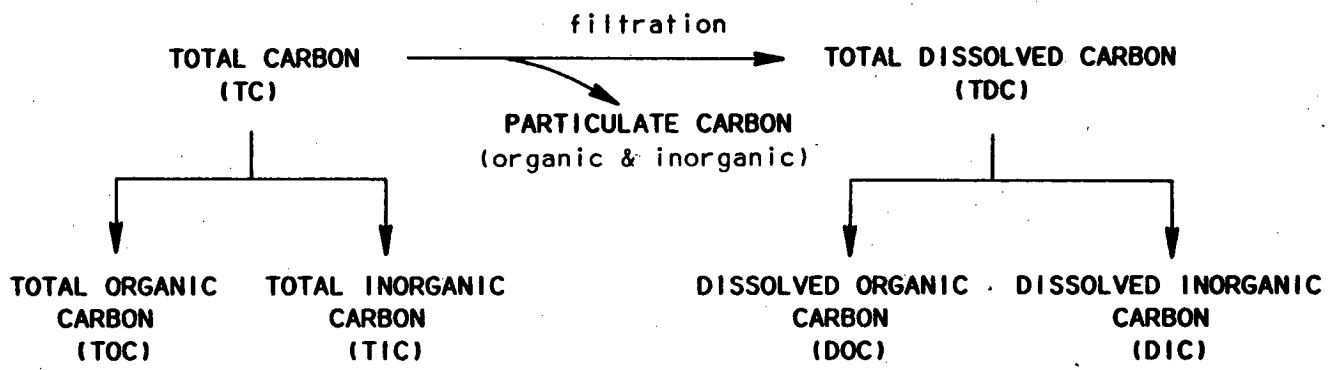


Figure 1. Terminology for carbon classifications used in carbon analysis.

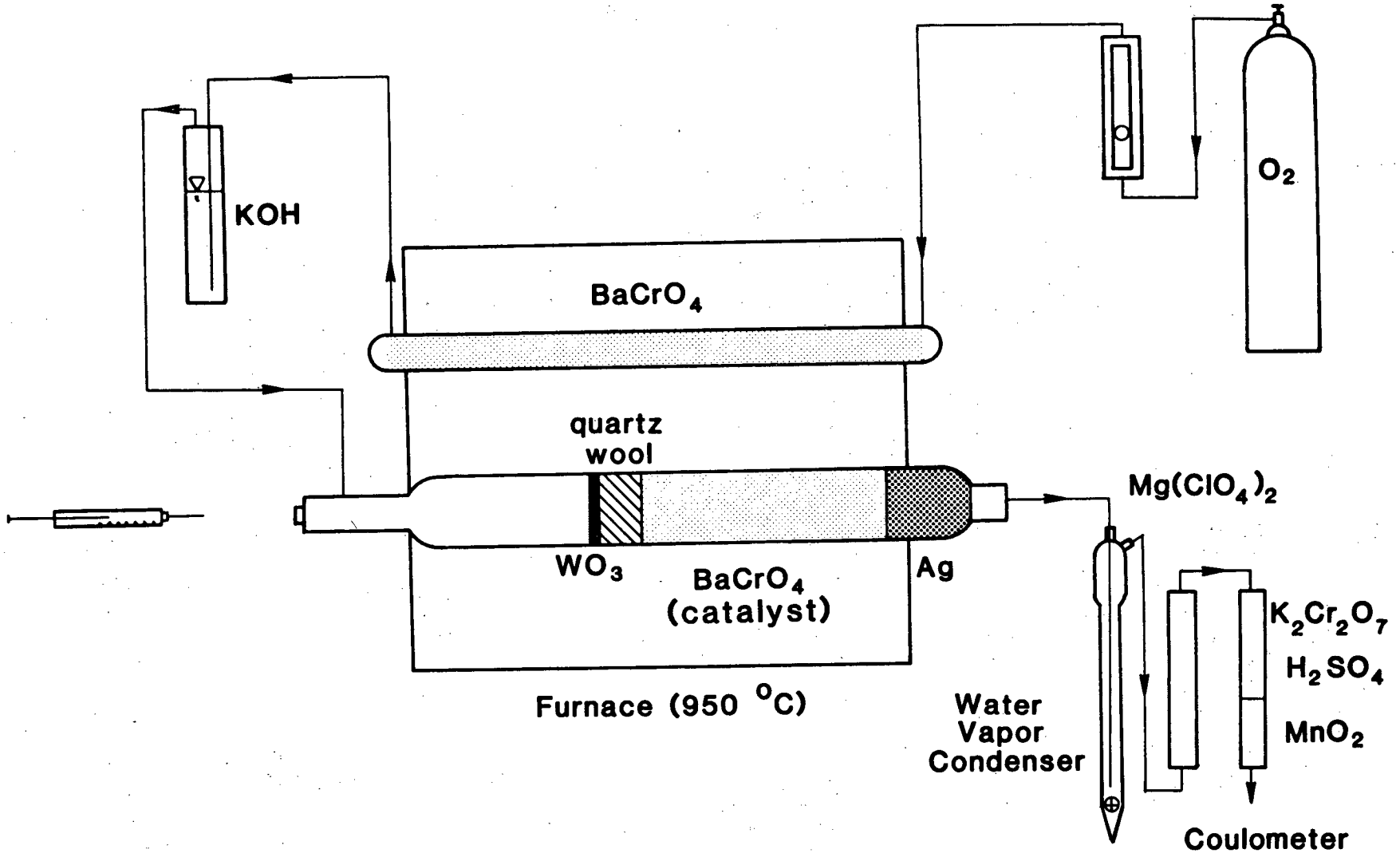


Figure 2. Schematic of Coulometrics high-temperature combustion apparatus.

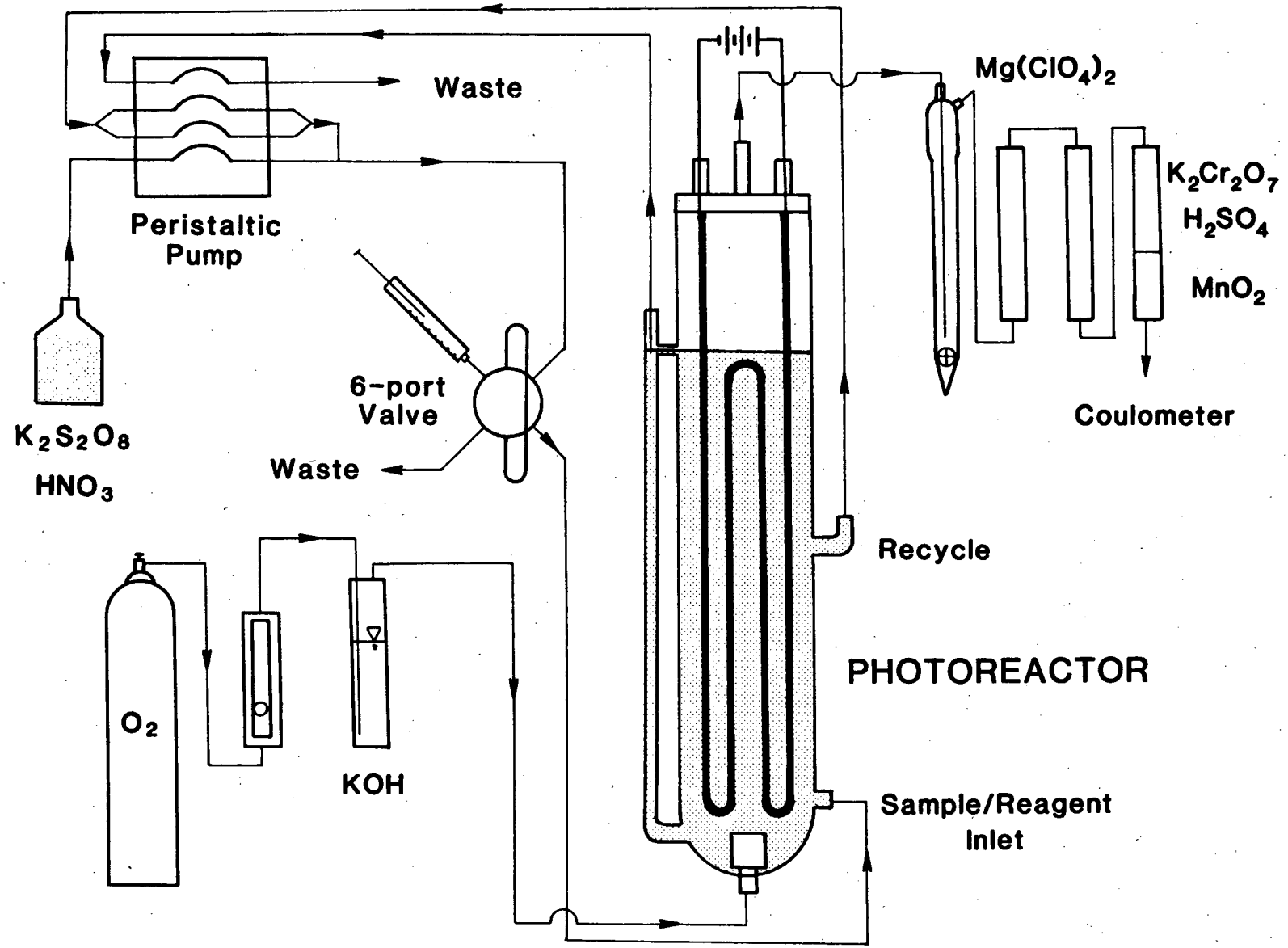


Figure 3. Schematic of UV-peroxydisulfate low-temperature oxidation apparatus.

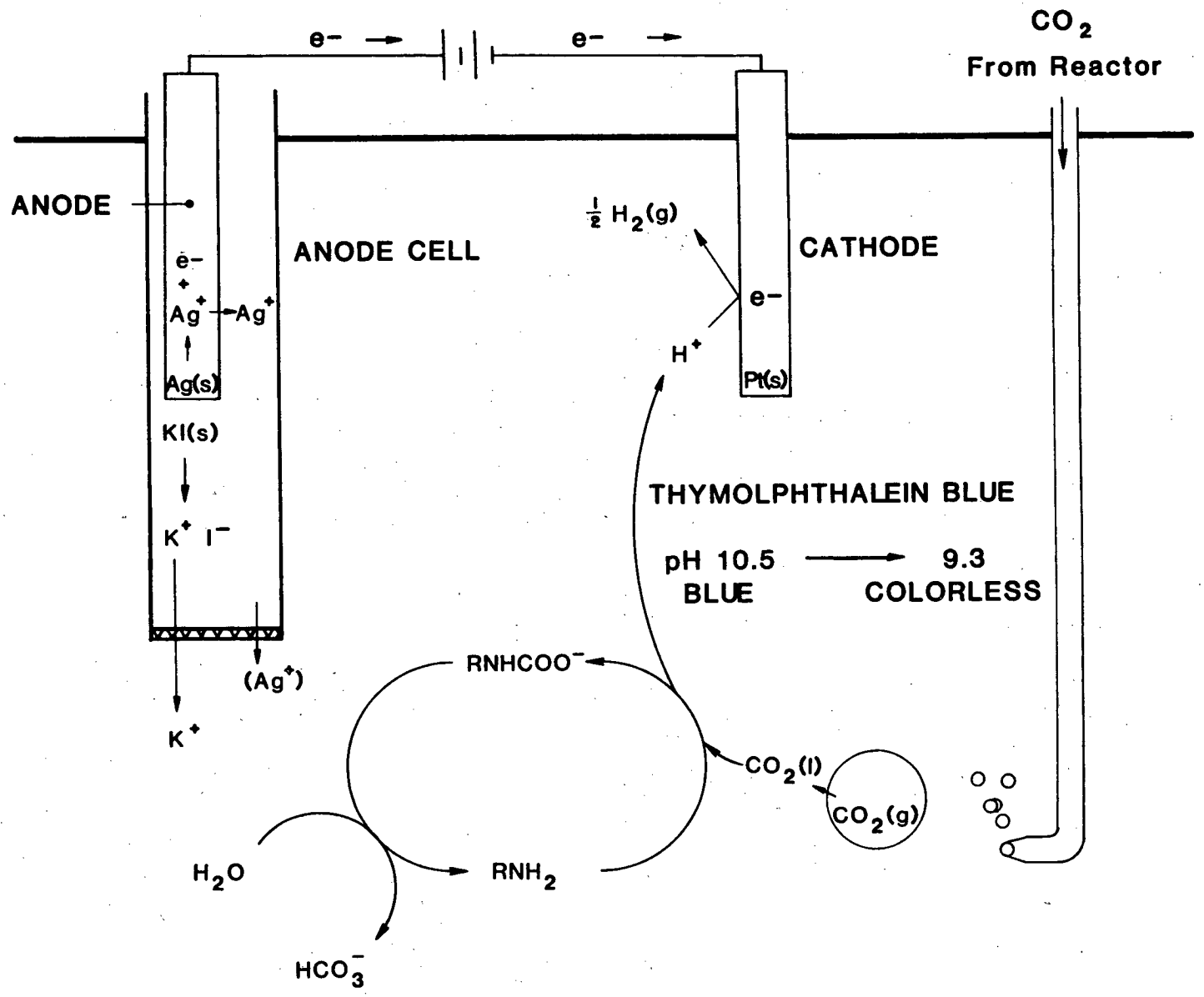


Figure 4. Proposed scheme for coulometric titration of carbon dioxide.

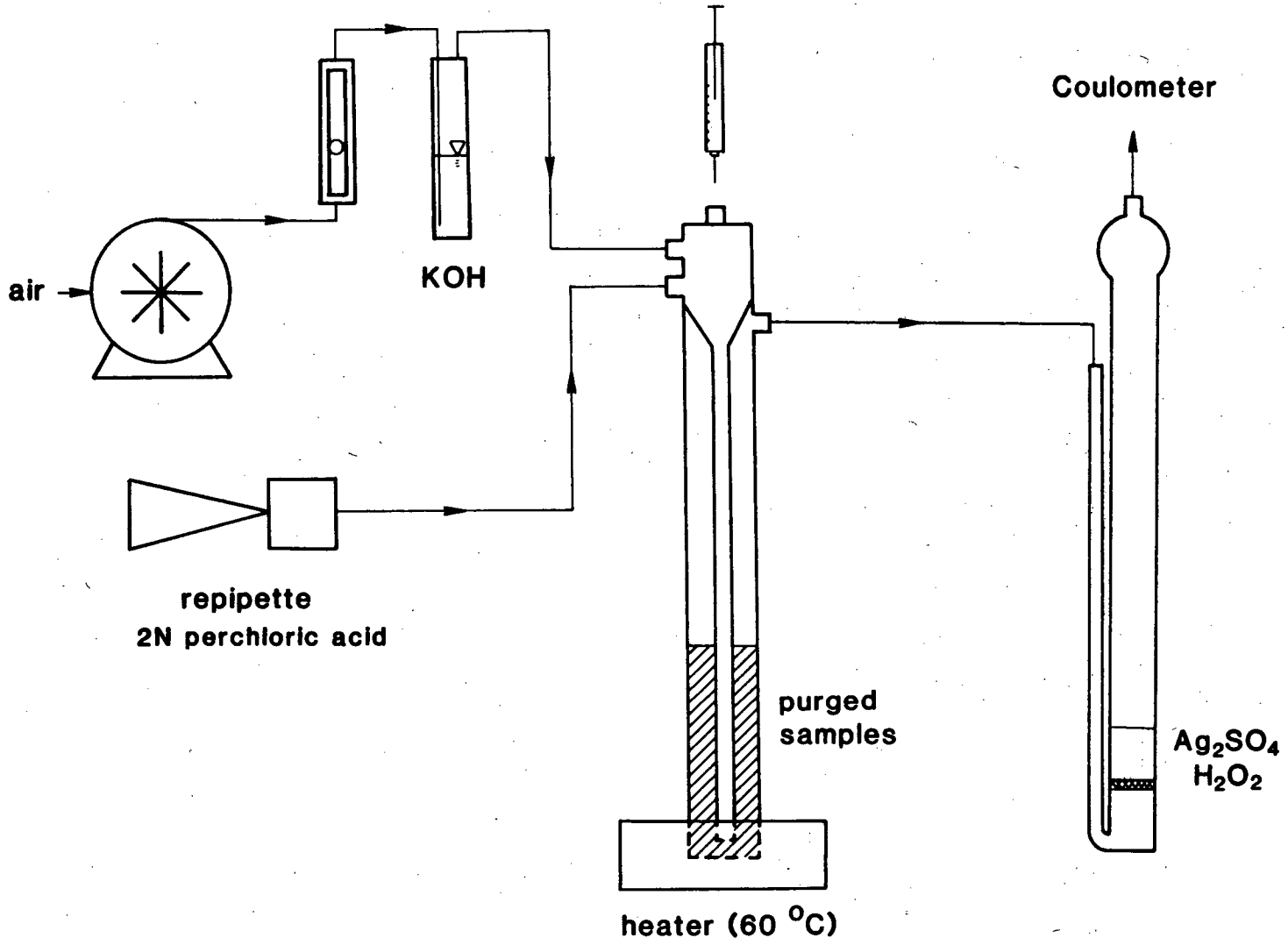


Figure 5. Schematic of Coulometrics acidification/purge apparatus for inorganic carbon.

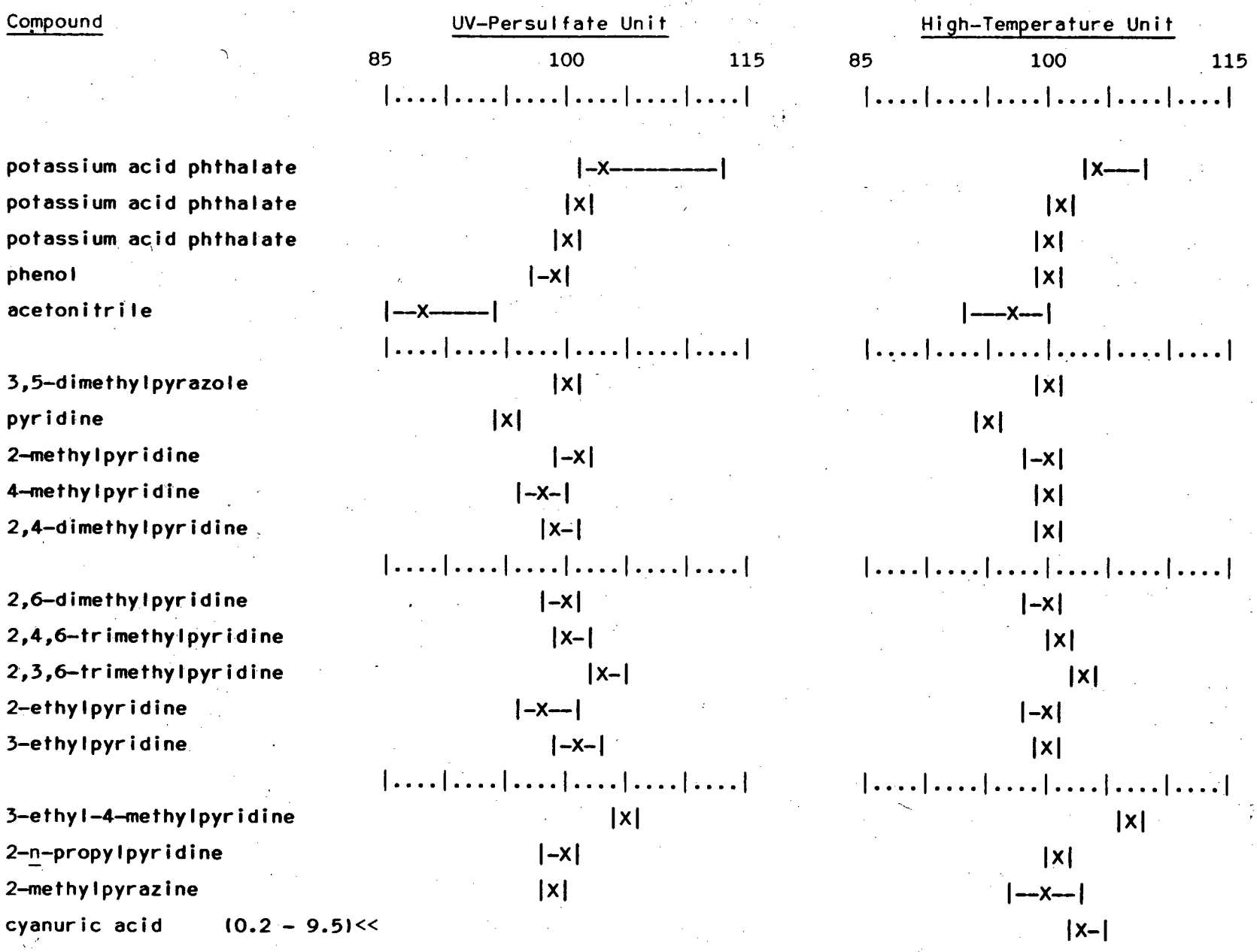


Figure 6. Comparison of percent recovery means (X) and ranges (---) for standard solutions; concentrations of compounds are identical to those presented in Tables II & III.

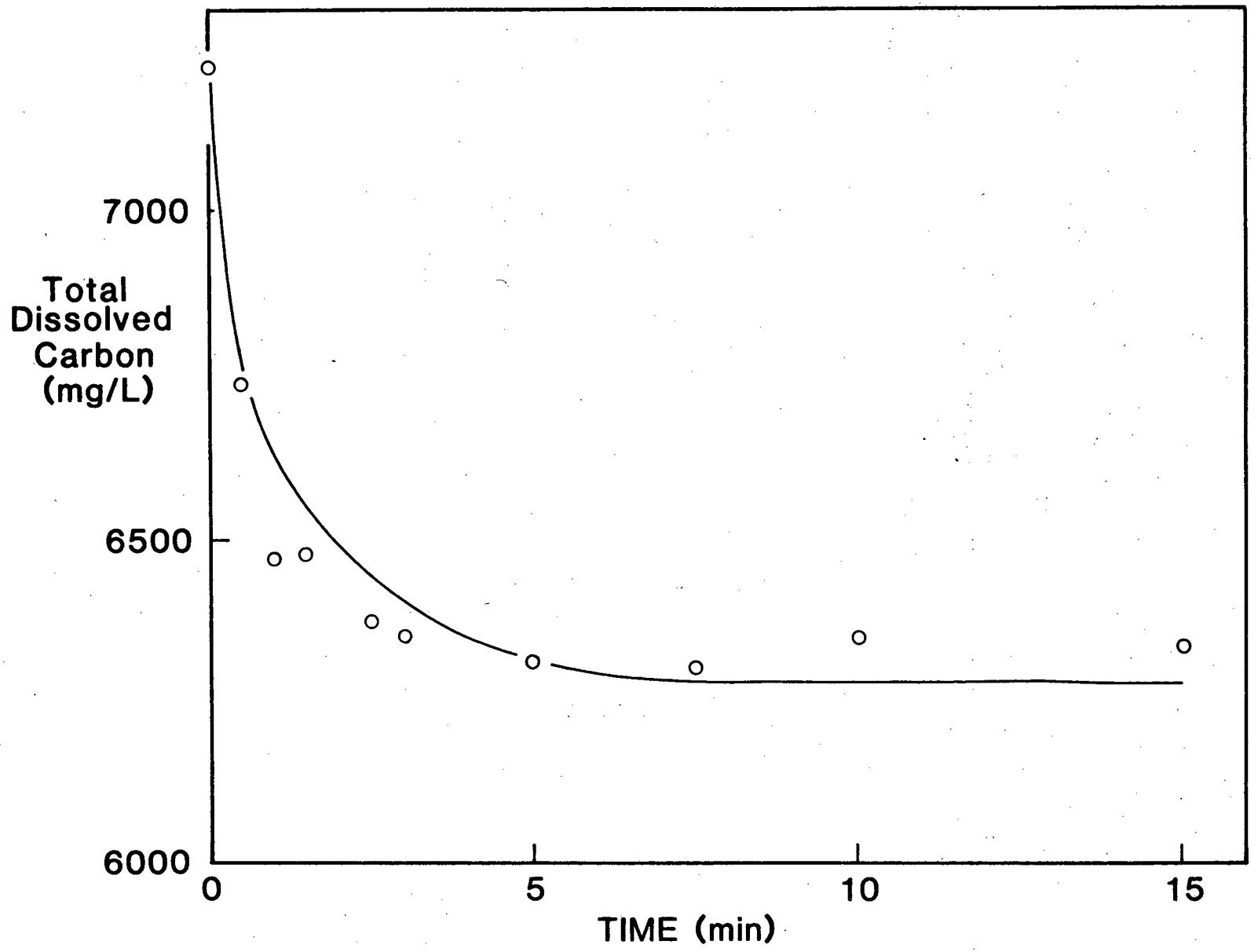


Figure 7. Time-course purge study for direct organic carbon determination.

PROTOCOL: DISSOLVED ORGANIC CARBON
(High-Temperature Combustion/Coulometric Titration)

A. START-UP

1. Turn the combustion furnace temperature control knob to 950 ^{o}C. Allow 0.5 to 1 hour for the furnace to reach temperature.
2. Increase the oxygen flow rate to 100 cm^{3}/min.
   a. ensure that the oxygen delivery pressure is 15 psig.
   b. ensure that at least 500 psig of oxygen is in the cylinder.
   c. if foaming occurs in the KOH scrubber, add a small amount of ASTM Type I water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.
3. Repack the magnesium perchlorate scrubber. This scrubber is positioned directly after the burette water-trap.
   NOTE: Using the old, wetted packing could result in formation of a plug, increasing the back-pressure.
   a. wash out the old packing, rinse the scrubber tube with ASTM Type I water, then air- or oven-dry the tube.
   b. repack and reconnect the tube.
4. Check the acid dichromate/manganese dioxide scrubber for exhaustion. This scrubber is positioned after the magnesium perchlorate scrubber.
   a. the acid dichromate packing will change from yellow-orange (oxidized) to green-orange (reduced) as it becomes exhausted. This color change will be seen as a front progressing in the direction of gas flow. When almost all of the acid dichromate has changed color, the entire scrubber must be repacked.
   b. when the manganese dioxide packing is exhausted, it will change from black to dark brown; the entire scrubber must then be repacked.
5. Assemble the coulometer cell.
   a. fill the coulometer cell with 75 mL of coulometer solution.
   b. add the stir bar.
   c. position the rubber stopper on the coulometer cell such that the anode, cathode, and gas line face the back wall of the cell (that portion of the beaker containing the volume graduations).
   d. add 3 pellets of potassium iodide to the anode compartment.
   e. add anode solution to the anode compartment. The anode solution level should be slightly higher than that of the coulometer cell solution.
   f. place the silver anode in the anode compartment. Ensure that the tip of the silver anode is wetted by the anode solution.
   g. place the assembled coulometer cell in the cell holder of the instrument; the volume graduations should face the rear of the instrument.
   h. plug the anode (red) and cathode (black) wires into the coulometer.

   DO NOT TURN ON THE ELECTROLYSIS CURRENT.

   i. connect the coulometer cell gas line to the one-way valve in-line from the nitrogen oxide scrubber. Check that the stopcock on the burette water trap is open. (The gas flow must be diverted from the coulometer cell during adjustment of cell transmittance per step A.8).
6. Connect the HP 97S to the coulometer interface cable.

a. turn on the HP 97S.
b. with the calculator in the RUN mode, load the "background" program as per the user instructions (Appendix A).

7. Turn on the main power supply. Allow a warm-up period of several minutes.
8. Adjust the coulometer cell transmittance.
   a. rotate the coulometer cell until a maximum transmittance is obtained.
   b. adjust the transmittance to 100% using the "100% adjust" knob.
   c. close the stopcock on the burette water trap.
   d. check that the gas flow into the coulometer cell does not deflect the 100% transmittance setting. If a deflection occurs, reposition the gas line to eliminate this interference; open the burette stopcock and repeat steps a-d.

   NOTE: The gas line must be submerged in the coulometer solution.
9. Turn on the electrolysis current and initiate the background program.
   a. allow several minutes for the titration of endogenous CO_2 in the coulometer solution.
   b. following this initial titration, check the coulometer stability. A stable background count of 1.0 to 1.2 mg/L per minute should be obtained when the range plug is set to display mg/L. Stabilization may take as long as 30 minutes.

B. SAMPLE PREPARATION -- TOTAL DISSOLVED CARBON (TDC)
1. Filter all samples through a 0.4-µm pore diameter polycarbonate membrane filter.
2. Dilute sample filtrates with ASTM Type I water to yield TDC concentrations between 40 and 200 mg/L.
3. Prepare a TDC sample blank; a 10-mL aliquot of ASTM Type I water should be processed with the TDC samples.
4. Refrigerate samples until analysis time.

C. SAMPLE PREPARATION -- DISSOLVED ORGANIC CARBON (DOC)
1. Filter all samples through a 0.4-µm pore diameter polycarbonate membrane filter.
2. Dilute sample filtrates with ASTM Type I water to yield DOC concentrations between 40 and 200 mg/L.
3. Prepare a DOC sample blank; a 10-mL aliquot of ASTM Type I water should be processed with the DOC samples.
4. Acidify the blank and DOC samples with 0.10 mL concentrated sulfuric acid (Analytical Reagent grade) per 10 mL of sample. A positive displacement pipette or a repipette should be used.
   NOTE: Samples should have pH values of 2 after acidification.
5. Refrigerate samples until analysis time.

D. PURGING OF DOC SAMPLES
1. Open the helium cylinder valve and set the delivery pressure to 10 psig. The needle valve on the helium flow meter is preset to deliver 775 cm^3/min at 10 psig.
2. Rinse the glass capillaries by submersion in the vials of concentrated HCl, and wipe dry.
3. Submerge each capillary in a sample and purge for 10 minutes.
4. Remove the capillaries, wipe dry, and repeat steps 2-3 for all samples.

5. After all the samples have been purged, repeat step 2 and place the capillaries in a clean, dry vial.
6. Turn off the helium cylinder valve.
   NOTE: Purging should be conducted in an enclosed compartment to prevent deposition of acidic aerosols on equipment.

E. PREPARATION OF STANDARDS
   (NOTE: Use Analytical Reagent grade chemicals only).
1. Prepare a stock solution of potassium acid phthalate (DOC = 1000.00 mg/L as C).
   a. weigh 1063.7 mg of dried potassium acid phthalate and quantitatively transfer to a 500-mL volumetric flask.
   NOTE: Glassware should be acid-washed.
   b. bring to volume with ASTM Type I water.
   c. acidify the standards as instructed in section C.4.
2. Prepare working standards of 50, 100, and 200 mg/L.
   NOTE: additional standard concentrations should be made if the sample DOC concentration is expected to be outside of this range.
   a. 50 mg/L : pipette 0.5 mL of stock solution into a 10-mL volumetric flask and bring to volume with ASTM Type I water.
   b. 100 mg/L : pipette 1.0 mL of stock solution into a 10-mL volumetric flask and bring to volume with ASTM Type I water.
   c. 200 mg/L : pipette 2.0 mL of stock solution into a 10-mL volumetric flask and bring to volume with ASTM Type I water.
   NOTE: Use air- or positive-displacement pipettes.
3. Prepare a stock solution of phenol for recovery determinations (DOC = 2371.7 mg/L as C).
   a. weigh 155.0 mg of phenol and quantitatively transfer to a 50-mL volumetric flask.
   b. bring to volume with ASTM Type I water.
   c. dilute this stock solution 1:10 with ASTM Type I water and acidify as instructed in section C.4 (DOC = 237.17 mg/L as C).
4. Prepare a stock solution of pyridine for recovery determinations (DOC = 1000 mg/L as C).
   a. dispense approximately 65 µL of pyridine into a tared 50-mL volumetric flask and record the exact weight.
   b. bring to volume with ASTM Type I water.
   c. acidify as instructed in section C.4.
   d. calculate the theoretical DOC of this solution:
      DOC (mg/L) = (15.18) x (mg pyridine added/0.050 L).
   e. dilute this stock solution 1:5 with ASTM Type I water (DOC = 200 mg/L as C).

F. SAMPLE ANALYSIS
1. Load the "Water Analysis" program into the HP 97S.
   a. turn off the coulometer main power.
   b. with the HP 97S in the RUN mode, run the program card through the HP 97S card reader.
   c. initiate the program as per the user instructions (Appendix B).
   d. turn on the coulometer main power.
2. If the Hamilton constant rate carbon analyzer syringe is used, check that it is set and locked at 200 µL. Recheck frequently during sample analysis.

NOTE: a sample injection volume of 200 µL is recommended; excessive injection volumes can result in damage to the quartz tube and are unsafe due to the combustive expansion of gases.

3. Rinse the syringe 10 times with the sample to be analyzed.
   a. insert the syringe needle into the sample injection port; ensure that the Luer fittings are seated.
   b. inject the sample and simultaneously initiate the sample program as per the user instructions (Appendix B).
   c. the syringe must remain in the injection port throughout the analysis; proper flushing by the oxygen will not occur if the port is not sealed.
   d. the suggested analysis time for each sample is 3 minutes.
   e. for replicates, rinse the syringe twice with the sample to be analyzed and repeat steps a-e.
   f. check the burette water trap. This trap should be emptied between analyses so that the gas line does not become submerged in the condensate. Collect the condensate in an acid-washed vial for later validation (i.e., by DOC) of complete combustion.
4. Repeat step 3 for each sample.
5. Samples should be analyzed in the following order:
   a. the sample blank; the mean DOC value from the blanks must be subtracted from the DOC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
   b. the acid phthalate standards.

   NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following in decreasing order of priority: accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; condition of combustion tube; coulometer performance.

   c. the recovery standards (phenol and pyridine).
   d. the DOC samples.

   NOTE: if a large number of DOC samples is to be analyzed, the series of standards should be analyzed at intervals throughout the DOC analyses.

   e. upon completion of the DOC analyses, the series of standards and blanks should be reanalyzed.
   f. the collected condensate sample should also be analyzed for DOC concentration. A DOC value greater than the background count indicates carry-over of uncombusted carbon.

G. SHUT-DOWN
1. Turn off the HP 97S.
2. Remove the syringe and replace the end-plug over the sample injection port.
3. Reduce the furnace temperature to approximately 750 ^{o}C.
4. Reduce the oxygen flow rate to 40 cm^3/min.
5. Open the stopcock on the burette water trap.
6. Disassemble the coulometer cell.
   a. turn off the electrolysis current.
   b. turn off the main power.
   c. unplug the anode and cathode wires from the coulometer.

d. disconnect the gas line at the one-way valve in-line between the coulometer cell and the nitrogen oxide scrubber.
e. remove the coulometer cell from the coulometer.
f. remove the silver anode, rinse with ASTM Type I water, and air-dry on a clean surface.
g. remove the rubber stopper from the coulometer cell and rinse the anode cell with acetone -- ensure that no potassium iodide deposits remain in the anode cell. Using a vacuum source and the perforated serum stopper, draw a small volume of acetone through the fritted-glass end of the anode cell.
h. rinse the exteriors of the anode, cathode, and gas line with ASTM Type I water and air-dry on a clean surface.
i. rinse the coulometer cell and stir bar several times with ASTM Type I water and air-dry on a clean surface.

H. DATA REDUCTION
1. Calculate the mean value for each set of DOC replicates:
a. $\bar{x}(DOC) = \Sigma(x_i - b)/n$
where x_i = each data point in a set of replicates;
b = the mean value of all DOC blank analyses
n = the number of replicates per sample.
2. The HP 97S "Water Analysis" program automatically calculates $(x_i - b)$ X (dilution factor) for each data point. Therefore the mean for a set of replicates equals the sum of data outputs divided by the number of replicates (n).
3. Determine whether suspected outliers should be discarded.
a. suspected outliers should be subjected to statistical analysis before being discarded (1).
b. if an outlying value is known to be the result of a mechanical or operator error, it may be rejected without statistical verification.

I. MAINTENANCE
1. Record the appropriate information in the C-Analyzer Log Book, including:
a. date and duration of usage.
b. number of injections (retort water and total) and sample dilutions.
c. symptoms of malfunctioning.
d. repairs.
e. initial all entries.

J. REFERENCES
1. Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1977; 1110 pp.

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton.

PROTOCOL: DISSOLVED ORGANIC CARBON
(UV-Enhanced Persulfate Oxidation/Coulometric Titration)

A. START-UP

NOTE: All chemicals are Analytical Reagent grade unless otherwise specified. The dipotassium salt of peroxydisulfuric acid is referred to as "persulfate".

1. Prepare the persulfate solution.
   a. weigh 20 g of persulfate and quantitatively transfer to 300 mL of ASTM Type I water in a 1000-mL volumetric flask.
   b. add 1.0 mL of concentrated nitric acid and bring to volume with ASTM Type I water.
2. Increase the oxygen flow rate to 190 cm^3/min.
   a. ensure that the oxygen delivery pressure is 15 psig.
   b. ensure that at least 500 psig of oxygen is in the cylinder.
   c. if foaming occurs in the KOH scrubber, add a small amount of ASTM Type I water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.
3. Repack the magnesium perchlorate scrubbers. These scrubbers are positioned directly after the burette water-trap.
   NOTE: Using the old, wetted packing could result in formation of a plug, increasing the back-pressure.
   a. wash out the old packing, rinse the scrubber tube with ASTM Type I water, then air- or oven-dry the tube.
   b. repack and reconnect the tube.
4. Check the acid dichromate/manganese dioxide scrubber for exhaustion. This scrubber is positioned after the magnesium perchlorate scrubber.
   a. the acid dichromate packing will change from yellow-orange (oxidized) to green-orange (reduced) as it becomes exhausted. This color change will be seen as a front progressing in the direction of gas flow. When almost all of the acid dichromate has changed color, the entire scrubber must be repacked.
   b. when the manganese dioxide packing is exhausted, it will change from black to dark brown; the entire scrubber must then be repacked.
5. Position the recirculation (0.063" id) and reagent delivery (0.031" id) Viton pump tubes and the silicone waste-line pump tube (1.0-mm id) in the peristaltic pump (Sage Instruments, model 375A) and close the platten lid.
   a. the Viton pump tubes require the 11-lb pressure plates (grey); the silicone pump tube requires the 2.12-lb pressure plate (tan).
6. Fill the UV reactor with the persulfate solution, connect the persulfate resevoir in line using the Omnifit Teflon fittings, and connect the waste line to an appropriate receptacle.
7. Turn on the pump.
   a. the pump setting should be preset to deliver approximately 0.6 mL/min of fresh persulfate solution to the reactor. The contents of the reactor should recycle at a rate of 3.0 mL/min through the valve. Combined flow of fresh and recycled reagent will be 3.6 mL/min. The contents of the reactor are pumped to waste at a rate of at least 0.6 mL/min.

NOTE: Check for leaks at all tubing connections during initial pumping; a misaligned sample injection valve rotor will increase back-pressure and cause leaking.

8. Assemble the coulometer cell.
   a. fill the coulometer cell with 75 mL of coulometer solution.
   b. add the stir bar.
   c. position the rubber stopper on the coulometer cell such that the anode, cathode, and gas line face the back wall of the cell (that portion of the beaker containing the volume graduations).
   d. add 3 pellets of potassium iodide to the anode compartment.
   e. add anode solution to the anode compartment. The anode solution level should be slightly higher than that of the coulometer cell solution.
   f. place the silver anode in the anode compartment. Ensure that the tip of the silver anode is wetted by the anode solution.
   g. place the assembled coulometer cell in the cell holder of the instrument; the volume graduations should face the rear of the instrument.
   h. plug the anode (red) and cathode (black) wires into the coulometer.

   DO NOT TURN ON THE ELECTROLYSIS CURRENT.

   i. connect the coulometer cell gas line to the one-way valve in-line from the nitrogen oxide scrubber. Check that the stopcock on the burette water trap is open (the gas flow must be diverted from the coulometer cell during adjustment of cell transmittance per step A.11).
9. Connect the HP 97S to the coulometer interface cable.
   a. turn on the HP 97S.
   b. with the calculator in the RUN mode, load the "background" program as per the user instructions (Appendix A).
10. Turn on the main power supply. Allow a warm-up period of several minutes.
11. Adjust the coulometer cell transmittance.
   a. rotate the coulometer cell until a maximum transmittance is obtained.
   b. adjust the transmittance to 100% using the "100% adjust" knob.
   c. close the stopcock on the burette water trap.
   d. check that the gas flow into the coulometer cell does not deflect the 100% transmittance setting. If a deflection occurs, reposition the gas line to eliminate this interference. Open the burette stopcock and repeat steps a-d.

   NOTE: The gas line must be submerged in the coulometer solution.
12. Turn on the electrolysis current.
13. Start the UV-lamp and initiate the background program.

   CAUTION: Although the ultraviolet radiation emitted by the lamp is greatly attenuated by the Pyrex glass of the reactor and by safety glasses, the intensity of the transmitted light can cause eye strain. The glare can be minimized by enclosing the reactor in 6-inch O.D. Plexiglass tubing which has been coated with reflective plastic film (e.g., solar control film). This allows for visual inspection of the reactor during operation.

   a. when the coulometer has stabilized, the background (counts per minute) should be approximately 2.0 to 3.2 mg/L. Stabilization should be complete within 0.5 to 2.0 hours.
   b. if high background counts persist (greater than 4 mg/L per minute) for more than two hours, replace the Viton pump tubing and repeat step 13.a.

NOTE: Should the high background persist after installing new Viton tubing, check for the following in decreasing order of priority: exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; contamination of persulfate solution; coulometer performance.

B. SAMPLE PREPARATION -- TOTAL DISSOLVED CARBON (TDC)
1. Filter all samples through a 0.4-µm pore diameter polycarbonate membrane filter.
2. Dilute sample filtrates with ASTM Type I water to yield TDC concentrations between 200 and 1000 mg/L.
3. Prepare a TDC sample blank; a 10-mL aliquot of ASTM Type I water should be processed with the TDC samples.
4. Refrigerate samples until analysis time.

C. SAMPLE PREPARATION -- DISSOLVED ORGANIC CARBON (DOC)
1. Filter all samples through a 0.4-µm pore size polycarbonate membrane filter.
2. Dilute sample filtrates with ASTM Type I water to yield DOC concentrations between 200 and 2000 mg/L.
3. Prepare a DOC sample blank; a 10-mL aliquot of ASTM Type I water should be processed with the DOC samples.
4. Acidify the blank and DOC samples with 0.10 mL concentrated sulfuric acid per 10 mL of sample. A positive displacement pipette or a repipette should be used.
NOTE: Samples should have pH values of 2 after acidification.
5. Refrigerate samples until analysis time.

D. PURGING OF DOC SAMPLES
1. Open the helium cylinder valve and set the delivery pressure to 10 psig. The needle valve on the helium flow meter is preset to deliver 775 cm^3/min at 10 psig.
2. Rinse the glass capillaries by submersion in the vials of concentrated HCl, and wipe dry.
3. Submerge each capillary in a sample and purge for 10 minutes.
4. Remove the capillaries, wipe dry, and repeat steps 2-3 for all samples.
5. After all the samples have been purged, repeat step 2 and place the capillaries in a clean, dry vial.
6. Turn off the helium cylinder valve.
NOTE: Purging should be conducted in an enclosed compartment to prevent deposition of acidic aerosols on nearby equipment.

E. PREPARATION OF STANDARDS
(NOTE: Use Analytical Reagent grade reagents only).
1. Prepare a stock solution of potassium acid phthalate (DOC = 1000.0 mg/L as C).
a. weigh 1063.6 mg of dried potassium acid phthalate and quantitatively transfer to a 500-mL volumetric flask.
NOTE: All glassware should be acid-washed.
b. bring to volume with ASTM Type I water.
c. acidify the standards as instructed in section C.4.
2. Prepare working standards of 100, 500, and 1000 mg/L.

NOTE: additional standard concentrations should be made if the sample DOC concentration is expected to be outside of this range.

a. 100 mg/L : pipette 1.0 mL of stock solution and 9.0 mL of ASTM Type I water into a DOC vial.

NOTE: use air- or positive-displacement pipettes suitable for analytical work.

b. 500 mg/L : pipette 5.0 mL of stock solution and 5.0 mL of ASTM Type I water into a DOC vial.

c. 1000 mg/L : pipette 10.0 mL of stock solution into a DOC vial.

3. Prepare a stock solution of phenol for recovery determinations (DOC = 2371.7 mg/L as C).
   a. weigh 155.0 mg of phenol and quantitatively transfer to a 50-mL volumetric flask.
   b. bring to volume with ASTM Type I water.
   c. Dilute this stock solution 1:5 with ASTM Type I water and acidify (DOC = 474.3 mg/L as C).
4. Prepare a stock solution of pyridine for recovery determinations (DOC = 1000 mg/L as C).
   a. dispense approximately 65 µL of pyridine into a tared 50-mL volumetric flask and record the exact weight.
   b. bring to volume with ASTM Type I water.
   c. acidify as instructed in section C.4.
   d. calculate the theoretical DOC of this solution:
      DOC (mg/L) = (15.18) x (mg pyridine added/0.050 L).
   e. dilute this stock solution 1:5 with ASTM Type I water (DOC = 200 mg/L as C).

F. SAMPLE ANALYSIS

1. Load the "Water Analysis" program into the HP 97S.
   a. turn off the coulometer main power.
   b. With the HP 97S calculator in RUN mode, run the program card through the HP 97S card reader.
   c. initiate the program as per the user instructions (Appendix B).
   d. turn on the coulometer main power.
2. Load samples into the 200-µL injection loop with a 1.0-mL gas-tight HPLC syringe.
   a. Rinse the syringe and sample loop with one milliliter of sample; this is required to exponentially dilute the persulfate reagent from the loop. Ensure that the injector waste line is connected to the proper receptacle.
   b. fill the syringe with a minimum of 0.6 mL of sample.
   c. insert the syringe needle into the injection valve port.
   d. position the rotary sample injection valve in the LOAD position.
   e. load the sample into the 200-µL injection loop. Leave the syringe in place to prevent introduction of air into the sample loop by capillary action.
   f. switch the rotary injection valve to the INJECT position and initiate the sample program on the HP 97S as per the user instructions (Appendix B).
   g. the syringe can now be removed from the injection port. The valve must remain in the INJECT position during sample analysis.
   h. the suggested analysis time for each sample is 5 minutes.
   i. for replicates, repeat steps a-h.

j. check the burette water trap. This trap should be emptied between analyses so that the gas line does not become submerged in the condensate.

3. Repeat step 2 for each sample.
4. Samples should be analyzed in the following order:
   a. the sample blank; the mean DOC value from the blanks must be subtracted from the DOC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
   b. the acid phthalate standards.

   NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following in decreasing order of priority: accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; condition of persulfate reagent; condition of UV lamp; coulometer performance.

   c. the recovery standards (phenol and pyridine).
   d. the DOC samples.

   NOTE: if a large number of DOC samples is to be analyzed, the series of standards should be analyzed at intervals throughout the DOC analyses.

   e. upon completion of the DOC analyses, the series of standards and blanks should be reanalyzed.

G. SHUT-DOWN

1. Turn off the HP 97S.
2. Turn off the main power supply to the UV lamp.
3. Drain the contents of the reactor vessel and discard.
4. Disconnect the persulfate reservoir and refrigerate.
5. Turn off the peristaltic pump after the lines have been pumped dry.
   a. disengage the platten and remove all tubing from the pump.
6. Turn off the oxygen cylinder valve.
7. Open the stopcock on the burette water trap.
8. Disassemble the coulometer cell.
   a. turn off the electrolysis current.
   b. turn off the main power.
   c. unplug the anode and cathode wires from the coulometer.
   d. disconnect the gas line at the one-way valve in-line between the coulometer cell and the nitrogen oxide scrubber.
   e. remove the coulometer cell from the coulometer.
   f. remove the silver anode, rinse with ASTM Type I water, and air-dry on a clean surface.
   g. remove the rubber stopper from the coulometer cell and rinse the anode cell with acetone; ensure that no potassium iodide deposits remain in the anode cell. Using a vacuum source and the perforated serum stopper, draw a small volume of acetone through the fritted-glass end of the anode cell.
   h. rinse the exteriors of the anode, cathode, and gas line with ASTM Type I water and air-dry on a clean surface.
   i. rinse the coulometer cell and stir bar several times with ASTM Type I water and air-dry on a clean surface.

H. DATA REDUCTION

1. Calculate the mean value for each set of DOC replicates:
   a. $\bar{x}(DOC) = \Sigma(x_i - b)/n$
      where x_i = each data point in a set of replicates;
      b = the mean value of all DOC blank analyses
      n = the number of replicates per sample.
2. The HP 97S "Water Analysis" program automatically calculates $(x_i - b)$ X (dilution factor) for each data point. Therefore the mean for a set of replicates equals the sum of data outputs divided by the number of replicates (n).
3. Determine whether suspected outliers should be discarded.
   a. suspected outliers should be subjected to statistical analysis before being discarded (1).
   b. if an outlying value is known to be the result of a mechanical or operator error, it may be rejected without statistical verification.

I. MAINTENANCE

1. Record the appropriate information in the C-Analyzer Log Book, including:
   a. date and duration of usage.
   b. number of injections (retort water and total) and sample dilutions.
   c. symptoms of malfunctioning.
   d. repairs.
   e. initial all entries.

J. REFERENCES

1. Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1977; 1110 pp.

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton.

PROTOCOL: INORGANIC CARBON
(Acidification-Purge/Coulometric Titration)

A. START-UP

1. Turn on the main power supply for the inorganic carbon apparatus.
   a. the main power switch controls both the air pump and the heating element.
2. The temperature control knob should be set at 60 (60°C).
3. Increase the air flow rate to 100 cm^3/min.
   a. if foaming occurs in the KOH scrubber, add a small amount of ASTM Type I water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.

   NOTE: Use Analytical Reagent grade chemicals only.
4. Refill the Ag_2SO_4 scrubber.
   a. the contents can be removed with a 9-inch pasteur pipette.
   b. refill with 3 mL of saturated Ag_2SO_4 solution containing 3% H_2O_2 (vol/vol).
5. Refill the perchloric acid ($HClO_4$) reservoir-dispenser if necessary (each analysis requires 2 mL of acid).

   NOTE: Take appropriate precautions when handling concentrated $HClO_4$. (e.g., read pertinent sections in "Prudent Practices for Handling Hazardous Chemicals in Laboratories"; "Guide for Safety in the Chemical Laboratory"; and "First Aid Manual for Chemical Accidents").
   a. to prepare a 2N solution of $HClO_4$ (70%), place 100 mL of ASTM Type I water in a 250-mL volumetric flask, followed by 43.0 mL of $HClO_4$, and bring to volume.
6. Check the neoprene slip-on septum.
   a. replace the septum if signs of oxidation (i.e., from $HClO_4$) are evident (e.g., dryness, cracking).
7. Check the silicone reducing connectors on the air- and acid-delivery lines.
   a. replace these fittings if they show signs of deterioration.
8. Assemble the coulometer cell.
   a. fill the coulometer cell with 75 mL of coulometer solution.
   b. add the stir bar.
   c. position the rubber stopper on the coulometer cell such that the anode, cathode, and gas line face the back wall of the cell (that portion of the beaker containing the volume graduations).
   d. add 3 pellets of potassium iodide to the anode compartment.
   e. add anode solution to the anode compartment. The anode solution level should be slightly higher than that of the coulometer cell solution.
   f. place the silver anode in the anode compartment. Ensure that the tip of the silver anode is wetted by the anode solution.
   g. place the assembled coulometer cell in the cell holder of the instrument; the volume graduations should face the rear of the instrument.
   h. plug the anode (red) and cathode (black) wires into the coulometer.

   DO NOT TURN ON THE ELECTROLYSIS CURRENT.
9. Connect the HP 97S to the coulometer interface cable.
   a. turn on the HP 97S.

b. with the calculator in the RUN mode, load the "background" program as per the user instructions (Appendix A).

NOTE: Analyses may be conducted without the HP 97S.

10. Turn on the coulometer main power supply. Allow a warm-up period of several minutes.
11. Adjust the coulometer cell transmittance.
    a. rotate the coulometer cell until a maximum transmittance is obtained.
    b. adjust the transmittance to 100% using the "100% adjust" knob.
    c. connect the coulometer cell gas line to the one-way valve in-line from the Ag_2SO_4 scrubber.
    d. check that the gas flow into the coulometer cell does not deflect the 100% transmittance setting. If a deflection occurs, reposition the gas line to eliminate this interference; disconnect the gas line and repeat steps a-d.

    NOTE: The gas line must be submerged in the coulometer solution.
12. Turn on the electrolysis current and initiate the background program.
    a. allow several minutes for the titration of endogenous CO_2 in the coulometer solution.
    b. following this initial titration, check the coulometer stability. A stable background count of 1.0 to 1.2 mg/L per minute should be obtained when the range plug is set to display mg/L. Stabilization may take as long as 30 minutes.

B. SAMPLE PREPARATION -- DISSOLVED INORGANIC CARBON (DIC)
1. Filter all samples through 0.4-µm pore diameter polycarbonate membrane filters.
2. Dilute sample filtrates with ASTM Type I water to yield DIC concentrations between 100 and 500 mg/L.
3. Prepare a DIC sample blank; a 10-mL sample of ASTM Type I water should be processed with the DIC samples.
4. Refrigerate samples until analysis time.

C. PREPARATION OF STANDARDS
1. Prepare a stock solution of sodium carbonate (DIC = 1000.00 mg-C /L).
    a. weigh 4414.5 mg of dried sodium carbonate and quantitatively transfer to a 500-mL volumetric flask.

    NOTE: Glassware should be acid-washed.

    b. bring to volume with ASTM Type I water.
2. Prepare working standards of 100, 250, and 500 mg/L.

    NOTE: additional standard concentrations should be made if the sample DIC concentration is expected to be outside of this range.

    a. 100 mg/L : pipette 1.0 mL of stock solution into a 10-mL volumetric flask and bring to volume with ASTM Type I water.
    b. 250 mg/L : pipette 2.5 mL of stock solution into a 10-mL volumetric flask and bring to volume with ASTM Type I water.
    c. 500 mg/L : pipette 5.0 mL of stock solution into a sample vial.

    NOTE: Use air- or positive-displacement pipettes.

D. SAMPLE ANALYSIS
1. Load the "Water Analysis" program into the HP 97S.
    a. turn off the coulometer main power.
    b. with the HP 97S in the RUN mode, run the program card through the HP 97S card reader.

c. initiate the program as per the user instructions (Appendix B).
d. turn on the coulometer main power.

2. If a gas-tight syringe (e.g., Unimetrics) is used, check that the constant-volume adaptor is set for 200 µL. Recheck frequently during sample analysis.
3. Rinse the syringe 10 times with the sample to be analyzed.
   a. insert the syringe needle through the septum injection port.
   b. inject the sample and withdraw the syringe.
   c. depress the plunger on the perchloric acid resevoir and initiate the program loop as per the user instructions (Appendix B). The repipette should be set to deliver 2.0 mL of acid.
   d. the suggested analysis time for each sample is 3 minutes.
   e. for replicates, rinse the syringe twice with the sample to be analyzed and repeat steps a-e.

   NOTE: If analyses are conducted without the HP 97S, a stopwatch should be used to measure the analysis time. The coulometer should be reset at the start of the analysis time.
4. Repeat step 2 for each sample.
5. Samples should be analyzed in the following order:
   a. the sample blank; the mean DIC value from the blanks must be subtracted from the DIC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
   b. the sodium carbonate standards.

   NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following in decreasing order of priority: accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; contamination of reactor tube; coulometer performance.
   c. the DIC samples.

   NOTE: if a large number of DIC samples is to be analyzed, the series of standards should be analyzed at intervals throughout the DIC analyses.
   d. upon completion of the DIC analyses, the series of standards and blanks should be reanalyzed.

E. SHUT-DOWN
1. Turn off the HP 97S.
2. Turn off the inorganic carbon apparatus.
   a. remove the reactor tube and rinse thoroughly with ASTM Type I water.
3. Disassemble the coulometer cell.
   a. turn off the electrolysis current.
   b. turn off the main power.
   c. unplug the anode and cathode wires from the coulometer.
   d. disconnect the gas line at the one-way valve in-line between the coulometer cell and the Ag_2SO_4 scrubber.
   e. remove the coulometer cell from the coulometer.
   f. remove the silver anode, rinse with ASTM Type I water, and air-dry on a clean surface.
   g. remove the rubber stopper from the coulometer cell and rinse the anode cell with acetone -- ensure that no potassium iodide deposits remain in the anode cell. Using a vacuum source and the

perforated serum stopper, draw a small volume of acetone through the fritted-glass end of the anode cell.

h. rinse the exteriors of the anode, cathode, and gas line with ASTM Type I water and air-dry on a clean surface.

i. rinse the coulometer cell and stir bar several times with ASTM Type I water and air-dry on a clean surface.

F. DATA REDUCTION

1. Calculate the mean value for each set of DIC replicates:

a. $\bar{x}(DIC) = \Sigma(x_i - b)/n$

where x_i = each data point in a set of replicates;
b = the mean value of all DIC blank analyses
n = the number of replicates per sample.

2. The HP 97S "Water Analysis" program automatically calculates $(x_i - b)$ X (dilution factor) for each data point. Therefore the mean for a set of replicates equals the sum of data outputs divided by the number of replicates (n).

3. Determine whether suspected outliers should be discarded.

a. suspected outliers should be subjected to statistical analysis before being discarded (1).

b. if an outlying value is known to be the result of a mechanical or operator error, it may be rejected without statistical verification.

G. MAINTENANCE

1. Record the appropriate information in the C-Analyzer Log Book, including:

a. date and duration of usage.

b. number of injections (retort water and total) and sample dilutions.

c. symptoms of malfunctioning.

d. repairs.

e. initial all entries.

H. REFERENCES

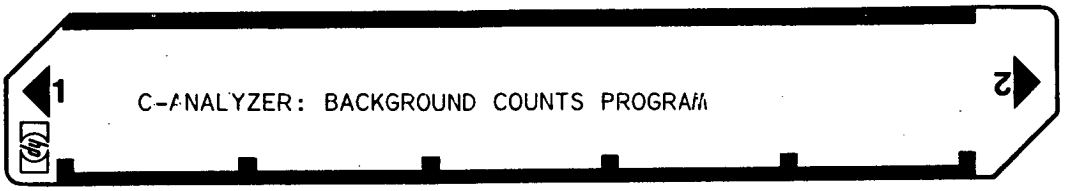
1. ASTM
Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1977; 1110 pp.

2. Lefevre, M.J.
First Aid Manual for Chemical Accidents; Dowden, Hutchinson and Ross, Inc.: Stroudsburg, PA, 1980; 218 pp.

3. Manufacturing Chemists Assosciation
Guide for Safety in the Chemical Laboratory; Van Nostrand Reinhold Company: New York, NY, 1972; 505 pp.

4. National Research Council
Prudent Practices for Handling Hazardous Chemicals in Laboratories; National Acadamy Press: Washington, D.C., 1981; 291 pp.

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton.



STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position, coulometer turned off.			
2	PRESS"E": display will go to 0.0		E	
3	PRESS "R/S": display will go to 0.		R/S	
4	ENTER NUMBER OF DATA INPUTS FROM COULOMETER (e.g., 20 inputs at 15 sec intervals = 5 min analysis time). PRESS "R/S".	"n"	R/S	
5	TURN COULOMETER ON.			
6	PRESS "R/S": prints number of inputs, spaces 2 lines, begins data acquisition loop.		R/S	"n"
7	When data input is completed (i.e., after the "n"th data input) the system background for the selected analysis time is printed; the data acquisition loop is automatically reentered.			Background value
8	PRESS "R/S" to terminate the program.		R/S	
9	To change the value for number of data inputs turn off the coulometer, PRESS "f" "e" and repeat instructions beginning at step 4.		f e	

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLE	21 15	
002	CF3	16 22 03	
003	R/S	51	
004	SF3	16 21 03	
005	*LBLe	21 16 15	
006	SF3	16 21 03	
007	DSP0	-63 00	
009	0	00	
009	R/S	51	
010	PRTX	-14	
011	SPC	16-11	
012	SPC	16-11	
013	1	01	
014	+	-55	
015	STOC	35 13	
016	R/S	51	
017	*LBLD	21 14	
018	DSP0	-63 00	
019	0	00	
020	RCLC	36 13	
021	STOI	35 46	
022	DSP1	-63 01	
023	SF0	16 21 00	
024	PSE	16 51	
025	*LBLB	21 12	
026	CF3	16 22 03	
027	CF0	16 22 00	
028	R/S	51	
029	*LBLH	21 11	
030	1	01	
031	0	00	
032	÷	-24	
033	DSZI	16 25 46	
034	GTOB	22 12	
035	CF3	16 22 03	
036	PSE	16 51	
037	SF3	16 21 03	
038	PRTX	-14	
039	GTOD	22 14	
040	R/S	51	
050			

STEP	KEY ENTRY	KEY CODE	COMMENTS
060			
070			
080			
090			
100			

LABELS

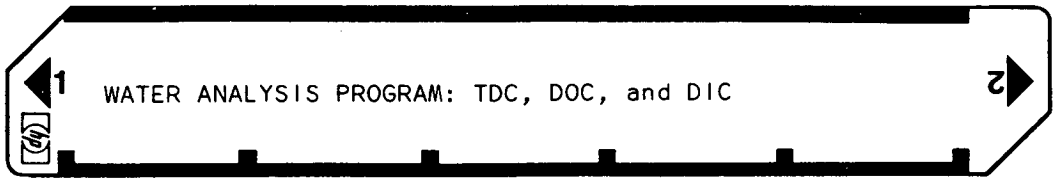
A DATA INPUT LOOP	B DATA LOOP	C	D INITIATE DATA LOOP	E INITIATE PROGRAM
a	b	c	d	e MANUAL DATA INPUT
0	1	2	3	4
5	6	7	8	9

REGISTERS

0	1	2	3	4	5	6	7	8	9
S0	S1	S2	S3	S4	S5	S6	S7	S8	S9

A	B	C # DATA INPUTS	D	E	I COUNTER FOR DATA INPUTS

APPENDIX B. User Instructions



STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position, coulometer turned off.			
2	PRESS "E": display will go to 0.0		E	
3	PRESS "R/S": display will go to 0.000		R/S	
4	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
5	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
6	ENTER BLANK VALUE	Value		
7	PRESS "R/S": prints blank value, display goes to 0.		R/S	Value
8	ENTER NUMBER OF DATA INPUTS FROM COULOMETER (e.g., 20 inputs at 15 sec intervals = 5 min analysis time).	"n"		
9	PRESS "R/S": prints number of inputs, spaces 3 lines, displays 0.		R/S	"n"
10	TURN COULOMETER ON.			
11	ENTER SAMPLE NUMBER (integer only)	Value		
12	PRESS "R/S": prints sample number, displays 0.0		R/S	Value
13	ENTER DILUTION FACTOR (e.g., "10" for a 1:10 dilution).	Value		
	NOTE: THE FOLLOWING STEP INITIATES THE DATA ACQUISITION LOOP; THE LOADED SAMPLE MUST BE INJECTED AT THIS STEP.			
14	INJECT SAMPLE, PRESS "R/S": prints dilution factor, starts analysis time.		R/S	Value

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	001 *LBLE	21 15	
	002 CF3	16 22 03	
	003 R/S	51	
	004 SF3	16 21 03	
	005 *LBLe	21 16 15	
	006 SF3	16 21 03	
	007 DSP3	-63 03	
	008 0	00	
	009 R/S	51	
010	010 PRTX	-14	
	011 STOA	35 11	
	012 DSP1	-63 01	
	013 0	00	
	014 R/S	51	
	015 PRTX	-14	
	016 STOB	35 12	
	017 DSP0	-63 00	
	018 0	00	
	019 R/S	51	
020	020 PRTX	-14	
	021 SPC	16-11	
	022 SPC	16-11	
	023 SPC	16-11	
	024 1	01	
	025 +	-55	
	026 STOC	35 13	
	027 1	01	
	028 -	-45	
	029 RCLB	36 12	
030	030 X≠Y	-41	
	031 ÷	-24	
	032 STO2	35 02	
	033 *LBLD	21 14	
	034 DSP0	-63 00	
	035 0	00	
	036 R/S	51	
	037 PRTX	-14	
	038 DSP1	-63 01	
	039 0	00	
040	040 R/S	51	
	041 PRTX	-14	
	042 STO1	35 01	
	043 RCLC	36 13	
	044 STOI	35 46	
	045 1	01	
	046 -	-45	
	047 STO3	35 03	
	048 DSP1	-63 01	
	049 SF0	16 21 00	
050	050 PSE	16 51	
	051 *LBLB	21 12	
	052 STOE	35 15	
	053 CF3	16 22 03	
	054 CF0	16 22 00	
	055 R/S	51	
	056 *LBLA	21 11	

STEP	KEY ENTRY	KEY CODE	COMMENTS
	057 1	01	
	058 0	00	
	059 ÷	-24	
0	060 DSZI	16 25 46	
	061 GTOB	22 12	
	062 CF3	16 22 03	
	063 PSE	16 51	
	064 SF3	16 21 03	
	065 STOD	35 14	
	066 RCLA	36 11	
	067 x	-35	
	068 RCLE	36 15	
	069 -	-45	
c	070 RCL2	36 02	
	071 -	-45	
	072 X>0?	16-44	
	073 GTOC	22 13	
	074 DSP1	-63 01	
	075 RCLD	36 14	
	076 ENT↑	-21	
	077 RCL2	36 02	
	078 ENT↑	-21	
	079 RCL3	36 03	
c	080 x	-35	
	081 -	-45	
	082 PRTX	-14	
	083 RCL1	36 01	
	084 x	-35	
	085 PRTX	-14	
	086 SPC	16-11	
	087 GTOD	22 14	
	088 *LBLC	21 13	
	089 ISZI	16 26 46	
	090 RCL3	36 03	
	091 1	01	
	092 +	-55	
	093 STO3	35 03	
	094 RCLD	36 14	
	095 GTOB	22 12	
	096 R/S	51	
100			

LABELS

A Data Input Loop	B Data Loop	C Reset for Add. Data	D Initiate Data Loop	E Initiate Program
a	b	c	d	e Manual Data Input
0	1	2	3	4
5	6	7	8	9

REGISTERS

0	1 Dilution Factor	2 Blank, Increment.	3 #Data Inputs	4	5	6	7	8	9
S0	S1	S2	S3	S4	S5	S6	S7	S8	S9

A Stability Factor	B Blank Value	C # Data Inputs	D Final Data Input Value	E Previous Data Input Value	I Counter for Data Inputs

User Instructions

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
15	When data input is completed (i.e., after the "n"th data input) the final value minus the blank is printed; this value is multiplied by the dilution factor and printed.			Final Value minus blank, multiplied by the dilution factor
16	To analyze the next sample repeat steps 11-14.			
17	To change the STABILITY FACTOR, BLANK, or NUMBER OF DATA INPUTS, turn off the coulometer, PRESS "f" "e", and repeat instructions beginning at step 4.		f e	

Chapter III

COLORIMETRIC AND TITRIMETRIC QUANTITATION OF AMMONIA IN OIL SHALE WASTEWATERS

C.G. Daughton, J. Cantor, B.M. Jones, and R.H. Sakaji

Introduction 2
Theory 2
Distillation/Titrimetry 2
Endpoint Detection by pH Electrode 3
Endpoint Detection by pH Indicator Dye 3
Colorimetry 4
Phenate Method 4
Interferences to Phenate Method 4
Protocol Summary 5
Acidimetric Titration Method 5
Colorimetric Phenate Method 6
Comparison Study: Results and Discussion 7
References 10
Tables
I. Comparison of Colorimetric Ammonia Determinations on Filtered and Unfiltered Oil Shale Wastewaters 11
II. Comparison of Colorimetric and Titrimetric Methods for Determining Ammonia in Unfiltered Oil Shale Wastewaters 12
III. Standard Additions Results 13
Protocol: Colorimetric Phenate Ammonia 14
Protocol: Distillation/Titrimetric Ammonia 17-22

INTRODUCTION

Ammonia gas is soluble in water as a hydrated form, and an equilibrium can be established with its protonated form, ammonium ion, as dictated by its pK_a (9.3 at 25 °C). Ammonia and ammonium ion are present in wastewaters from oil shale retorting at concentrations generally greater than several thousand parts per million. Methods for the quantitation of these species in synfuel wastewaters are necessary for research, treatment monitoring, and establishment of regulatory standards. The method of choice should be sufficiently simple to find applicability in the routine wet-chemistry laboratory. Published methods for determining ammonia in water, however, may be applicable only to the particular sample matrix for which they were developed or tested, and even conventional "standard methods" should not be used for oil shale wastewaters without validation because these methods often require modification.

Only three approaches are available to a routine wet-chemistry laboratory for quantitating the aqueous equilibrium pair as total ammonia: (1) colorimetry, (2) titrimetry, and (3) direct ammonia-selective electrode; other, less routine methods, include ion chromatography, gas-liquid chromatography, pyrochemiluminescence, and highly specific enzymatic assays. Methods that employ these routine approaches are numerous, and most of them give excellent results for particular sample matrices.

Only these three routine methods were extensively evaluated for oil shale wastewaters. Methods for colorimetry and acidimetric titrimetry proved comparable for "accuracy". While both methods were very reproducible, the titrimetric method, when automated, was superior for precision. Gas-sensing electrodes from two manufacturers proved to be extremely unreliable even though they could possibly provide the fastest and easiest means of quantitation. Ammonia-sensing electrodes generate unstable response curves in oil shale retort waters, probably because their membranes become easily fouled and because surfactants (e.g., fatty acids) increase the membrane permeability to other interfering solutes. One electrode gave extremely stable readings in standard solutions, exhibited severe drift when immersed in oil shale wastewater samples, and subsequently failed to produce stable readings when reimmersed in standard solutions. Changes in the slope of the response curve made frequent recalibration prohibitively time consuming. Erratic response of ammonia electrodes has also been reported by Wallace et al. (1982). Ammonia-sensing electrodes also respond to organic amines (Lopez and Rechnitz, 1982). We strongly recommend that ammonia electrodes not be used for these waters although they may be useful in range-finding to facilitate the subsequent application of other methods. For these reasons, a study was performed for the statistical comparison of only two methods, colorimetry and automated acidimetric titrimetry, on nine oil shale wastewaters.

THEORY

Distillation/Titrimetry

Ammonia quantitation by titrimetry requires the quantitative distillation of the ammonia from an aqueous sample to eliminate interference of other titratable species (e.g., carbonate alkalinity) and color. This is a straightforward method that makes use of the pK_a of the ammonium-ammonia equilibrium. If a sample is maintained at a sufficiently high pH and temperature, the equilibrium is shifted toward free hydrated ammonia, allowing

quantitative distillation of the hydrated ammonia as ammonia gas until the remaining ammonium ion has completely dissociated. If the adjusted pH is too high, however, hydrolysis of cyanates or organically bound nitrogen (e.g., urea) may occur and contribute a positive interference; samples are therefore buffered at about pH 9.5 during distillation to retard hydrolysis in the distilland. Volatile alkaline compounds such as amines, ketones, alcohols, and aldehydes also can distill and interfere. The latter three can possibly be eliminated by predistilling at a low pH before distilling at high pH.

The distilled ammonia gas is captured in a weak acidic receiving solution (e.g., boric acid), and the pH of the receiving solution is thereby increased. The two routine methods for quantitating the captured ammonia are colorimetry and acidimetric titrimetry. The former is more involved and is not the preferred finish following distillation. Acidimetric titrimetry simply involves the titration of the receiving solution with a standardized strong acid (e.g., sulfuric acid) until the pH is lowered to equal that of the original receiving solution. The titration endpoint can be most easily detected by measurement with a pH electrode (ASTM, 1980) or by color-matching when a pH indicator dye is used.

Endpoint detection by pH electrode.

If nondistilled blanks, distilled blanks, and distilled samples are diluted to equal volumes, then the equivalents of standardized acid titrant required for lowering the pH of the distillates to match the value of the nondistilled blanks is identical to the equivalents of ammonia present in the distillates. The endpoint is on the steep portion of the titration curve when boric acid is used to capture the ammonia, and it therefore is easily detected. A complicating factor, however, is the importance of ensuring that the volumes of all samples are also equal after titration. If the final volumes are not equal, then the pH values are no longer comparable. This problem is best avoided by ensuring that the quantity of ammonia in each distillation flask is about the same and that the volumes of the diluted distillates are large when compared with the titrant volumes. The use of a pH electrode to follow the titration is most easily accomplished with an automatic titrator; spurious problems have been encountered, however, with electrode stability in boric acid.

Endpoint detection by pH indicator dye.

There are no pH indicators that give sharp endpoints when the equivalency point (approximately pH 4.8) is reached during titration of ammonia in boric acid. Methyl red is the most widely used indicator, and it exhibits little salt error (Stover and Sandin, 1931). Protonation of the benzoic azo-nitrogen of methyl red yields the red form in the acid region of the pH range of 4.2 to 6.2; at the basic end of this range, the nonprotonated form is yellow. The titration of distillates that contain ammonia therefore proceeds from the higher end of the range (yellow) to the lower end (red). The intermediate colors of the two forms are gradations of pink, and the endpoint can only be determined by carefully comparing the distillate color with that of a nondistilled blank (Meeker and Wagner, 1933). Visual detection of the endpoint can be ameliorated by addition of a blue "enhancer" dye such as methylene blue (Johnson and Green, 1930), whose color does not change with pH. The color that results when methylene blue is combined with methyl red is green in basic solutions and violet in acidic solutions. Regardless of whether such an enhancer dye is used, experience and patience are required for accurate and reproducible color-matching titration. This is an inferior endpoint detection method except when the detection is performed by determining the absorbance of the solution and matching it to that of the blank; this is best done by automated colorimetry. The problem of unequal sample volumes during titration, which was

noted for endpoint detection by pH electrode, is also applicable to detection by indicator dye. Furthermore, uniformity of ionic strength and pH of the distillates is important because of their effects on salt error and color intensity of indicator dyes (Stover and Sandin, 1931).

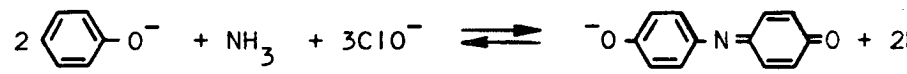
Colorimetry

Ammonia quantitation by direct colorimetry (i.e., without distillation) involves the formation of an ammonia reaction product whose chromophore has a molar absorptivity sufficiently high to alleviate interferences by endogenous chromophores. Routine colorimetric methods include Nesslerization, pyridine/pyrazole, oxidation to nitrite followed by nitrite quantitation, and various adaptations of the indophenol-blue procedure, which is also called the "phenate", "phenolate", "alkaline phenol-hypochlorite", or "Berthelot reaction" methods.

The Nessler reagent can only be used for relatively clean waters and involves the use of mercury. The pyridine/pyrazole method is highly specific for ammonium ion, but it is cumbersome. All of the colorimetric methods usually require distillation of the ammonia if the sample contains interferences. Distillation can be avoided by oxidation of the sample to yield nitrite. The nitrite can then be quantitated by diazotization, which is highly sensitive; the oxidation step, however, is very time consuming. The phenate method, in contrast, is simple to perform, and it was the only rapid colorimetric method that was applicable to the highly complex sample matrix of oil shale wastewater. The phenate method, as described by Weatherburn (1967), was evaluated for direct quantitation of ammonia in several of these waters, and it was statistically compared with the distillation/titrimetric method.

Phenate method.

The phenate method is based on the Berthelot color reaction catalyzed by nitroprusside (Scheiner, 1976):



Ammonia is converted to monochloramine between pH values of 9.7 and 11.5. Losses to dichloramine, nitrite, and other species occur above pH 11.5. Monochloramine then reacts with phenol in the presence of chlorine to form indophenol blue via intermediates (Hampson, 1977) such as quinonechloramine (Ngo et al., 1982).

Interferences to phenate method.

Hampson (1977) states that nitrogen from nitroprusside can enter the indophenol blue molecule and cause high blank absorbance values. High blank values were not found to be a problem in the 10- to 1000-mg/L NH_3-N range, but if blank values are found to be too high for determination of low concentrations of ammonia, other catalysts (e.g., ferrocyanide or manganese [II] ion) should be considered. We have found that high background values will also result from screwcaps that have been washed in acid; subsequent contact with water will continually leach a yellow substance (possibly a substituted phenol) from the plastic.

Although Weatherburn (1967) does not discuss interferences with the phenol-hypochlorite reaction, other authors have investigated the subject. Aliphatic amines and nitrite suppress color development significantly when present in excess of the quantity of ammonia in the sample (Hampson, 1977). Ngo et al. (1982) tested the effect of 72 compounds (10 mM) on the recovery of ammonia (0.5 mM). All primary and secondary aliphatic amines strongly inhibit the development of indophenol, possibly by nucleophilic addition to the

quinonechloramine intermediate at the ortho position, resulting in steric hindrance; sulfide and thiols also inhibit indophenol formation. Zadarojny, Saxton, and Finger (1973) describe the effects of amino acids, urea, and 24 inorganic electrolytes on the analysis of a 0.2-ppm NH_3-N solution. Apparent recoveries of 109 to 214 percent resulted from additions of 100 ppm of KCN, KSCN, Na_2S, NaF, $CuSO_4$, $CoCl_2$, and NiCl; recoveries of 38 to 93 percent were reported for additions of $NaNO_2$, $Na_2S_2O_2$, $HgCl_2$, and $AlCl_3$ at 100 ppm, and of L-cystine, L-methionine, and L-phenylalanine at 0.2 ppm amino acid-N. Interference was not evident below 5 ppm of electrolyte.

Gravitz and Gleye (1975) report the formation of an interfering substance having an absorbance maximum at 640 nm when the standards are exposed to sunlight. Standards so affected fail to follow Beer's law with respect to ammonia concentration, and absorbance values for blanks are high. The interfering substance has been tentatively identified as indophenol, formed with nitrogen from the nitrosyl group of the nitroprusside molecule. In our laboratory, the test has been performed routinely under fluorescent lighting, and these problems have not been encountered. If desired, light may be excluded from the reaction mixture by wrapping the test tubes in aluminum foil. This procedure may be helpful for samples with low ammonia concentrations. Use of a vacuum-operated flow-through spectrophotometer cuvette minimizes light exposure during sample transfer. Verdouw, van Echteld, and Dekkers (1978) recommend potassium ferrocyanide as the catalyst for better color stability in light and dark.

It must be emphasized that observations on one variation of the phenate method may not be applicable to another. Presumably, certain interferences (including color) are minimized in Weatherburn's version by the 500-fold dilution of the sample by the reagents. In our laboratory, this method has yielded results for ammonia concentrations in oil shale process waters statistically comparable to those obtained by distillation/titrimetry, and no color interference has been observed. Distillable bases, especially aliphatic amines, will cause interference for both methods.

PROTOCOL SUMMARY

Detailed laboratory protocols for determining ammonia by the colorimetric phenate and distillation/titrimetric methods are appended. The importance of representative subsampling, especially with regard to loss of ammonia by degassing, cannot be overemphasized. Degassing is especially a problem with oil shale wastewaters because of the high concentrations of ammonia coupled with high pH. Normally, it is best to acidify wastewater samples to pH values below 2.0 prior to storage. With oil shale wastewaters, however, the high alkalinity necessitates addition of large amounts of acid which then causes other problems such as foaming and precipitation (see: Chapter II).

Acidimetric Titration Method

Samples are diluted to give ammonia-N masses for a subsample in the range of 0.1 to 5 milligrams. The appropriate volume (generally 5 to 300 milliliters) is added to a 500-mL Kjeldahl distillation flask that contains an alkaline borate buffer solution; the amounts of ammonia in each flask should be as equal as possible. Upon the addition of sufficient sodium hydroxide to maintain the pH of the solution at about 9.5, the flask is immediately connected to a distillation/condensation apparatus. Immediate connection is important to avoid loss of ammonia to the headspace; losses can be further minimized by carefully adding the dense caustic down the inside of the flask so that it underlays the

sample solution. The flask contents (about 300 mL) are thoroughly mixed after connection to a spray-trap and condenser, the contents are heated to a boil, and the first 100 mL of distillate is collected by bubbling it through 50 mL of a 4-percent acidic boric acid solution. The quantity of boric acid in the receiving solution is important for good precision; it is most conveniently measured with a tilting dispenser, although highest precision is obtained with a volumetric pipette or repipette. A mixed indicator (methyl red indicator and methylene blue dye) is added to the boric acid receiving solution if an indicator endpoint is followed, but it is omitted if the endpoint is determined with a pH electrode. This acidic solution ensures quantitative capture of the ammonia gas as nonvolatile ammonium ion, with a concomitant stoichiometric rise in pH. Although over 90 percent of the ammonia is distilled in the first couple minutes, the distillation is continued until over 100 mL of distillate is collected.

The distillates are diluted to equivalent final volumes with water and titrated with standardized sulfuric acid. When mixed indicator/dye is used in the receiving solution, the distillates that contain sufficient ammonia are initially bluish-green. During titration, the color proceeds through a transition point of dirty gray followed by gradations of more intense shades of violet. Storage of the boric acid solution in glass containers should be avoided since alkali from the glass has been suggested as a cause of slowly rising pH, making the boric acid unusable (Meeker and Wagner, 1933). It is extremely important that the spray-traps and condenser tubes be steamed clean before and after each series of distillations; otherwise, deposits tend to accumulate in the condenser tubes.

This method is not very accurate or precise when performed manually. Automation can be easily accomplished, however, with an automatic titrator interfaced with either a pH electrode or colorimeter. The distilled samples are then titrated to either the pH of the nondistilled blanks or to the absorbance of the nondistilled blanks (at about 545 nm) if indicator dye is used.

Colorimetric Phenate Method

Samples are diluted to give ammonia-N concentrations in the ranges of 10 to 100 mg/L or 100 to 1000 mg/L. If the samples have been acidified, the pH values must be raised and made consistent. Five milliliters of phenol/nitroprusside reagent (5 g phenol and 25 mg nitroferricyanide dihydrate diluted to 500 mL with water) is added per tube to 20- X 150-mm Pyrex culture tubes with Teflon-lined screwcaps. Samples or standards (prepared from ammonium sulfate) are withdrawn with 20-µL glass capillaries, calibrated "to-contain" (end-to-end). The entire capillary is carefully added to the reagent mixture, and the tube is sealed and shaken vigorously. Immediately after adding 5 mL of alkaline hypochlorite solution (2.5 g sodium hydroxide and 4.2 mL of 5% sodium hypochlorite diluted to 500 mL), the tubes are resealed, shaken vigorously, and immersed in a 37°C bath for 20 minutes. The tubes are cooled to room temperature and the samples' absorbance values are read versus a reagent blank at 635 nm or 520 nm for low or high concentration ranges, respectively, using a spectrophotometer equipped with a 1-cm path length micro flow-through cell.

The use of glass capillaries (e.g., Drummond Microcaps) can be simplified by not using the dispenser which is usually supplied by the manufacturer. Standards or samples should be prepared in a small wide-mouthed container, such as a scintillation vial. The capillary should be grasped near its top with forceps, placed in the container, and leaned against the rim. When the container is tilted, the capillary becomes filled as it approaches the horizontal. Adequate headspace must be left in the container to avoid spilling

the sample, but it is imperative to work quickly because degassing of ammonia could be extensive in samples of high pH such as oil shale wastewaters. The capillary is removed from the container with forceps and kept horizontal. This ensures that the capillary remains filled from end to end. Excess liquid on the outer surface of the capillary should be removed by gently scraping its entire length against the rim of the container. The capillary should not be tilted from the horizontal until this step is completed. Tilting the capillary will result in the formation at its lower end of a drop of liquid which will be lost during the scraping step. Relative standard deviations of less than 1.5 percent can be easily achieved using this method.

The colorimetric method has been evaluated in our laboratory using only Drummond Microcaps. It is not known whether the use of other brands of capillary pipettes having different dimensions would necessitate variations in operator technique to achieve good reproducibility.

A spectrophotometer cell with a 1-cm path length has been used in the protocol reported here. A longer path length may be required for determination of lower ammonia concentrations. The lower detection limit for the phenate method as adapted for oil shale process wastewaters is about 10 mg/L. This limit could be reduced only by increasing the sample size, which would in turn increase the background. For the determination of ammonia concentrations less than 10 mg/L, other methods are available (see: Jenkins, 1977), including several other variations of the phenate method.

COMPARISON STUDY: RESULTS AND DISCUSSION

Ammonia concentrations in nine oil shale wastewaters were determined by the phenate colorimetric method for homologous unfiltered and filtered (0.4-μm pore-diameter polycarbonate membranes, Bio-Rad Laboratories, Richmond, CA) samples (Table I). The phenate ammonia values for unfiltered waters were no more than 5 percent higher than the values for filtered waters, with the exception of TV process water, and Oxy-6 and 150-Ton retort waters. Since only slight differences were observed between filtered and unfiltered samples, the time-consuming pressure filtration step was eliminated; particulates apparently did not interfere with the absorbance of the samples nor did they release interfering compounds.

Ammonia concentrations determined by a newly automated version of distillation/titrimetry (using the basic method of Standard Methods..., 1976) and colorimetry (using the method of Weatherburn, 1967) for nine oil shale process wastewaters are presented in Table II. Also presented are results from a "composite" water which was composed of equal volumes of each of the nine waters. Each sample was diluted to give an ammonia-N concentration of about 500 mg/L to minimize differences between waters for subsequent statistical analysis, and five replicate subsamples were analyzed by either method.

The average ammonia-N values for the nine waters were 6536 and 6286 mg/L for the titrimetric and colorimetric methods, respectively, compared with 6564 and 6225 mg/L for the composite water (Table II); both values for the composite water were within one percent of the respective averages. For all but one of the waters (Oxy-6 gas condensate), the titrimetric values were one to seven percent higher than the respective colorimetric values (Table II). To determine if a significant difference existed between the two methods for the determination of ammonia in this series of oil shale wastewaters, a two-way analysis of variance (anova) was conducted on reciprocal-transformed replicate data ($n = 5$). For the variability between methods, the calculated F-value was less than 1, which was less than the critical F-value of 3.92 at $\alpha = 0.05$.

Therefore, there was no significant difference ($P>0.05$) between the colorimetric and titrimetric methods for determining ammonia in these wastewaters.

The precision for both methods was excellent (Table II). The relative standard deviations (rsd values) for titrimetry ranged from 0.08 to 1.3 percent; those for colorimetry ranged from 0.7 to 1.4 percent, with the exception of the composite water (4.0 percent). The seemingly better precision of the titrimetric method was probably a result of automation of the titrimetric step.

The question of accuracy is more difficult to address. Although the two methods employed entirely different approaches and achieved comparable ammonia concentrations for each of the waters, they are subject to similar interferences (e.g., distillable amines). Accuracy was partly addressed by running standard additions recovery series on three of the waters: Paraho, composite, and Oxy-6 gas condensate. Each water was diluted to yield a concentration of about 500 mg/L ammonia-N, and then an equal portion of one of each of four ammonium sulfate standards was added to duplicate samples: 100, 300, 600, and 800 mg/L. The least-squares linear regression lines of total ammonia-N recovered versus ammonia-N added to each spiked sample yielded the data shown in Table III. In general, the recoveries of ammonia spikes from these three waters were greater than 100 percent for the titrimetric method and lower than 100 percent for the colorimetric method. If these recoveries were used to correct the apparent concentrations in Table II, the difference between the two methods would be even less. An EPA quality control nutrient sample that contained 1.52 mg/L NH_3-N was also analyzed. Recoveries were 102.0 percent (rsd = 3.2 percent) and 104.6 percent (rsd = 1.1 percent) for the titrimetric and colorimetric methods, respectively.

Mean concentrations determined on different days for a sample type were found to vary because subsamples were obtained from different lots and because of degassing during storage; this was the cause of the discrepancies between the two sets of colorimetric data in Tables I and II. Degassing is probably a major cause of problems in interlaboratory comparisons. Interlaboratory comparison results reported by Fox, Farrier, and Poulson (1978) for Omega-9 retort water gave NH_3-N concentrations that ranged from 2650 to 3457 mg/L for different methods. Wallace (1982) reported values from different methods of 3300 to 3600 for Omega-9. The values reported here for Omega-9 were 3551 to 3690 mg/l. Interlaboratory comparison values for other waters are not available.

The alkalinity of these waters apparently does not interfere with the colorimetric method. Alkalinity concentrations greater than 500 mg per liter, however, do interfere with the standard phenate method, in which the sample is diluted very little by the reagents (Standard Methods..., 1976, p 466). A major advantage of the phenate method is that of sample throughput. In our laboratory, an analyst can perform over 90 determinations per day (excluding sample preparation) versus 20 for the titrimetric method. This number can be processed in three batches of 30 samples each. The rate of color development at ambient temperature is sufficiently great that when more than about 10 samples are being prepared, considerable color-development for the samples prepared earliest will have occurred by the time the entire batch is ready to be immersed in the warm water bath. Studies to determine whether this affects final absorbance values have been inconclusive, but as a precaution, premature development may be prevented by maintaining the reaction mixtures at 0 ^{o}C during preparation. Cooling may also increase the time allowable for sample preparation, as reagent stability limits the number of samples that may be prepared in one batch. Tubes need not be cooled until the sample is added to the phenol/nitroprusside reagent and the tubes shaken; this will minimize

condensation of atmospheric moisture in the test tubes, which could measurably dilute the samples.

The success of the colorimetric method when applied directly to retort waters results from its low inherent minimum detection limit (i.e., 10 ppb); this allows for extensive dilution of the sample by the reagents, which effectively eliminates interference by endogenous chromophores. The disadvantage of any of the colorimetric methods is that several range-finding dilutions may be required to bring the absorbance of an unknown sample within the standard-curve range. Precision and accuracy are sacrificed when dilutions are performed, but more significantly, the large exposed-surface to volume ratio of samples contained in measuring devices (e.g, pipettes, syringes) exacerbates the loss of ammonia by degassing; this problem is magnified with the necessity of measuring small volumes and by the transient headspace pressure reduction during sample withdrawal. We have found that the most satisfactory solution to this problem is the use of to-contain glass capillaries for accurate volume measurement while sampling. If filtration of samples is required, vacuum filtration should never be used, as extensive degassing will occur. Range-finding is also required for the titrimetric method; the appropriate normality of the titrant must be determined for each unknown, or the unknown must be appropriately diluted before distillation. This problem can be alleviated by using an autotitrator that is capable of delivering microliter-volumes of titrant. Automation of the colorimetric method would be possible by segmented-flow or flow-injection analysis.

Several problems were encountered with the distillation/condensation apparatus that was used in this study. The spray traps had been connected to the condenser tubes with silicone-rubber tubing and fitted with silicone rubber stoppers for the Kjeldahl flasks. The extreme permeability of silicone rubber to gases (Brookes, 1969) necessitates that all connections be made with neoprene rubber and Tygon or Teflon tubing. Another, more serious problem was the inability to recover more than 89 percent of the ammonia from numerous replicates of standard solutions, and rsd values always exceeded 8 percent. The cause of this incomplete recovery was finally traced to the scavenging of ammonia by the block-tin condenser tubes. Whether these losses were caused by absorption into the pitted inner surfaces of the tin or by formation of insoluble complexes with volatile compounds from wastewater samples that had precipitated onto the tin from previous use is not clear. The problem was solved by replacing the condenser tubes with heavy-walled Pyrex tubing.

REFERENCES

ASTM
"Standard Test Method for Total Kjeldahl Nitrogen in Water", 1980 Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1980, D 3590-77, p 746.

Brookes, R.
"Properties of Materials Suitable for the Cultivation and Handling of Micro-organisms", in Methods in Microbiology; Norris, J.R. and Ribbons, D.W., Eds.; Academic Press: New York, NY, 1969; pp 21-75.

Fox, J.P.; Farrier, D.S.; Poulson, R.E.
"Chemical Characterization and Analytical Considerations for an In Situ Oil Shale Process Water", Laramie Energy Technology Center, 1978, LETC/RI-78/7.

Gravitz, N.; Gleye, L.
"A Photochemical Side Reaction that Interferes with the Phenolhypochlorite Assay for Ammonia", Limnol. Oceanogr. 1975, 20, 1015-7.

Hampson, B.L.
"The Analysis of Ammonia in Polluted Sea Water", Water Res. 1977, 11, 305-8.

Jenkins, D.
"The Analysis of Nitrogen Forms in Waters and Wastewaters", Prog. Water. Tech. 1977, 8, 31-53.

Johnson, A.H.; Green J.R.
"Modified Methyl Red and Sodium Alizarin Sulfonate Indicators", Indus. Eng. Chem. (Anal. Ed.), 1930, 2, 2-4.

Lopez, M.E.; Rechnitz, G.A.
"Selectivity of the Potentiometric Ammonia Gas Sensing Electrode", Anal. Chem., 1982, 54, 2085-9.

Meeker, E.W.; Wagner, E.C.
"Titration of Ammonia in Presence of Boric Acid", Indus. Eng. Chem. (Anal. Ed.), 1933, 5, 396-8.

Ngo, T.T.; Phan, A.P.H.; Yam, C.F.; Lenhoff, H.M.
"Interference in Determination of Ammonia with the Hypochlorite-Alkaline Phenol Method of Berthelot", Anal. Chem. 1982, 54, 46-9.

Scheiner, D.
"Determination of Ammonia and Kjeldahl Nitrogen by Indophenol Method", Water Res. 1976, 10, 31-6.

"Standard Methods for the Examination of Water and Wastewater", 14th ed.; American Public Health Association: Washington, D.C., 1976; p 407-18.

Stover, N.M.; Sandin, R.S.
"Use of Boric Acid in Micro-Kjeldahl Determination of Nitrogen", Indus. Eng. Chem. (Anal. Ed.), 1931, 3, 240-2.

Verdouw, H.; van Echteld, C.J.A; Dekkers, E.M.J.
"Ammonia Determination Based on Indophenol Formation with Sodium Salicylate", Water Res. 1978, 12, 399-402.

Wallace, J.R.; Alden, L.; Bonomo, F; Sexton, E.
"Analytical Methods for Oil Shale Wastes", in Fifteenth Oil Shale Symposium Proceedings; Gary, J.H., Ed.; Colorado School of Mines: Golden, CO, 1982, p 573-80.

Weatherburn, M.W.
"Phenol-Hypochlorite Reaction for Determination of Ammonia", Anal. Chem. 1967, 39, 971-4.

Zadorojny, C.; Saxton, S.; Finger, R.
"Spectrophotometric Determination of Ammonia", J. Water Pollut. Control Fed., 1973, 45, 905-12.

Table I. Comparison of Colorimetric Ammonia Determinations on Filtered and Unfiltered Oil Shale Wastewaters[1]

Process Water	Filtered mean	Filtered rsd (%)	Unfiltered mean	Unfiltered rsd (%)	unfiltered: filtered (% diff)[2]
Paraho	23 750	0.7	24 655	0.7	3.7
150-Ton	11 180	1.1	10 516	1.1	-6.3
Oxy-6 gas condensate	6 933	1.0	6 994	0.7	0.9
S-55	4 005	2.0	4 188	1.5	4.4
Omega-9	3 551	4.9	3 638	1.4	2.4
TV	2 346	6.9	2 097[3]	1.4	-11.9
Geokinetics	1 505	1.8	1 589	0.6	5.3
Oxy-6 retort water	1 136	0.9	1 117	0.9	-1.7
Rio Blanco sour water	1 032	1.1	1 061	1.1	2.7

[1] mg/L NH_3-N; n=10 for each sample

[2] (colorimetric unfiltered mean) - (colorimetric filtered mean) divided by (colorimetric unfiltered mean)

[3] second operator

Table II. Comparison of Colorimetric and Titrimetric Methods for Determining Ammonia in Unfiltered Oil Shale Wastewaters[1]

	Titrimetric		Colorimetric	
Process Water	mean	rsd (%)	mean	rsd (%)
Paraho	26 385	1.3	24 689	1.0
150-Ton	10 838	1.3	10 662	0.7
Oxy-6 gas condensate	7 157	0.1	7 202	0.7
Composite	6 564	0.5	6 225	4.0
S-55	4 079	0.08	4 047	1.4
Omega-9	3 690	0.4	3 583	1.1
TV	2 401	0.1	2 292	1.2
Geokinetics	1 991	1.0	1 905	0.7
Oxy-6 retort water	1 161	0.5	1 127	0.9
Rio Blanco sour water	1 118	0.3	1 065	0.9
average[2]	6 536		6 286	

[1] mg/L NH_3-N; n=5 for each sample

[2] mean for nine waters, excluding Composite

Table III. Standard Additions Results[1]

	Colorimetric					Titrimetric				
Process Water	m	b	r^2	zero spike[2]	percent recovery[3]	m	b	r^2	zero spike[2]	percent recovery[3]
Paraho	0.967	484	1.000	485	96.9	1.065	543	1.000	537	105.3
Composite	0.987	490	1.000	504	101.5	1.037	534	0.998	532	103.3
Oxy-6 gas condensate	0.964	478	1.000	484	97.6	1.040	518	0.999	514	103.2

[1] ammonia-N spike levels of 100, 300, 600, and 800 mg/L (n = 2)

[2] ammonia concentration of diluted sample (i.e., without spike)

[3] (zero spike) divided by b/m X 100

m = regression coefficient (slope)
b = y-intercept
r^2 = coefficient of determination

PROTOCOL: COLORIMETRIC PHENATE AMMONIA

I. Apparatus
   A. Glassware (one each per sample)
      1. 20- X 150-mm or 16- X 150-mm culture tube with Teflon-lined screwcap; Parafilm "M" may be used instead of screwcaps. The culture tubes should be soap-washed followed by acid washing in 35-percent nitric acid. Rinse with ASTM Type I water. Screwcaps should be soap washed, then rinsed several times with ASTM Type I water (Never contact screwcaps with acid). Do not use screwcapped tubes with chipped rims, as they will leak when shaken.
      2. Drummond Microcap 20-microliter capillary (Drummond Scientific Co., Broomall, PA); substitutes must be calibrated "to contain".
   B. Repetitive dispensing device (e.g., 8.5-mL Manostat Pistolpet, Manostat, New York, NY) or automatic pipette (e.g., 5-mL Gilson Pipetman, Rainin Instrument Co., Emeryville, CA).
   C. Water bath with heater (e.g., Haake D-1, Haake Inc., Saddle Brook, NJ).
   D. Forceps, preferably flexible.

II. Reagents (Note: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality).
   A. Phenol/Nitroprusside Solution: dissolve 5 g phenol and 25 mg sodium nitroferricyanide (nitroprusside) dihydrate [$Na_2Fe(CN)_5NO \cdot 2H_2O$] in water in a 500-mL volumetric flask and bring to volume with water. Store in amber or foil-covered bottle (4 °C). Discard after one month.
   B. Alkaline Hypochlorite Solution: dissolve 2.5 g sodium hydroxide in water in a 500-mL volumetric flask, add 4.2 mL of 5 percent sodium hypochlorite solution (J.T. Baker or equivalent) and bring to volume with water. Store in amber or foil-covered bottle at 4 °C. Discard after one month or when high blank values or change in slope of calibration curve are observed.
   C. Ammonia-Nitrogen Standard Stock Solution (1000 ppm-N): dissolve 0.4717 g ammonium sulfate in water and dilute to 100 mL, or use Hellige standard solution R-2434 (1 mL = 1 mg N) (Hellige, Inc., Garden City, NY) or equivalent. Store at 4 °C.

III. Protocol
   A. Dilute sample concentration to within a 10- to 100-ppm or 100- to 1000-ppm NH_3-N range.
   B. Prepare working standards.
      1. For the 10- to 100-ppm and 100- to 1000-ppm ranges, add the following quantities of the Standard (II.C.) and water to a suitable container (e.g., scintillation vials):

working standard (mg-N/L)	Standard (mL)	water (mL)
blank	0.00	10.00
10.0	0.100	9.90
20.0	0.200	9.80
50.0	0.500	9.50
100	1.000	9.00
200	2.000	8.00
500	5.000	5.00
1000	10.00	0.00

C. Place tubes for reagent blank, standards and samples in test tube rack. Samples and standards should be prepared and developed together.
D. Adjust water-bath temperature to 37 °C.
E. Add 5 mL phenol/nitroprusside solution to each tube; an automatic pipette or repipette is recommended for convenience.
F. Fill capillary (held in forceps) with sample or standard and drop into tube containing phenol/nitroprusside solution without contacting rim of tube.
NOTE: see "Protocol Summary: Colorimetric Phenate Method" for discussion on filling capillaries.
1. seal tube immediately and shake vigorously about 10 times. (No more than 1/2 hour should be allowed to elapse before immersion of all samples in the water bath; the number of samples that can be prepared in this interval will depend on the operator's speed). Repeat for all tubes.
NOTE: breakage of the capillary during shaking is inconsequential.
G. Immediately after adding 5 mL alkaline hypochlorite solution to each tube, replace cap and shake vigorously.
NOTE: vortex mixing is inadequate to flush the capillaries.
H. Immerse test tube rack in water bath for 20 minutes.
I. Cool to approximately room temperature with cold tap water and promptly read absorbance of each sample against a water blank (to allow detection of high blanks) at 635 nm (for 10- to 100-ppm NH_3-N range) or at 520 nm (for 100- to 1000-ppm range).
NOTE: in our laboratory, absorbance of blanks is usually less than 0.015 AU at 520 nm. A spectrophotometer with a flow-through cell is recommended.

IV. Waste Disposal

A. Ensure that the contents of the assay tubes are separated from the capillaries before disposal.

V. Data Reduction

Subtract absorbance of the blank from that of each standard or sample. Calculate least-squares regression equation for standards. Over the ranges of 10 to 100 ppm and 100 to 1000 ppm NH_3-N, response should be sufficiently linear to allow use of the regression equation for determining sample values (routinely $r^2 = 0.999+$). Between 1000 and 3000 ppm, the curve is usable, though non-linear, and curve fitting methods are recommended. Multiply result by appropriate dilution factor.

Example:

	A_{520}	blank subtracted
Blank	.008	---
100 mg NH_3-N/L	.064	.056
200	.122	.114
500	.316	.308
1000	.628	.620

NH_3-N (mg/L) = 1589(A_{520}) + 14, $(r^2 = 0.999+)$

sample absorbance	blank subtracted	NH_3-N (mg/L) calculated from regression equation
.472	.464	751
.464	.456	739
.469	.461	747

Protocol prepared by: J. Cantor, B.M. Jones, R.H. Sakaji, and C.G. Daughton

PROTOCOL: DISTILLATION/TITRIMETRIC AMMONIA

I. **Apparatus**

A. Glassware (one each per sample)
   1. 500-mL Kjeldahl flask (discard any Kjeldahl flask with star-shaped cracks)
   2. 250-mL Erlenmeyer flask (or sampling cup for autotitrator); the masses of these containers should be within several grams of each other

B. 50-mL tilting dispenser

C. Volumetric flasks (1-L, 250-mL; Class A)

D. 100-mL graduated cylinder (TD)

E. Magnetic stirring bar (one each per sample for manual titration)

F. Top-loading electronic balance (e.g., Mettler PC 2000)

G. pH meter with pH probes (e.g., Corning model 135); for detection of pH endpoint during manual titration

H. Titration equipment
   1. Option 1: Automatic titrator: e.g., Sybron/Brinkmann (Westbury, NY) Autotitrator (Metrohm model 655 Dosimat, E 526 titrator, 643 control unit/624 auto sampler, and appropriate electronic burette, e.g., 10- or 20-mL).
      a. combination pH electrode (Metrohm EA 157); for automated titration to pH endpoint, or
      b. submersible colorimeter probe with 1-cm path length probe tip, 545 nm filter, and colorimeter (Brinkmann PC 800) for automated titration to indicator endpoint.
   2. Option 2: 50-mL precision-bore burette.

I. Kjeldahl distillation apparatus (e.g., 12-unit Labconco combination digestion/distillation); it is highly recommended that Pyrex condenser tubes with Teflon ferrules and fittings be used rather than block tin condenser tubes.

J. Zetex insulated gloves

IMPORTANT: The Kjeldahl distillation unit must **never** be plumbed with silicone rubber stoppers or tubing because of the permeability of this rubber to ammonia gas; neoprene Kjeldahl stoppers are highly recommended. It is also very important to ensure that ammonia vapors are not present in the lab.

CAUTION: Safety face-shields or glasses with side-shields should be worn while performing this assay.

II. **Reagents** (Note: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality).

A. Borate Buffer Solution: add 88 mL of 0.1 N NaOH (prepared from Hellige standard R-1226C) to approximately 500 mL of 0.025 M sodium tetraborate solution (5.0 g $Na_2B_4O_7$ or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ per L) in a 1-L volumetric flask and bring to volume with water; store in polyethylene or polypropylene.

B. Boric Acid Receiving Solution: dissolve 40 g of H_3BO_3 in water in a 1-L volumetric flask and bring to volume with water; store in polyethylene or polypropylene.
   1. Indicator/Boric Acid Receiving Solution (optional for titration to indicator endpoint): add 10 mL of indicator (II.C.) before bringing to volume.

C. Mixed-Indicator Solution (for titration to indicator endpoint): dissolve 300 mg of methyl red (acid) indicator and 200 mg of methylene blue in 250 mL of 95-percent ethanol. Fresh indicator should be made every month.

D. 0.02 N H_2SO_4 Titrant: use commercially prepared concentrate (e.g., Hellige R-1241C); alternatively, prepare a 0.1N sulfuric acid stock solution (e.g., from Hellige R-1238C) and dilute 250 mL (quantitatively transferred from 250-mL volumetric flask) of this solution to 1 L.

E. Phenolphthalein Indicator: dissolve 0.5 g phenolphthalein in 50 mL of 95-percent ethanol, add 50 mL water, and mix.

F. Sodium Hydroxide Solution (6N): carefully dissolve 240 g NaOH in 800 mL of water (1-L volumetric flask) and bring to volume; store in screwcapped polypropylene bottle.

III. Protocol

A. Preparation of apparatus and glassware

1. Steam apparatus clean; prior to each run, place 300 to 400 mL water and 1 or 2 boiling chips or glass beads in each Kjeldahl flask and attach to distillation apparatus. With cooling water off, distill approximately 150 mL; collect distillate in any convenient container and discard.
2. all glassware should be soap-washed followed by acid washing in 35-percent nitric acid. Rinse with ASTM Type I water.

B. Receiving flasks: measure 50.0 mL of boric acid solution (tilting dispenser) into each 250-mL Erlenmeyer flask (or sampling cup) and place on Kjeldahl rack so that the distillation effluent tube-tips are completely submerged in the solution; use boric acid solution for pH endpoint or indicator/boric acid solution for indicator endpoint.

C. Samples: place boiling chips or glass beads and appropriate volume of sample in Kjeldahl flask. Sample volume may be adjusted to optimize titrant volume based on expected sample concentration; a 5-mL sample containing 1000 ppm ammonia-N should require 17.86 mL of 0.02 N H_2SO_4 titrant; the mass of ammonia-nitrogen added to the sample flask should not exceed 5 mg, nor should it be lower than 0.1 mg. The quantities of ammonia in each flask should be equalized as best as possible.

1. Add water to bring the total volume to approximately 300 mL; add 2 drops of phenolphthalein and 10 mL of borate buffer solution (automatic pipette recommended for convenience, but the volume is not critical).
2. Randomize flasks by consulting the "Ten Thousand Random Digit" Table; (Table "0" in Statistical Tables, F.J. Rohlf and R.R. Sokal, W.H. Freeman and Co.: San Francisco, CA; pp. 152-156, 1969).
   a. randomly select a group of five digits from the table.
   b. pick any two adjacent digits within the group of five.
   c. for two-digit random numbers, begin with the pair selected, move horizontally, and record the next two consecutive digits; continue this process in a consistent manner until two-digit numbers are generated for each flask.
   d. record the random numbers and the corresponding sample number.
   e. relabel each flask with its assigned random number.

3. Slowly add several drops of NaOH solution down the inner side of each Kjeldahl flask so that it layers beneath the aqueous sample, and **immediately** connect to distillation/condenser apparatus. Place flasks on distillation unit in ascending order of assigned random numbers. Ensure that the stopper is firmly seated. Swirl the flask contents to mix.

D. Standards: use Hellige 1000-ppm ammonia-N standard R-2434 or equivalent (e.g., ammonium sulfate) and prepare as in III.C.

E. Blank (distillation): omit sample (substitute water) and prepare the flask contents as in III.C.

F. Blanks (nondistilled): prepare three as in III.B. using the Boric Acid Receiving Solution prepared in II.B., but do not connect to distillation tubes; these will be used to determine either the pH endpoint (IV.C.2.i.) or the colorimetric indicator endpoint (IV.B.2.i.). Allow these blanks to sit under the same conditions as the receiving flasks (i.e., exposed to the air).

G. Distillation

1. Turn on condenser cooling water and set heating mantles to maximum temperature. After samples begin to boil, reduce heat as necessary to control boiling and foaming.
2. Collect approximately 100 mL of distillate. Though considerable variation in the volume collected is allowed, the distillate volumes must be equalized before titration. Distillation must not progress too long because the distilland can become concentrated and viscous; this can lead to explosive bumping over into the condenser tubes.

NOTE: ensure that the distilland retains a pinkish hue during distillation.

3. Upon completion of the distillation, remove the effluent tubes from the receiving-container solutions and rest the tubes on the flask rims to prevent sample drawback during cooling. Turn off heat and continue collection of distillate until distillation ceases.
4. Place each receiving vessel on the balance and add water until the contents of each is within a gram in weight (e.g., 200 g); alternatively, dilute all samples to equivalent volumes (e.g., 200 mL).

H. Manual Titration (also see option at: IV. Automatic Titration)

1. Endpoint detection by pH indicator
   a. add stirring bar to each flask
   b. if the indicator/boric acid receiving solution (II.B.1.) was not used, add 0.2 mL of mixed-indicator solution (II.C.) to each 200-mL distillate and nondistilled blank.
   c. titrate with 0.02 N H_2SO_4 titrant until the color matches that of the nondistilled blanks. Samples containing sufficient ammonia will begin bright green and progress through darker shades of blue-green until the grayish transition point is reached; the sample will then gradually develop more intense hues of violet. The blank should require no more than one drop of titrant.

NOTE: the endpoint is **not** sharp.

2. Endpoint detection by pH electrode

NOTE: do not use indicator in the receiving container solutions.

a. add stirring bar to each flask.

b. determine the average pH of the three nondistilled blanks prepared in III.F.; this value is approximately 4.8 if the samples have been diluted to 200 mL.
c. titrate samples with 0.02 N H_2SO_4 titrant until the pH of the blanks is reached.

IV. **Automatic Titration** (instructions for Sybron/Brinkmann Metrohm Autotitrator)

A. Setup

1. Fill rinse-water reservoir with water and plug solenoid into outlet "J" on the back of the 643 control unit.
2. Install burette containing 0.02 N H_2SO_4.
3. Connect HP 97S calculator to external power supply and connect Amphenol connector to 643 control unit.
4. Switch 643 control unit to "manual".
5. Turn on the following units in sequence: titrator E 526, Dosimat 655, control unit 643, and calculator.

B. Indicator Endpoint Calibration

1. E 526 titrator:
   a. plug shorting strap into the reference and indicator electrode inputs
   b. set: thumbwheels to 0000,
      calibration knob to "calibr",
      selector knob to +mV
   c. turn "U" knob until analog meter is nulled

   NOTE: it is important that the setting of the "U" knob not be touched once it is set.
   d. disconnect shorting strap
2. PC 800 Colorimeter:
   a. connect colorimeter leads; black to reference electrode input and red to indicator electrode input.
   b. install 545-nm filter and turn on colorimeter power switch to %T; allow at least 5 minutes for warmup.

   Warning: NEVER turn on the colorimeter without a filter in the filter block.
   c. ensure that the colorimeter probe tip is the appropriate path length.
   d. adjust colorimeter to 100 %T with coarse/fine knobs.
   e. thoroughly rinse colorimeter probe.
   f. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise. Avoid vortex formation to prevent air bubbles from becoming entrapped in the colorimeter probe tip.
   g. use sample cup that contains the most ammonia (i.e., brightest green) to set 100% T on colorimeter.
   h. determine transmittance (545 nm) of three nondistilled blanks from III.F.
   i. average these transmittance values and set on titrator thumbwheels (e.g., for an average transmittance value of 40.2 percent, the thumbwheels are set to read 0402); this setting will be about 0400-0440.
   j. set calibration knob to first -mV setting.
3. Proceed to IV.D.

C. pH Endpoint Calibration

NOTE: do not use indicator in the receiving boric acid solutions.

1. E 526 Titrator:
   a. set selector knob to "pH" and calibration knob to "calibr".
   b. connect pH electrode lead to indicator electrode inlet jack of E 526 titrator.
2. pH Calibration:
   a. open filler port on pH electrode.
   b. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise.
   c. set thumbwheels to 0700, raise sample cup containing pH 7.00 buffer, turn mixer on, and null analog meter with "U" knob.
   d. lower sample cup, stop mixer, rotate table, and rinse electrode.
   e. set thumbwheels to 0401 and raise sample cup containing pH 4.01 buffer.
   f. null meter using the slope correction knob (located with the temperature knob on the far right of the control panel; slope correction is the inner knob).
   g. set calibration knob to "pH 0" and turn mixer on.
   h. lower sample cup, turn off mixer, and rinse electrode.
   i. set thumbwheels to match the average pH of the three nondistilled blanks (III.F.); this setting will be about 0482, which corresponds to a pH of 4.82.
3. Proceed to IV.D.

D. Operation

1. Reconnect the mixer to the 643 control unit and rotate mixer knob to the "on" position. Adjustment can be made once the control unit is engaged (IV.D.9.).
2. Close control panel cover.
3. Ensure that the turntable surface is clean.
4. Place samples on turntable and record their positions; the 624 control unit only holds 10 samples and the calculator program can only accommodate 10 samples before it must be restarted.
5. Load "Basic Titration Program" and depress "E" button to initiate.
6. Set titration rate knob on 655 Dosimat between "5" and "6".
   a. if samples contain low concentrations of ammonia, then the speed must be accordingly decreased to prevent overtitration.
7. Set 643 control unit to "auto".
8. Ensure that the titrator delivery-line diffuser is installed.
9. Press "Start" switch on 643 control unit.

NOTE: The titration sequence can be stopped between samples by switching the 643 control unit to "stop"; to restart, depress the calculator "E" button.

E. Shutdown: reverse order of sequence at IV.A.5.

V. Data Reduction

$$\text{ammonia nitrogen (mg/L)} = \frac{(\text{sample titrant volume (mL)} - \text{blank titrant volume (mL)}) \times \text{normality of acid (meq/mL)} \times 1000 \text{ mL/L} \times 14 \text{ mg/meq}}{\text{sample volume (mL)}}$$

Protocol prepared by: C.G. Daughton, G. Harris, B.M. Jones, and R.H. Sakaji

Chapter IV

COLORIMETRIC AND TITRIMETRIC QUANTITATION OF CHEMICAL OXYGEN DEMAND IN OIL SHALE WASTEWATERS

B.M. Jones, R.H. Sakaji, and C.G. Daughton

Introduction 2
Theory 2
Chemical Oxygen Demand 2
Oxidant 2
Interferences 3
Digestion 4
Quantitation by Redox Titrimetry 4
Quantitation by Colorimetry 5
Specific COD 5
Oil Shale Wastewaters and Theoretical COD 6
Protocol Summary 7
Macro-Titrimetric COD 7
Micro-Colorimetric COD 8
Comparison Study 9
References 11
Tables
I. Reported COD Recoveries from Nitrogen and Oxygen Heterocycles 13
II. Specific COD of Oil Shale Process Waters 14
III. Comparison of Reagent Concentrations for Four COD Methods 15
IV. Advantages and Disadvantages of Five COD Methods 16
V. Comparison of Macro-Titrimetric and Micro-Colorimetric Methods for Determination of COD in Filtered Oil Shale Process Waters 17
VI. COD Recovery from Potassium Hydrogen Phthalate Standards 18
VII. Standard Additions Results 19
Figures
1. Absorbance Scans of Digested Phthalate Standards (mg/L COD) 20
2. Absorbance Changes around the Isosbestic Point (A_{535}) during Titration of Dichromate Ion in the Presence of Ferroin Indicator 21
3. COD Standard Curve at A_{600} 22
Protocol: Macro-Titrimetric COD 23
Protocol: Micro-Colorimetric COD 29
Appendix A
Autotitration Program-COD 32-35

INTRODUCTION

The chemical oxygen demand (COD) test was first applied as a rapid estimator of the biochemical oxygen demand (BOD) of wastewater organic material (Moore, Kroner, and Ruchhoft, 1949). Of numerous possible oxidants, four have been used for determining the oxygen demand of aqueous effluents: potassium permanganate, potassium dichromate, ceric acid, and iodic acid. Of these four, potassium dichromate in sulfuric acid is the prescribed method in the United States. Specific correlations have been developed to relate the COD of a waste to its five-day BOD (Rhame, 1947). The correlations between BOD and COD must be applied with caution because potassium dichromate does not oxidize ammonia; therefore, the COD value does not reflect the contribution of ammonia to ultimate BOD. The routine method for COD determination has been revised (i.e., by the addition of silver as a catalyst) to yield oxidation of certain biodegradable compounds (i.e., aliphatic hydrocarbons, straight-chain alcohols, and fatty acids) that are not fully susceptible to the chemical oxidant. In addition to saving time, the COD test was recognized as being extremely valuable for determining the oxygen demand of wastes that are toxic to microorganisms. Chemical oxygen demand is often determined in lieu of BOD and has achieved an intrinsic meaning separate from its biochemical counterpart.

THEORY

Chemical Oxygen Demand

The numerical value obtained from a COD test reflects in theory both the carbon concentration and the overall oxidative state of the oxidizable organic material. The COD of a sample is defined by the absolute amount of hexavalent chromium that is reduced during two hours of digestion by potassium dichromate in a solution of 50-percent sulfuric acid. Ideally, organic compounds are completely oxidized to carbon dioxide and water with the simultaneous stoichiometric reduction of the orange hexavalent dichromate ion (Cr +VI) to the green trivalent chromic ion (Cr +III). The degree of reduction is quantified either by colorimetry (i.e., by determination of the remaining dichromate ion or, alternatively, by determination of the newly produced chromic ion) or by titrimetry (i.e., redox titration of the remaining dichromate ion). This value is then related stoichiometrically to oxygen equivalents. The reduction of each mole of Cr(VI) to Cr(III) is equivalent to the consumption of 1.5 moles of O_2; the results therefore can be expressed in terms of milligrams of O_2 per liter ($mg\text{-}O_2/L$) even though oxygen has no direct role in the chemical oxidation process.

Soluble COD (SCOD), the COD of dissolved species, is of primary interest because of (i) the availability of dissolved organic compounds to microorganisms and (ii) the minimization of sampling error. For these reasons, our laboratory has limited itself to the determination of SCOD. An extensive discussion of the problems associated with filtration is included in Chapter II.

Oxidant.

Potassium dichromate is used both as a source of Cr(VI) and as a primary standard for determining the normality of the titrant in the COD test. Potassium dichromate is preferred over other oxidants because it can be highly purified, it is not hygroscopic, and it can be dried without decomposition (Waser, 1966). In aqueous solution, two forms of hexavalent chromium exist: chromate ion CrO_4^{2-} and dichromate ion $Cr_2O_7^{2-}$ (Latimer and Hildebrand,

1951). The equilbrium between the two species is represented by:

$$2\ CrO_4^{2-} + 2\ H^+ \rightleftharpoons Cr_2O_7^{2-} + H_2O \qquad K = 1.2 \times 10^{14}$$

The highly acidic sulfuric acid solution of the COD digestion reagent ensures that the predominant species is the very potent oxidant dichromate ion (half-reaction potential = 1.33 volts). The acidic environment and elevated temperatures of the COD assay are essential for dichromate to mineralize carbonaceous material to CO_2 and H_2O (Sawyer and McCarty, 1967).

Organic compounds, however, are not equally susceptible to dichromate oxidation; aliphatic hydrocarbons, straight-chain organic acids, and alcohols, for instance, are resistant to oxidation unless silver is added as an oxidation catalyst (Standard Methods, 1981). Even then heterocyclic compounds, such as many nitrogen and oxygen heterocycles, are incompletely oxidized by the prescribed procedure (Table I) (Gibbs, 1979; Standard Methods, 1981).

Interferences.

Certain inorganic compounds consume oxidant and therefore exert a COD. Nitrites, sulfites, sulfides, and ferrous ions are oxidized by dichromate (ASTM, 1980) and are calculated as part of the oxygen demand. Each mole of thiosulfate loses eight equivalents of electrons when completely mineralized (Wong and Mercer, 1979); this is equivalent to the consumption of two moles of oxygen per mole of thiosulfate.

$$S_2O_3^{2-} + 2\ O_2 + H_2O \longrightarrow 2\ SO_4^{2-} + 2\ H^+$$

Chloride represents the most serious inorganic interference to the determination of COD. Chloride ion will precipitate the silver catalyst as silver chloride; this both reduces the effectiveness of the catalyst and obscures the titrimetric endpoint (Dobbs and Williams, 1963). Secondly, chloride ion is oxidized by dichromate, giving a positive interference to the assay (Moore et al., 1949). This oxidation is not stoichiometric in the presence of organic material (Dobbs and Williams, 1963; Gibbs, 1979); moreover, the oxidation of chloride can generate a second oxidant, gaseous chlorine, which in turn may react with organic components in a sample and thereby lower the apparent COD (Dobbs and Williams, 1963). Although reduced nitrogen such as ammonia does not exert a COD (Foulds and Lunsford, 1968), the combination of chloride ion and high concentrations of ammonia, or even organic amines or nitrogenous compounds, can lead to the continuous reduction of dichromate by a cyclic series of changes from chloride to chlorine (Dobbs and Williams, 1963). Chlorine that is produced from oxidation of chloride ion by dichromate reacts with ammonia to form chloramines; if the molar ratio of chlorine to ammonia exceeds unity, chloride ion is produced (Jenkins et al., 1976) and reinitiates further reduction of dichromate. Therefore, the combination of these two inorganic constituents in a sample can lead to a tremendous positive interference.

To minimize the involved interactions of chloride ion, mercuric sulfate is added to complex the chloride, essentially protecting it from oxidation and preventing its interaction with silver ion (Standard Methods, 1981). It has been noted that mercuric sulfate is not completely soluble in the cooled digestion mixture (Gibbs, 1979; Jirka and Carter, 1975; unpublished observations). The precipitate that develops can interfere with quantitation by clouding the titrimetric endpoint or by contributing severe noise to the spectrophotometric determination (Jirka and Carter, 1975; unpublished observations).

Digestion.

The COD digestion mixture includes sample, dichromate reagent, and acid/catalyst solution. Volatile compounds may be lost from the sample during the addition of acid/catalyst because of the heat of mixing, unless certain precautions are followed. The acid must be added carefully by allowing it to run down the inside of the flask or tube, and the contents should not be mixed until connected to a reflux condenser or capped. For two hours, the mixture is then either maintained at 150 °C (micro-colorimetric COD) or refluxed (macro-titrimetric COD). With the latter method, the sample and reagents reach a rapid boil and a portion of the volatile gases are captured during refluxing. It has been postulated that this refluxing may lead to more complete mixing of the sample and therefore better digestion of solids if total COD is being determined (Messenger, 1981). A major disadvantage of the macro reflux/titrimetric method is the loss of volatile compounds during refluxing. This loss can be eliminated by the use of sealed tubes, but in the absence of refluxing, unoxidized volatile compounds can still escape from the digestion mixture into the headspace, and the mixing of particulates is impeded.

Quantitation by redox titrimetry.

At the conclusion of two hours of digestion, the extent of Cr(VI) reduction can be determined by either redox titration or spectrophotometry. The titrimetric procedure involves the reduction of the remaining Cr(VI) with ferrous ion, which is provided as the standardized titrant, ferrous ammonium sulfate (FAS): $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$.

$$6\ Fe^{2+} \longrightarrow 6\ Fe^{3+} + 6\ e^-$$

$$Cr_2O_7^{2-} + 14\ H^+ + 6e^- \longrightarrow 2\ Cr^{3+} + 7\ H_2O$$

The COD is calculated by subtracting the volume of FAS required for sample titration from that required for a digested blank. The difference is a measure of the amount of Cr(VI) that was reduced by the sample. This difference is multiplied by the product of (i) the normality (N) of the FAS (determined daily by titration of a nondigested blank, i.e., normalization blank), (ii) the molecular weight of oxygen, and (iii) 1000 mg/g. The entire product is then divided by the product of the sample volume (S) and the electron equivalents transferred per mole of oxygen.

$$COD = \frac{N \times (\text{blank titrant} - \text{sample titrant}) \times 32\ g\text{-}O_2/mol \times 1000\ mg/g}{S \times 4\ eq/mol}$$

Recognition of the endpoint is assisted by the addition of a redox indicator, 1,10-phenanthroline, which forms a red undissociated cation when complexed with divalent metal ions (e.g., ferrous ion). At the completion of titration, when dichromate is no longer present to oxidize excess ferrous ion from the titrant, the ferrous ion-phenanthroline complex forms, and the color of the reaction mixture abruptly changes from green (typical of Cr +III) to red. Formation of sufficient ferrous ion-phenanthroline complex to color the solution red is actually indicative of overtitration; the true endpoint, which is gray, exists when all of the dichromate has been reduced, but before excess ferrous ion has been added. The amount of ferrous ion required to go from transition to endpoint, however, is very small.

The indicator is added to the digestates as the ferrous-chelated form of 1,10-phenanthroline (known commercially as "ferroin"). The ferroin should be

added in reproducible quantities since the chelated ferrous ion will reduce dichromate. In addition, the oxidation of one mole of phenanthroline would consume 14 moles of oxygen (0.15 mL of ferroin would have a COD of 253 mg/L); the oxygen demand of the nitrogenous heterocycle indicator would probably be minimal because of the lack of susceptibility of this class of chemical compounds to dichromate oxidation.

The COD titration lends itself easily to automation. The molar absorptivity of the two chromium species is equivalent at the isosbestic point (535 nm) (Fig. 1 and inset); therefore, at this wavelength, any change in absorbance during the titration of Cr(VI) must result from the appearance of another chromophore. The appearance of the ferrous ion-phenanthroline complex at the endpoint immediately changes the absorbance (Fig. 2), indicating completion of titration. A colorimeter whose wavelength is set near the isosbestic point can detect this change in absorbance and can control titrant addition, if connected to an autotitrator (e.g., Sybron/Brinkmann, Westbury, NY; Metrohm model 655 Dosimat with appropriate accessories).

Quantitation by colorimetry.

An alternative method for quantitating COD is by direct colorimetry. The COD of an unknown can be interpolated directly from a standard curve that is constructed from the absorbance of either the amount of orange Cr(VI) remaining (440 nm) or the amount of green Cr(III) produced (600 nm) from the digestion of standards. Although the volume of each digestate must be reproducible in such a colorimetric analysis, the quantity of Cr(VI) added to each sample is not critical when Cr(III) is quantitated, while it is when Cr(VI) is measured.

For samples with low chemical oxygen demand (e.g., 0 to 250 mg/L), the disappearance of Cr(VI) should be quantitated directly. The micro-colorimetric COD method is eight times more sensitive for the detection of Cr(VI) in the 400-nm range than for the detection of Cr(III) in the 600-nm range because of differences in the molar absorptivities of the two species (see: Fig. 1). For the high-range samples (250 to 850 mg/L), the appearance of Cr(III) should be followed (i.e., A_{600}).

Two significant problems have been encountered with the colorimetric determination of COD: (i) the appearance of catalyst-induced precipitates of mercuric and silver salts (Gibbs, 1979; Jirka and Carter, 1975; Messenger, 1981; unpublished observations) and (ii) "stratification" of the acid-water mixture following digestion (Messenger, 1981). When a temperature gradient exists in the digestate, schlieren lines form. This severely interferes with colorimetric quantitation because the differential refractive indices cause substantial drift and inaccurate spectrophotometric readings (unpublished observations). We have found that extensive mixing of the tube contents following digestion and a prolonged cooling period reduces the appearance of schlieren lines and allows the analyst to sample the tube contents without aspirating the catalyst-induced precipitates.

A semi-micro titrimetric method that uses sealed tubes instead of refluxing flasks has been reported (Himebaugh and Smith, 1979). This method affords many of the advantages of the micro-colorimetric method while permitting titrimetric quantitation.

Specific COD

Chemical oxygen demand is often misinterpreted as an estimate of the total concentration of organic solutes. Rather, in the absence of oxidizable inorganic solutes, it is a vague measure of both the quantity of organic solutes and their associated oxidative states. When SCOD is normalized to dissolved organic carbon (DOC) concentration, however, the ratio yields considerable

information about the types of chemicals that are present in a heterogeneous sample matrix. The amount of COD yielded per unit of organic carbon is defined as "specific COD" (Daughton, Jones, and Sakaji, 1981). The higher the average reductive state of the compounds, the higher will be the specific COD. The two extremes for this concept are represented by CHOOH and CH_4, which contain the same amount of carbon on a molar basis yet their theoretical specific COD values span the range available to organic material, i.e., 1.33 and 5.33, respectively. The specific COD values for oil shale process waters range from 3.05 to 5.01 (Table II).

The specific COD of a heterogeneous sample may be higher than 5.33; this would indicate that inorganic constituents of the sample matrix have contributed a "positive interference" to the COD test or that there was a severe problem with the DOC determination (e.g., incomplete recovery). An abnormally low specific COD indicates either that (i) a substantial portion of the sample was resistant to oxidation by dichromate but was susceptible to combustion or oxidation for the DOC determination or (ii) inorganic carbon was mistakenly included in the determination of the organic carbon.

Oil Shale Wastewaters and Theoretical COD

The determination of the COD of oil shale process waters may be confounded by the particulate turbidity and numerous chemical classes that typify these wastewaters. Process waters can contain thiosulfate, chloride, and ammonia; up to 20 percent of the COD of a retort water was reportedly contributed by thiosulfate (Wong and Mercer, 1979). The characteristic organoleptic properties of these waters are contributed by nitrogen and oxygen heterocycles, which are only partially accounted for by the COD test because of their resistance to dichromate digestion (Table I). Heterocyclic triazines are particularly resistant to oxidation (see: Chapter II).

For any COD analysis, the anticipated oxygen demand must be estimated so that the capacity of the oxidant is not exceeded. It is advisable to run several dilutions of a sample if the COD is unknown. Alternatively, if the DOC is known, the COD can be estimated by assuming a specific COD of about 3.6 for oil shale wastewaters. To determine the theoretical COD, the compound of interest is assumed to be mineralized completely to CO_2, H_2O, SO_4^{2-}, and other mineral species. Examples:

Potassium Hydrogen Phthalate:

$$C_8H_5KO_4 + 7.5\ O_2 + H^+ \longrightarrow 8\ CO_2 + 3\ H_2O + K^+$$

1 mole of KHP theoretically consumes 7.5 moles of O_2;
100 mg/L of KHP should exert an oxygen demand of 118 mg/L.

Glucose:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

1 mole of glucose theoretically consumes 6 moles of O_2;
100 mg/L of glucose should exert an oxygen demand of 107 mg/L.

Pyridine:

$$C_5H_5N + 6.25\ O_2 \longrightarrow 5\ CO_2 + 2.5\ H_2O + 0.5\ N_2$$

1 mole of pyridine theoretically consumes 6.25 moles of O_2;
100 mg/L of pyridine should exert an oxygen demand of 252.8 mg/L.

The calculations of theoretical COD for nitrogen heterocycles are complex because it is unclear if the hetero atom is oxidized by dichromate; the literature is not in agreement. For example, as to the fate of nitrogen in pyridine, Dobbs and Williams (1963) assume that the nitrogen in pyridine is reduced to ammonia, whereas the values calculated by Moore et al. (1949) presume that the nitrogen would be released as nitrogen gas. The actual theoretical COD is impossible to verify because pyridine is not oxidized by dichromate, and other nitrogen heterocycles (e.g., quinoline) are only partially oxidized (Table 1).

PROTOCOL SUMMARY

Laboratory protocols for determining COD by reflux/titrimetric and colorimetric methods are appended. The importance of representative subsampling and laboratory safety cannot be overemphasized. The use of safety equipment and proper technique for dispensing reagents (i.e., mouth pipetting is absolutely unacceptable) and the proper disposal of the digestate following titration should be primary concerns to the analyst.

Macro-Titrimetric COD

Accurate and precise addition of the dichromate reagent and careful titration are critical for accurate and reproducible results. Two glass beads and 12.00 mL of dichromate reagent are added to each reflux flask. In contrast to the protocol detailed in Standard Methods (1981) it is better to dissolve the mercuric sulfate in the dichromate reagent rather than dispensing it as a solid. This minimizes the hazard of inhaling mercurial dust and increases the precision of the mercuric sulfate addition. Twenty milliliters of an appropriate dilution of the sample is added. The COD of a 20-mL sample should be between 50 and 500 mg/L for best results. Acid/catalyst solution consists of concentrated sulfuric acid and 10 g/L silver sulfate; 28 mL is added very carefully to the sample and the flask is **immediately** connected to the appropriate condenser to minimize the loss of volatile components. The addition of the acid/catalyst solution through the condensers, as recommended by Standard Methods (1981), is very awkward and potentially dangerous; a double-necked flask would be preferred. The contents of the flask should then be very carefully mixed to prevent localized superheating which could result in violent expulsion of the flask contents (U.S. EPA, 1979). Two sets of blanks are also prepared. The normalization blanks are not digested and are used to determine the normality of the titrant. The digestion blanks are heated with the samples and are used for determining the oxygen demand of the dilution water and reagent contaminants. It is important to randomize the positions of all the flasks on the hot plate to eliminate bias from the temperature differential across the plate surface. The samples and digestion blanks are then refluxed for two hours and the amount of dichromate that remains in the cooled digestates is determined by titration with 0.10N FAS to the gray ferrous ion-phenanthroline endpoint.

A standard of a known concentration of potassium hydrogen phthalate should also be analyzed with each series of digestions. The complete recovery of COD from this easily digested organic compound verifies adequate digestion and correct titration. In addition to standards, with each new type of sample, a potassium hydrogen phthalate-fortified sample should be analyzed to ensure that matrix effects have not influenced the oxidation process.

The macro-titrimetric COD method requires extensive bench space and substantial quantities of glassware and expensive reagents (silver and mercuric salts). It also produces large quantities of hazardous, acidic wastes that require special handling. In addition, the open configuration of the reflux condensers allows for the escape of volatile organic compounds. This macro-titrimetric COD method offers the advantage of reduced scale in comparison to the method outlined in Standard Methods (1981) (waste volumes are approximately 150 vs. 315 mL/assay, respectively).

Micro-Colorimetric COD

The method reported here is an adaptation of the method of Jirka and Carter (1975). It is imperative that the reagents are dispensed in a precise manner because absorbance values are a function of volume; accurate reagent volume dispensation is important when A_{440} is followed. The method uses the same reagents, and the reagents and sample are added in the same order as in the macro-titrimetric COD method.

The acid/catalyst reagent should be added quickly to a screwcapped Pyrex culture tube (ensure that rims are not chipped), and the tube should be capped immediately with a Teflon-lined cap to minimize the loss of volatile organic compounds. The contents are vortexed and placed in a fluidized sand bath at 150 ^{o}C for two hours. A sand bath has the advantage over a water bath in that the tops of the tubes remain cool; this encourages refluxing and minimizes the possibility of leakage of steam through the cap seals leading to irreproducible losses in the volumes of the digestate and inaccurate colorimetric quantitation. At the conclusion of digestion, it is essential that the tube digestates equilibrate with the ambient air-temperature to prevent schlieren lines from forming. When the tubes have cooled, the sample contents are repeatedly mixed by vortexing, any precipitate is allowed to settle, and the sample COD values are quantitated colorimetrically. The importance of the cooling and mixing steps cannot be overemphasized.

The absorbance of the digestates is best determined by sampling into a spectrophotometer micro-flow-through cell. Pouring the digestates into cuvettes is both messy and hazardous, and the sampling of particulates then cannot be avoided. Flow-through cells that use vacuum for sample introduction are not well-suited for the high viscosity and density of sulfuric acid; a low-speed peristaltic pumping system therefore is recommended (e.g., Bausch & Lomb 2000).

The quantity of Cr(VI) remaining (440 nm) or the quantity of Cr(III) produced (600 nm) is determined against the appropriate blank (250-mg/L standard or water, respectively). For quantitation at 600 nm, the digested reagent blank should not be used in the reference cell because the slow and uneven heating of the solution at the incident-light side of the cell produces schlieren lines resulting in severe drift. Fortunately, the absorbance of the blank is close to zero with respect to water. For quantitation at 440 nm, the reference standard should be repeatedly mixed to minimize the production of schlieren lines.

The COD values are determined by interpolation from a standard curve prepared from reference standards (e.g., potassium hydrogen phthalate) that are treated in parallel with the digested samples. The slope of the A_{600} standard curve will be positive with a y-intercept close to zero absorbance (Fig. 3). In

contrast, the slope of the A_{440} standard curve is negative, and the y-intercept should be close to the absorbance of the reagent blank (e.g., about 0.53 AU). It is imperative that the colorimetric method be validated by determining the actual COD of the standards with the reflux-titrimetric method every time new stock solutions of standards are made.

In contrast to the macro-titrimetric method, the micro-colorimetric COD method of Jirka and Carter (1975) requires a minimum of bench space and can be performed using either laboratory reagents prepared in screwcapped tubes or prepackaged ampules (e.g., Hach Chemical, Loveland, CO). The colorimetric method consumes only seven percent of the reagents used by the macro-titrimetric COD method and thereby reduces the attendant waste disposal problems (Table III). An additional benefit of the reduced scale is the minimization of sample consumption; this may be critical when assessing the progress of bench-scale batch treatment experiments that have small volumes. As mentioned earlier, the use of screwcapped tubes minimizes the escape of volatile gases, but it does not improve the recovery of COD from nitrogen heterocycles (Jirka and Carter, 1975). The advantages and disadvantages of the five COD methods (standard, macro-titrimetric, semi-micro-titrimetric, and two micro-colorimetric methods) are listed in Table IV.

COMPARSION STUDY

Two methods (macro-titrimetric and micro-colorimetric) were compared for the determination of COD in oil shale process waters. The results from nine oil shale wastewaters and from a composite sample of these nine waters showed that both methods were very precise; the precision of the macro-titrimetric method, however, was superior to the micro-colorimetric method (Table V). The results from a two-way analysis of variance (anova) on log-transformed data showed that there was no significant difference ($P>0.05$) between COD methods: $F_s(2.50)<F_{.05}(3.92)$. The anova data also indicated that there was no significant interaction ($P>0.05$) between methods and waters: $F_s(1.04)<F_{.05}(2.01)$. Although it has been hypothesized in the literature that sealed-tube digestion methods have improved COD recovery because of the capture of volatile compounds that would be lost during refluxing (Jirka and Carter, 1975), the statistical analysis of the comparison study data did not reflect any difference between the two methods for oil shale process waters.

The accuracy of the two COD procedures were assessed for high (192.7-mg/L) and low (10.4-mg/L) EPA quality control standards. For the titrimetric method, the recoveries were within four percent of the theoretical COD and within one percent of the empirical value reported by EPA; the relative standard deviations (rsd values) for five replicates ranged from 2.4 to 5.7 percent for the high and low samples, respectively. For the colorimetric method, the recoveries were within five percent of the theoretical COD and within one percent of the empirical value reported by EPA (rsd = 13.9 percent) for the high-range standard. The colorimetric procedure was inaccurate and imprecise for the low-range standard.

The accuracy and precision of the macro-titrimetric COD method also was determined by analyzing potassium hydrogen phthalate standards in triplicate. The COD recovery from five different digestions was within three percent of theoretical, and the precision was excellent (rsd values generally below one percent) (Table VI).

The "accuracy" of COD values is impossible to validate for a complex matrix such as oil shale process waters; each wastewater is an unknown mixture of hundreds of organic compounds each of which may be oxidized by a COD method to

varying degrees. From one perspective, the accuracy of the method for a complex waste is merely an academic exercise because the "true" value for colligative properties, such as COD, can be defined simply as the value actually obtained by the careful application of the prescribed method.

The incomplete recovery of a spike of an easily mineralized organic compound, such as potassium hydrogen phthalate, from a waste gives an indication of matrix effects. Composite oil shale process water was diluted 1:40, and one of four levels of potassium hydrogen phthalate spike was added to duplicate samples: 100, 200, 300, and 400 mg/L (COD). The least-squares linear regression line of total COD found versus COD added to each spiked sample yielded the data shown in Table VII. The COD value determined by standard additions using the colorimetric procedure for this composite water was five percent higher than that from the titrimetric procedure (25,835 vs. 24,886 mg/L), and the percent recoveries were equivalent.

REFERENCES

ASTM
"Standard Test Method for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water" in 1980 Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1980, D 1252-78, pp 665-9.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.
"Analytical Problems in Monitoring Retort Water Treatment", in Energy and Environment Division 1980 Annual Report, Lawrence Berkeley Laboratory Report LBL-11989, 1981.

Dobbs, R.A.; Williams, R.T.
"Elimination of Chloride Interference in the Chemical Oxygen Demand Test", Anal. Chem. 1963, 35, 1064-7.

Foulds, J.M.; Lunsford, J.V.
"An Analysis of the COD Method", Water Sew. Works 1968, 115, 112-5.

Gibbs, C.R.
"Introduction to Chemical Oxygen Demand", Technical Information Series-Booklet No. 8; Hach Chemical Co.: Loveland, CO; 1979 (19 pp.).

Himebaugh, R.R.; Smith, M.J.
"Semi-Micro Tube Method for Chemical Oxygen Demand", Anal. Chem. 1979, 51, 1085-7.

Jenkins, D; Snoeyink, V.L.; Ferguson, J.F.; Leckie, J.O.
Water Chemistry Laboratory Manual, Association of Environmental Engineering Professors, 1976; VI-4c, pp 1-2.

Jirka, A.M.; Carter, M.J.
"Micro Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand", Anal. Chem. 1975, 47, 1397-402.

Latimer, W.M.; Hildebrand, J.H.
Reference Book of Inorganic Chemistry, 3rd ed., Macmillan Company: New York, NY, 1951; p 377.

Medalia, A.I.
"Test for Traces of Organic Matter in Water", Anal. Chem. 1951, 23, 1318-20.

Messenger, A.L.
"Comparisons of Sealed Digestion Chamber and Standard Method COD Tests", J. Water Pollut. Control Fed. 1981, 53, 232-6.

Moore, W.A.; Kroner, R.C.; Ruchhoft, C.C.
"Dichromate Reflux Method for Determination of Oxygen Consumed", Anal. Chem. 1949, 21, 953-7.

Rhame, G.A.
"Determination of B.O.D. Values by Chemical Oxidation", Water Sew. Works 1947, 94, 192-4.

Sawyer, C.N.; McCarty, P.L.
Chemistry for Sanitary Engineers, 2nd ed., McGraw-Hill Book Company: New York, NY, 1967; p 414.

"Standard Methods for the Examination of Water and Wastewater", 15th ed.; American Public Health Association; American Water Works Association; Water Pollution Control Federation: Washington, D.C., 1981; pp 489-93.

U.S. EPA
Methods for Chemical Analysis of Water and Wastes; United States Environmental Protection Agency: Cincinnati, OH, 1979, EPA-600/4-79-020, pp 410.1-3.

Waser, J.
Quantitative Chemistry, revised edition, W.A. Benjamin, Inc.: New York, NY, 1966; p 326.
Wong, A.L.; Mercer, B.W.
"Contribution of Thiosulfate to COD and BOD in Oil Shale Process Wastewater", presented at ASTM Symposium on Analysis of Waters Associated with Alternate Fuel Production; Pittsburg, PA; June 4-5, 1979.

Table I. Reported COD Recoveries[1] from Nitrogen and Oxygen Heterocycles

Compound	Recovery	Method	Reference
Pyridine	nil	macro[2]	Standard Methods (1981)
	nil	macro[2]	Moore et al. (1949)
	nil	micro	Jirka and Carter (1975)
3-Methylpyridine	27	macro	Jirka and Carter (1975)
	32	micro	Jirka and Carter (1975)
Quinoline	44	macro[3]	Medalia (1951)
	86	macro	ASTM (1980)
2-Furoic Acid	86	macro	Moore et al. (1949)

[1] percentage of theoretical
[2] no addition of silver or mercuric salts
[3] no additionof mercuric salts

Table II. Specific COD of Oil Shale Process Waters

Process Water	Specific COD[1]
Omega-9	5.01
Rio Blanco sour water	4.41
Geokinetics	4.34
S-55	4.12
Oxy-6 gas condensate	3.60
Paraho	3.60
150-Ton	3.39
TV	3.37
Oxy-6 retort water	3.05

[1] specific COD = macro-titrimetric COD ÷ DOC (direct/UV-persulfate)

Table III. Comparison of Reagent Concentrations for Four COD Methods

	Reagent volumes (mL) per Assay Mixture			
	Standard Method[1]	Macro-Titrimetric[2]	Semi-Micro-Titrimetric[3]	Micro-Colorimetric[2]
sample	50	20	5.0	2.5
dichromate reagent	25	12	3.0	1.5
acid/catalyst reagent	75	28	7.0	3.5
water	150	75	0.0	0.0
titrant (ca)	15	15	4	0.0
total waste	315	150	19	7.0
	Concentrations in the Digestate			
H_2SO_4	50%	50%	50%	50%
Ag^+	15.87 mM	14.81 mM	14.81 mM	14.81 mM
Hg^{2+}	22.47 mM	22.45 mM	22.49 mM	22.45 mM
$Cr_2O_7^{2-}$	6.945 mM	6.945 mM	6.667 mM	6.945 mM

[1] Standard Methods (1981)
[2] as described in the appended protocols
[3] Himebaugh and Smith (1979)

Table IV. Advantages and Disadvantages of Five COD Methods

Method	Advantages	Disadvantages
Macro-Titrimetric and Standard Method	-reflux achieved -spectrophotometer not required	-low sample throughput -large volumes of waste -extensive space and glassware requirement -expense of reagents -escape of volatile compounds
Semi-Micro-Titrimetric	-spectrophotometer not required - method applicable if precipitates and schlieren lines make colorimetric finish impossible	-larger scale than micro-colorimetric
Micro-Colorimetric (screwcapped tubes)	-minimal space and glassware -minimal expense for reagents -minimal waste disposal -high sample throughput -less analyst skill required	-standards must be validated by titrimetric method -requires spectrophotometer with micro-flow-through sipper cell -precipitate and schlieren lines interfere
Micro-Colorimetric (commercial ampules)	-need only colorimeter -all advantages of screwcap tube method without hazards associated with dispensing toxic chemicals	-need sealer and ampule breaker -expense of pre-prepared reagents

Table V. Comparison of Macro-Titrimetric and Micro-Colorimetric Methods for Determination of COD in Oil Shale Process Waters[1]

	Macro-Titrimetric		Micro-Colorimetric	
Process Water	Mean	rsd (%)	Mean	rsd (%)
Paraho	151 600	0.79	141 379	4.5
Composite	22 114	0.59	23 282	2.5
150-Ton	11 048	1.1	11 662	2.4
S-55	9 414	0.35	9 781	2.4
TV	9 193	0.47	9 459	2.3
Oxy-6 retort water	8 967	0.52	8 990	0.86
Geokinetics	7 191	0.81	7 578	1.5
Omega-9	3 596	1.2	3 729	1.2
Oxy-6 gas condensate	2 308	1.0	2 074	6.4
Rio Blanco sour water	912.4	0.33	924.8	0.74
average[2]	22 692		21 731	

[1] mg/L COD; n=5 for each sample
[2] average of nine waters, excluding Composite

Table VI. COD Recovery from Potassium Hydrogen Phthalate Standards

day of analysis	Recovery[1]	rsd (%)[2]
a	100.55	0.17
b	98.72	0.68
c	98.81	1.01
d	97.69	0.21
e	98.46	0.48

[1] percentage of theoretical
[2] n=3

Table VII. Standard Additions Results[1]

	Titrimetric					Colorimetric				
Process Water	m	b	r^2	zero spike[2]	percent recovery[3]	m	b	r^2	zero spike[2]	percent recovery[3]
Composite	0.953	297	1.000	296	95.11	0.946	306	0.996	311	96.30

[1] COD spike levels of 100, 200, 300, and 400 mg/L (n=2)
[2] COD of diluted sample (i.e., without spike)
[3] (zero spike) divided by b/m X 100

m = regression coefficient (slope)
b = y-intercept
r = coefficient of determination

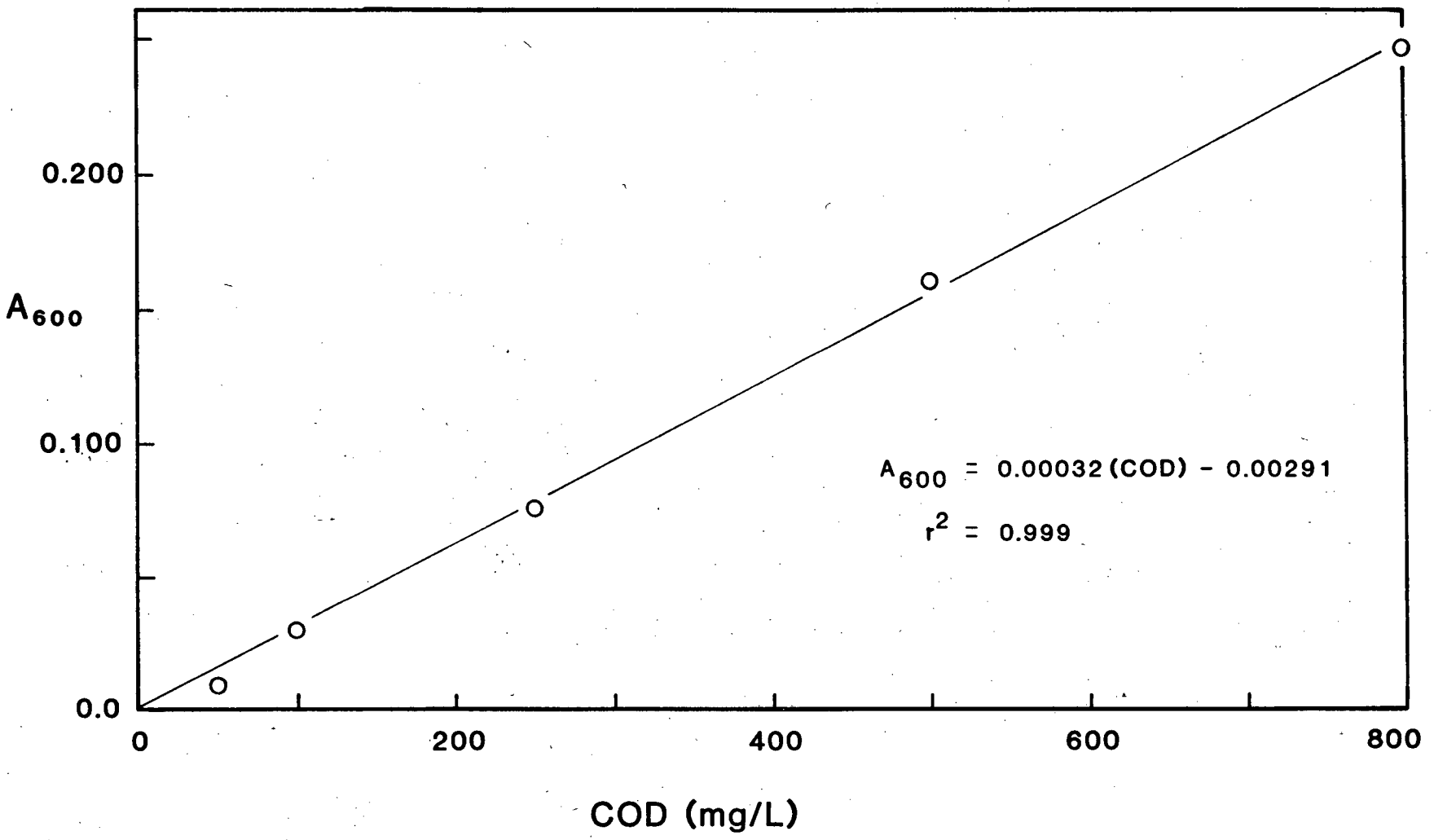


Figure 1. Absorbance Scans of Digested Phthalate Standards (mg/L COD) (Inset: detail around isosbestic point at 535 nm).

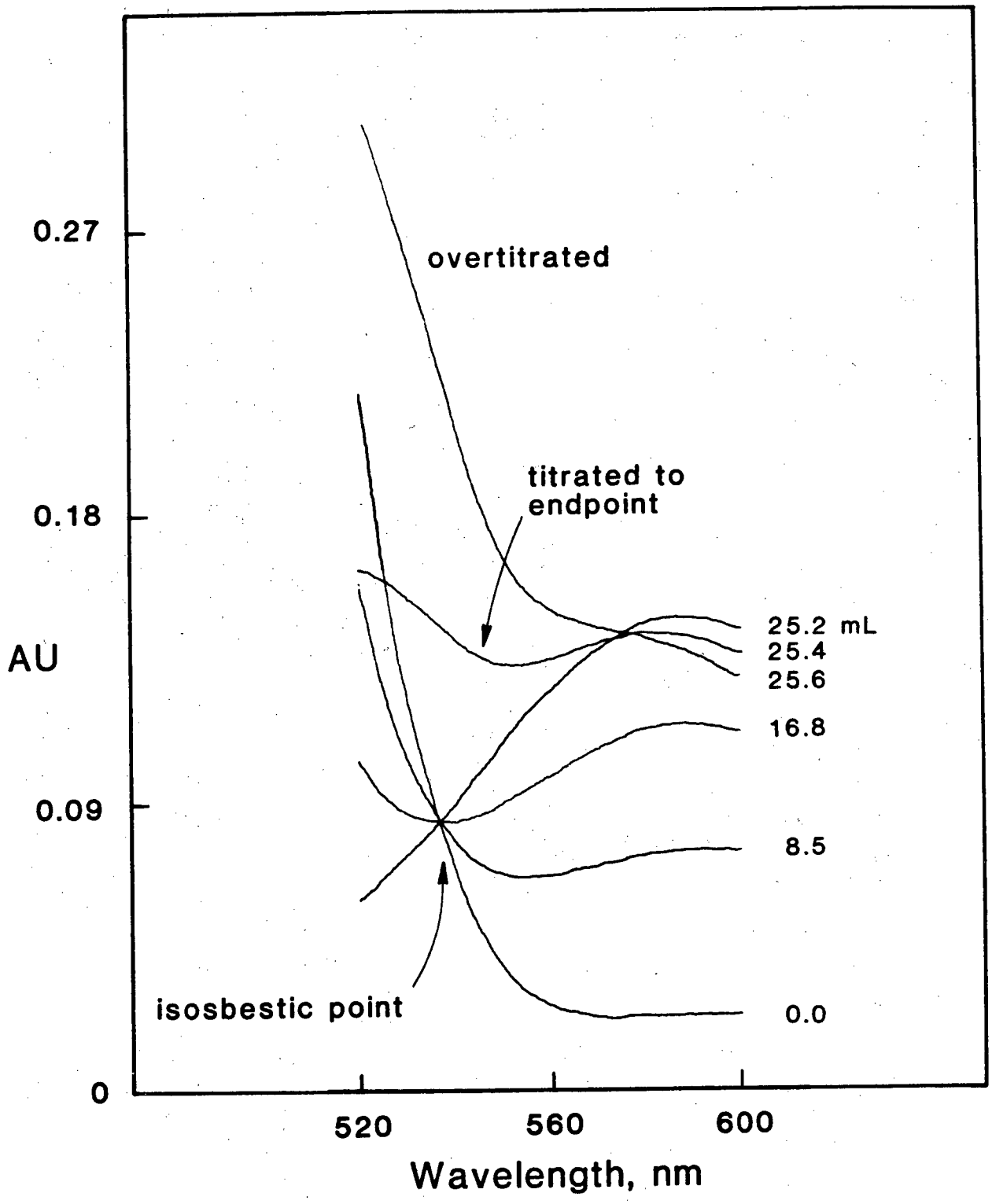


Figure 2. Absorbance Changes around the Isosbestic Point (A_{535}) during Titration of Dichromate Ion in the Presence of Ferroin Indicator. Milliliters of 0.10N FAS titrant added to flask indicated at right. Scan at titration endpoint shows the shift in absorbance at the isosbestic point as a result of ferrous-ion/phenanthroline complex.

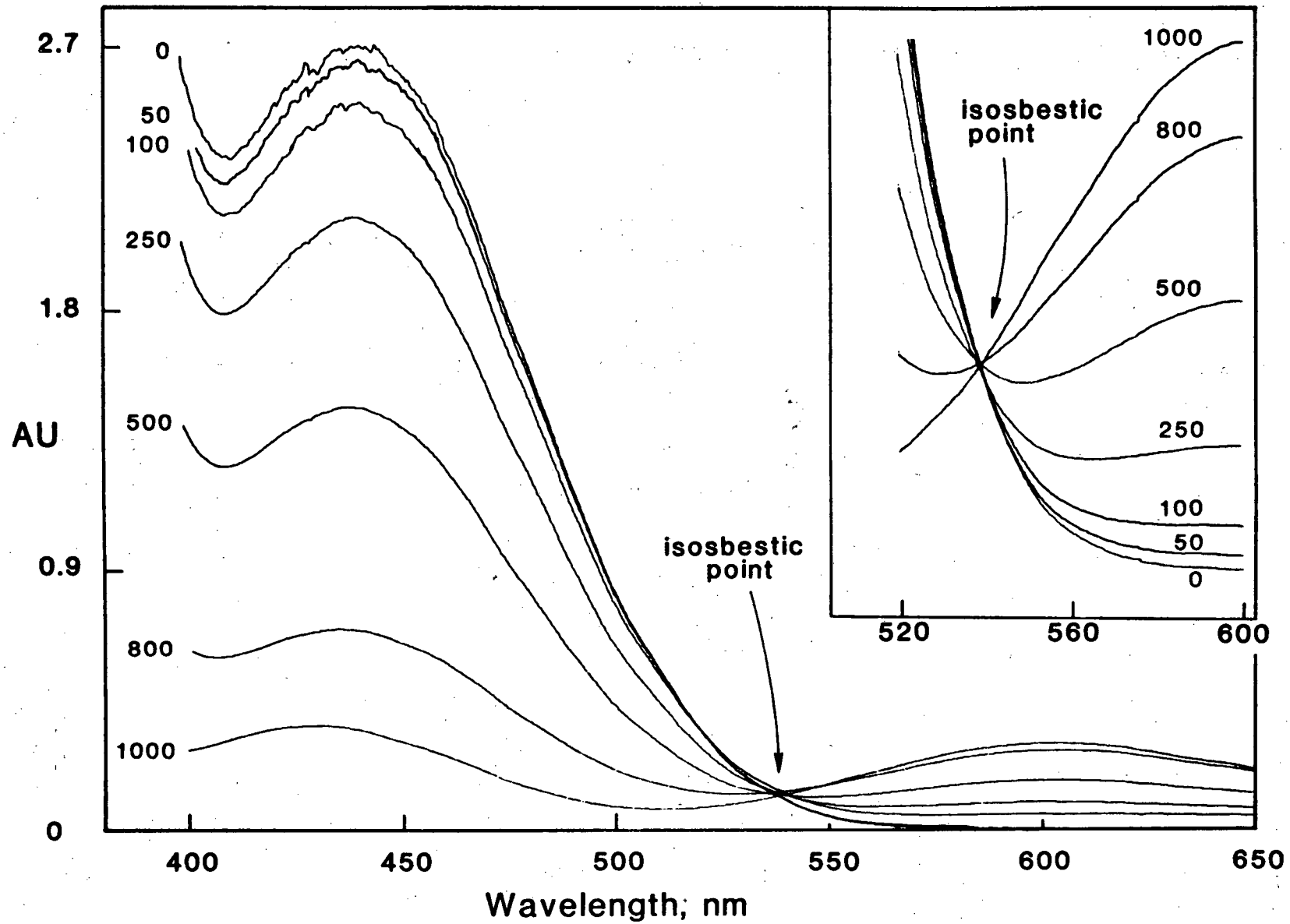


Figure 3. COD Standard Curve at A_{600}. Data points are averages of duplicates.

PROTOCOL: MACRO-TITRIMETRIC COD

I. **Apparatus**
   A. Glassware (one unit per sample)
      1. 250-mL Erlenmeyer flask; 24/40 ȿ outerjoint
      2. 50-mL beaker
      3. two glass beads
      4. 30-cm West condenser, 24/40 ȿ outerjoint at top, 24/40 ȿ inner drip joint at bottom; cover outerjoint with 50-mL beaker.
   B. Titration equipment
      1. Option 1: Automatic titrator: e.g., Sybron/Brinkmann (Westbury, NY) Autotitrator (Metrohm model 655 Dosimat, E 526 titrator, 643 control unit/624 auto sampler, and appropriate electronic burette, e.g., 10- or 20-mL).
         a. submersible colorimeter probe with 1-cm path length probe tip, 545 nm filter, and colorimeter (Brinkmann PC 800) for automated titration to indicator endpoint.
      2. Option 2: 50-mL precision-bore burette.
   C. Pipettes (calibrated): air-displacement, e.g., 5.0-mL digital adjust Gilson; positive displacement, e.g., Scientific Manufacturing Industries (SMI), Emeryville, CA, Micro/Pettors, digital adjust "F" (100 to 500 µL) and fixed volumes "H" (2.0-2.5-3.0 mL) and "K" (4.0-5.0-6.0 mL)
   D. Volumetric flasks (1-L, 500-mL; class A)
   E. Repipette (50-mL; Labindustries, Berkeley, CA)
   F. Tilting dispensers (25- and 50-mL): e.g., catalog #K759300, Kontes, Vineland, NJ
   G. Graduated cylinders (100- and 500-mL)
   H. Forceps
   I. Teflon-coated magnetic stir bars (one per Erlenmeyer flask)
   J. Magnetic stirring plate with light source
   K. Cold water bath
   L. 3600 W hot plate (model #53015, Lindberg, Watertown, WI)
   M. Polyethylene carboy (5 gal.) for disposal of waste
   N. Safety face-shield or glasses with side shields
   O. Zetex safety gloves
   P. Semi-micro analytical balance

II. **Reagents** (Note: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.)
   A. Potassium Hydrogen Phthalate (KHP) Standard: dissolve 850.2 mg of KHP (dried at 105 °C) in 300 mL of water in a 1-L volumetric flask and bring to volume with water; solution has a COD of 1000 mg/L (i.e., 1 mL = 1 mg COD).
   B. Dichromate Reagent:
      1. Potassium dichromate ($K_2Cr_2O_7$) (dried at 105 °C), 10.216 g
      2. Concentrated H_2SO_4 (sp gr = 1.84), 167 mL
      3. Mercuric sulfate, 33.3 g
      4. Add the above to 500 mL of water in a 1-L volumetric flask and bring the cooled solution to volume with water.
   C. Acid/Catalyst Reagent: add 22 g of Ag_2SO_4 to a 4.09-kg (9-lb) bottle of concentrated H_2SO_4; mix on stir plate with a large Teflon-coated stir bar until completely dissolved (six to eight hours).

D. Titrant:
   1. 0.25N FAS stock solution: dissolve 100 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (FAS) in 800 mL of water in a 1-L volumetric flask; add 20 mL concentrated H_2SO_4 and bring to volume with water.
   2. 0.10N FAS titrant solution: add 400 mL of 0.25 N FAS stock solution to a 1-L volumetric flask and bring to volume with water (titrant solution should be discarded when the concentration falls below 0.08N).

E. Ferroin Indicator:
   1. 1.49 g of 1,10-phenanthroline monohydrate
   2. 0.70 g of $FeSO_4 \cdot 7H_2O$
   3. Dissolve the above in 100 mL of water.

   Note: this indicator may be purchased commercially.

III. Protocol

A. Glassware preparation

   Note: analyst should wear protective eye-wear during all steps of this procedure.
   1. Wash flasks and beakers in a 35-percent nitric acid bath, and rinse with water. Dry thoroughly.
   2. Each flask should be covered with a 50-mL beaker until connected to the condenser.

B. Apparatus preparation
   1. Turn on hot plate at least 15 minutes prior to the beginning of the digestion.
   2. Turn on water to condensers. The water flow-rate should be sufficient to ensure that the condensers are cool to the touch.

C. Normalization blank preparation
   1. Prepare two normalization blanks for each series of digestions. These are required for determining the normality of the FAS titrant.
   2. To each flask add:
      a. 12.00 mL dichromate reagent (use "K" SMI pipette)
      b. 70.00 mL water (use 100-mL graduated cylinder)

D. Digestion blank preparation
   1. Prepare two digestion blanks for each series of digestions. These are required for assessing the contribution of the reagents to the sample COD values.
   2. To each flask add:
      a. 2 glass beads
      b. 12.00 mL dichromate reagent (use "K" SMI pipette)
      c. 20.00 mL water (use "K" SMI or 5.0-mL Gilson pipette)

E. Standard preparation
   1. Prepare at least one standard for each series of digestions.
   2. To the flask add:
      a. 2 glass beads
      b. 12.00 mL dichromate reagent (use "K" SMI pipette)
      c. 10.00 mL water (use "K" SMI or 5.0-mL Gilson pipette)
      d. 10.00 mL KHP standard solution (use "K" SMI or 5.0-mL Gilson pipette)

F. Sample preparation

Note: samples should be well-mixed and filtered (to eliminate the affect of particulates). The resulting value will be soluble COD (i.e., SCOD).

1. The reagents should be added in the order specified to minimize the interaction of chloride ion in the sample with the silver catalyst.
2. To each flask add:
   a. 2 glass beads
   b. 12.00 mL dichromate reagent (use "K" SMI pipette)
   c. add 20.00 mL of sample or appropriate amount of sample and dilution water so that the total addition equals 20.00 mL (use "K" SMI or 5.0-mL Gilson pipette). The maximum COD concentration that could theoretically be determined on a 20-mL sample is 1000 mg/L; it is advisable to dilute each sample so that the COD is in the range of 500 mg/L or less. The minimum COD that can be determined accurately is 10 to 15 mg/L.

G. Fortified sample preparation

1. One fortified sample should be analyzed with each digestion.
2. To each flask add:
   a. 2 glass beads
   b. 12.00 mL dichromate reagent (use "K" SMI or 5.0-mL Gilson pipette)
   c. 20.00 mL of a combination of known volume of sample solution, known volume of KHP, and water (use "K" SMI or 5.0-mL Gilson pipette). COD of this combination should not exceed 500 mg/L; the COD of the KHP addition should be approximately 50 percent of the total COD.

H. Flask randomization and digestion

1. Randomize flasks by consulting the "Ten Thousand Random Digit" Table; (Table "0" in Statistical Tables, F.J. Rohlf and R.R. Sokal, W.H. Freeman and Co.: San Francisco, CA; pp 152-6, 1969).
   a. randomly select a group of five digits from the table.
   b. pick any two adjacent digits within the group of five.
   c. for two-digit random numbers, begin with the pair selected, move horizontally, and record the next two consecutive digits; continue this process in a consistent manner until two-digit numbers are generated for each flask, except for one normalization blank if autotitration is used.
   d. record the random number and the corresponding sample number.
   e. relabel each flask with its random number.
2. Add 28.00 mL acid/catalyst reagent to each flask (use 50-mL repipette)
   a. acid/catalyst reagent should be introduced by carefully touching the repipette tip to the inside of the flask neck.
3. **Immediately** connect each flask (except normalization blanks) to a condenser; then raise the flask and condenser off of the hot plate and mix the contents by swirling to avoid localized superheating, which may result in violent expulsion of the flask contents. Replace the flask on the hot plate and ensure

that the bottom fully contacts the surface.

4. Place flasks on hot plate in ascending order of assigned random numbers.

Caution: If a flask should break on the hot plate, try to turn off hot plate and evacuate the lab immediately. Personnel should avoid breathing SO_3 fumes and should not re-enter the laboratory until fumes have been vented.

5. Note the time when five flasks have come to a rapid boil. This is considered the beginning of heated digestion.
6. At the end of 2 hours of heated digestion, turn off the hot plate and allow flasks to stand for at least 30 minutes. A fan will expedite cooling.
7. After 30 minutes of cooling, the flasks can be raised onto inverted 50-mL beakers while still connected to their condensers.
   a. alternatively, the flasks can be raised onto inverted 50-mL beakers immediately at the conclusion of the digestion without turning off the hot plate; this facilitates cooling and allows a second digestion to proceed without delay.
8. When the flasks are cool to touch, wash residual condensate into each flask by adding 50 mL of water to the top of the condenser with a 50-mL tilting dispenser.
9. Remove flasks from the hot plate, cover with 50-mL beakers, and place in cooling bath.

I. Manual titration (also see option at: IV. Automatic Titration)

1. Fill titrant reservoir with 0.10N FAS solution; ensure that the solution is mixed.
2. Add a Teflon-coated stir bar and 150 µL of ferroin indicator with a positive displacement pipette to each flask immediately prior to manual titration.
3. Titrate to gray endpoint. A transient red color indicates approach of endpoint, while persistent red indicates overtitration. Record encoded sample number and volume of FAS that was required to titrate the sample to the endpoint.

IV. Automatic Titration (instructions for Sybron/Brinkmann Metrohm Autotitrator)

A. Setup

1. Fill rinse-water reservoir with water and plug solenoid into outlet "J" on the back of the 643 control unit.
2. Install burette containing 0.10N FAS titrant solution.
3. Connect HP 97S calculator to external power supply and connect Amphenol connector to 643 control unit.
4. Switch 643 control unit to "manual".
5. Turn on the following units in sequence: titrator E 526, Dosimat 655, control unit 643, and calculator.

B. Sample transfer

1. Quantitatively transfer the contents of the Erlenmeyer flasks to the sample cups using 25 mL of water (tilting dispenser) to rinse the flasks.
2. Transfer the encoded number of the flask to appropriate sample cup.

C. Indicator endpoint calibration

1. E 526 titrator:

a. plug shorting strap into the reference and indicator electrode inputs
b. set: thumbwheels to 0000,
calibration knob to "calibr",
selector knob to +mV
c. turn "U" knob until analog meter is nulled
NOTE: it is important that the setting of the "U" knob not be touched once it is set.
d. disconnect shorting strap

2. PC 800 Colorimeter:
a. connect colorimeter leads; black to reference electrode input and red to indicator electrode input.
b. install 545-nm filter and turn on colorimeter power switch to %T; allow at least 5 minutes for warmup.
Warning: NEVER turn on the colorimeter without a filter in the filter block.
c. ensure that the colorimeter probe tip is the appropriate path length.
d. adjust colorimeter to 100 %T with coarse/fine knobs.
e. thoroughly rinse colorimeter probe.
f. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise. Avoid vortex formation to prevent air bubbles from becoming entrapped in the colorimeter probe tip.
g. use normalization blank to set 100% T on colorimeter.
h. add 150 µL of ferroin to a digestion blank and titrate manually to the gray endpoint; determine transmittance (545 nm) and record volume of titrant required.
i. set titrator thumbwheels for a value slightly (4 percent) above endpoint transmittance to compensate for overtitration error by autotitrator (e.g., endpoint is generally 88%, the thumbwheels are set to read approximately 0920); this setting will be about 0900-0940.
j. set calibration knob to first -mV setting.

D. Operation
1. Reconnect the mixer to the 643 control unit and rotate mixer knob to the "on" position. Adjustment can be made once the control unit is engaged (IV.D.10.).
2. Close control panel cover.
3. Ensure that the turntable surface is clean.
4. Place samples on turntable and record their positions; the 624 control unit only holds 10 samples and the calculator program can only accommodate 10 samples before it must be restarted.
5. Load "COD Titration Program" (see: Appendix A), and depress "E" button to initiate.
6. Set titration rate knob on 655 Dosimat between "5" and "6".
7. Set 643 control unit to "auto".
8. Ensure that the titrator delivery-line diffuser is installed.
9. Add 150 µL of ferroin to each sample cup.
10. Press "Start" switch on 643 control unit.

D. Shutdown: reverse order of sequence at IV.A.5.

V. **Data Reduction**

A. Calculate the normality of the FAS titrant:

$$N = \frac{(12.00 \text{ mL } K_2Cr_2O_7) \times (0.208 \text{ eq-}K_2Cr_2O_7/L)}{V_n}$$

$$= \frac{2.50 \text{ mL-eq/L}}{V_n}$$

where V_n = average mL of FAS required for normalization blanks
N = normality of FAS (eq/L).

B. Calculate the COD (mg/L) for each sample, standard, and fortified sample using the following equation:

$$\text{COD (mg/L)} = \frac{N \times (V_b - V_s) \times 32 \text{ g-}O_2\text{/mol} \times 1000 \text{ mg/g}}{S \times 4 \text{ eq/mol}}$$

$$\text{COD (mg/L)} = \frac{N \times (V_b - V_s) \times 8000 \text{ mg/eq}}{S}$$

where N = normality of FAS (eq/L)
V_b = mL FAS required for digestion blank
V_s = mL FAS required for sample
S = mL of sample added to flask.

V. **Waste Disposal**

A. Carefully decant the contents of each flask into the waste carboy. Retrieve stir bars and glass beads and rinse them in water. The silver from the catalyst reagent can be reclaimed.

Protocol prepared by: B.M. Jones, G.W. Langlois, R.H. Sakaji, and C.G. Daughton

PROTOCOL: MICRO-COLORIMETRIC COD

I. Apparatus
- A. Glassware (one each per sample)
  1. Culture tubes: Pyrex (16-mm O.D. X 150 mm) with Teflon-lined screwcaps
- B. Pipettes (calibrated): air-displacement, e.g., 5.0-mL digital adjust Gilson; positive displacement, e.g., Scientific Manufacturing Industries (SMI), Emeryville, CA, Micro/Pettors, digital adjust "K" (2.0 to 6.0 mL) and fixed volume "H" (2.0-2.5-3.0 mL)
- C. Vortex mixer
- D. Heating unit: Tecam fluidized sandbath SBL-1 (Techne, Inc., Princeton, NJ) with overflow flange (accessory #1133).
- E. Circular stainless-steel 42-place test-tube rack for sand bath.
- F. Spectrophotometer: e.g., Bausch & Lomb Spectronic 2000 with micro flow-through cell, 1-cm path length; ensure that Viton O-rings are used. Flow-through cells must use a peristaltic pumping system rather than a vacuum system because of the density and viscosity of the sulfuric acid.
- G. Volumetric flasks (1-L, 10-mL; class A)
- H. Polyethylene carboy for disposal of waste
- I. Safety face-shield or glasses with side shields
- J. Semi-micro analytical balance

II. Reagents (Note: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.)
- A. Potassium Hydrogen Phthalate (KHP) Stock Solution: dissolve 850.2 mg of KHP (dried at 105 °C) in 300 mL of water in a 1-L volumetric flask and bring to volume with water; this solution has a COD of 1000 mg/L (i.e., 1 mL = 1 mg COD).
- B. Dichromate Reagent:
  1. Potassium dichromate ($K_2Cr_2O_7$) (dried at 105 °C), 10.216 g
  2. Concentrated H_2SO_4 (sp gr = 1.84), 167 mL
  3. Mercuric sulfate, 33.3 g
  4. Add the above to 500 mL of water in a 1-L volumetric flask, and bring the cooled solution to volume with water.
- C. Acid/Catalyst Reagent: add 22 g of Ag_2SO_4 to a 4.09-kg (9-lb) bottle of concentrated H_2SO_4; mix on stir plate with a large Teflon-coated stir bar until completely dissolved (six to eight hours).

III. Protocol

Note: Analyst should wear protective eye-wear during all steps of this procedure.
- A. Apparatus preparation
  1. Turn on sandbath. Adjust temperature setting and air flow rate (maximum delivery pressure = 35 psig) to stabilize temperature at 150 °C. Place a thermometer in a test tube containing silicone oil and place test tube in the sand bath to monitor the temperature.
  2. Use only culture tubes without chipped rims (to ensure a gas-tight seal). Wash tubes in a 35-percent nitric acid bath, and rinse with water. Dry thoroughly. Do not acid wash caps; rinse thoroughly only in water.

B. Digestion blank preparation
   1. Prepare three digestion blanks for each series of digestions.
   2. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette)
      b. water, 2.50 mL (use "H" SMI pipette)
      c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette)
      Caution: add acid/catalyst reagents carefully; mouth of tube should be facing away from analyst when adding this reagent in the event of explosive bumping.
      d. Seal tubes **tightly** with Teflon-lined screwcaps.
      e. Vortex
   3. Digestion blanks are heated with samples (see: III.E.).

C. Standard curve preparation
   1. For each of the following COD concentrations, add the respective volume of KHP to a 10-mL volumetric flask and bring to volume with water:

800 mg/L:	8.00 mL
500 mg/L:	5.00 mL
250 mg/L:	2.50 mL
100 mg/L:	1.00 mL
50 mg/L:	0.50 mL

   Note: use positive displacement pipettes whenever possible.
   2. Prepare duplicates of each standard concentration for each series of digestions.
   3. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette)
      b. appropriate standard solution, 2.50 mL (use "H" SMI pipette)
      c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette); solution should be carefully added down the inside of the tube so that the acid forms a layer on the bottom. See **Caution** at III.B.2.c.
      d. **Immediately** seal tubes **tightly** with Teflon-lined screwcaps.
      e. Vortex
   4. Standards are heated with samples (see: III.E.).

D. Sample preparation
   1. Samples should be well-mixed and filtered (to eliminate the affect of particulates). The resulting value will be soluble COD (i.e., SCOD).
   2. The reagents should be added in the order specified to minimize the interaction of chloride ion in the sample with the silver catalyst.
   3. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette).
      b. sample (diluted or neat), 2.50 mL (use "H" SMI pipette). The maximum COD concentration that could theoretically be determined for a 2.50-mL sample is 1000 mg/L. It is advisable to dilute each sample so that the COD is in the upper portion of the standard curve (i.e., 600-800 mg/L for A_{600} or up to 200 mg/L for A_{440}); this increases the absorbance values for A_{600} and serves to decrease the variance of replicate readings.

c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette) see **Caution** at III.B.2.c.
d. **Immediately** seal tubes **tightly** with Teflon-lined screwcaps.
e. Vortex

E. Sample digestion
1. Place tubes in sandbath rack. Heat at 150 °C for 2 hours.
2. Remove sample tubes, brush off sand, and cool to ambient air temperature.

F. Quantitation

IMPORTANT: follow these instructions exactly to obtain accurate and precise results.

1. Vortex each tube repeatedly to ensure complete mixing.
2. If a precipitate forms, allow crystals to settle. Ensure that digestate remains mixed after this settling period. Schlieren lines must be totally absent from sample digestates.
3. Read the absorbance of the blanks, duplicate series of standards, and samples at 600 nm against a water reference. Avoid aspiration of the precipitate into the cell.
4. If COD values are low (i.e., 0 to 250 mg/L), read the absorbance at 440 nm against the 250-mg/L standard.

Note: ensure that the standard in the reference cell remains well-mixed.

IV. Data Reduction

A. Determine the linear regression equation of the COD standard curve by least-squares analysis. Calculate the coefficient of determination (r^2).

B. Interpolate the COD of each sample using the least-squares standard curve equation. For A_{600}, either subtract the mean blank absorbance value from each sample absorbance or include the coordinate (0, blank A_{600}) in the least-squares equation. For A_{440}, include the coordinate (0, blank A_{440}) in the equation.

$$\text{COD (mg/L)} = (m)(A_{600 \text{ or } 440}) + b$$

where m = regression coefficient (i.e., slope)
b = y-intercept

Typical values are:

A_{600}	m =	0.00032
	b =	-0.00291
A_{440}	m =	-0.00193
	b =	0.51936

C. If the sample solution has been diluted, multiply the result by the appropriate dilution factor.

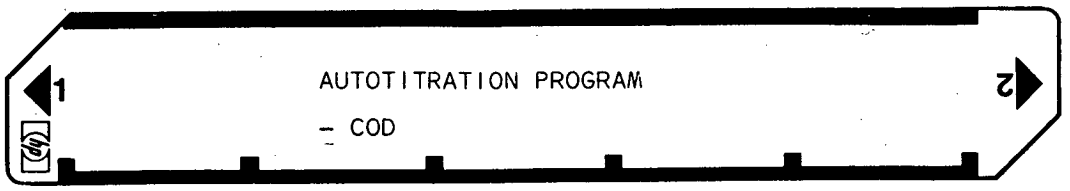
V. Waste Disposal

A. Carefully decant the contents of each tube into the waste carboy. The silver from the catalyst solution can be reclaimed, if desired.

Protocol prepared by: B.M. Jones, G.W. Langlois, R.H. Sakaji, and C.G. Daughton

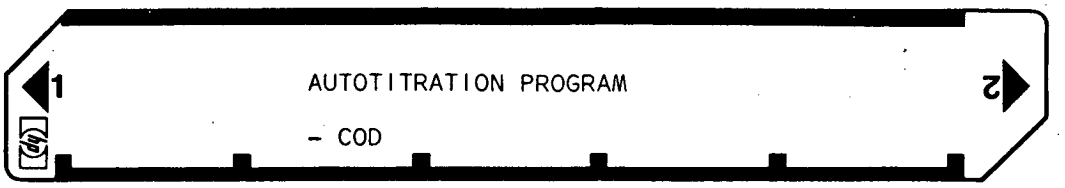
APPENDIX A

User Instructions



STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: Calculator in RUN mode, Print			
	in MANUAL position.			
2	PRESS B		B	
3	ENTER mL DICHROMATE	Value		
4	PRESS R/S: Prints mL Dichromate		R/S	Value
5	ENTER DICHROMATE NORMALITY	Value		
6	PRESS R/S: Prints Dichromate Normality		R/S	Value
7	ENTER VOLUME OF SAMPLE	Value		
8	PRESS R/S: Prints volume of sample and stores		R/S	Value
	it in corresponding memory.			
9	REPEAT STEPS 7-8 FOR ALL 6 SAMPLES (the first			
	four samples consist of 2 for the determination			
	of FAS normality and 2 blanks). Following			
	input of last sample volume, display will go to			
	-1.00.			
10	PLACE SAMPLES IN AUTOSAMPLER TRAY AS FOLLOWS:			
	2 FAS samples, 2 blanks, 6 samples.			
11	START AUTOTITRATOR: with controller in AUTO			
	mode, press start switch. Data printout will be as			
	follows:			
	Sample 1: prints mL titrant, FAS normality.			
	Sample 2: prints mL titrant, FAS normality,			
	and $\bar{x}$ FAS normality.			
	Sample 3: prints mL titrant.			
	Sample 4: prints mL titrant, $\bar{x}$ mL titrant for			
	blanks.			
	Samples 5-10: prints mL titrant, COD value.			

User Instructions



STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
12	Autotitration stops after 10th sample, display goes to 0.00. To continue, repeat steps 7-8 for 6 additional samples. (NOTE: 6 values must be entered).			
13	PRESS START SWITCH ON CONTROLLER: prints mL titrant and COD value for each sample.			

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBL8	21 12	
002	CF3	16 22 03	
003	DSP2	-63 02	
004	P≠S	16-51	
005	R/S	51	
006	PRTX	-14	
007	STO1	35 01	
008	R/S	51	
009	PRTX	-14	
010	STO2	35 02	
011	P≠S	16-51	
012	*LBLa	21 16 11	
013	R/S	51	
014	PRTX	-14	
015	STO4	35 04	
016	R/S	51	
017	PRTX	-14	
018	STO5	35 05	
019	R/S	51	
020	PRTX	-14	
021	STO6	35 06	
022	R/S	51	
023	PRTX	-14	
024	STO7	35 07	
025	R/S	51	
026	PRTX	-14	
027	STO8	35 08	
028	R/S	51	
029	PRTX	-14	
030	STO9	35 09	
031	SPC	16-11	
032	*LBLE	21 15	
033	CF0	16 22 00	
034	CF1	16 22 01	
035	1	01	
036	CHS	-22	
037	STOI	35 46	
038	CF3	16 22 03	
039	R/S	51	
040	*LBLA	21 11	
041	CF3	16 22 03	
042	STOA	35 11	
043	ISZI	16 26 46	
044	PSE	16 51	
045	RCLA	36 11	
046	1	01	
047	0	00	
048	÷	-24	
049	FRC	16 44	
050	1	01	
051	0	00	
052	x	-35	
053	10^x	16 33	
054	RCLA	36 11	
055	1	01	
056	0	00	
057	0	00	
058	÷	-24	
059	INT	16 34	
060	X≠Y	-41	
061	÷	-24	
062	DSP2	-63 02	
063	PRTX	-14	
064	STOB	35 12	
065	1	01	
066	ENT↑	-21	
067	RCLI	36 46	
068	X≤Y?	16-35	
069	GSBb	23 16 12	
070	3	03	
071	X≠Y	-41	
072	X≤Y?	16-35	
073	GSBc	23 16 13	
074	GSB1	23 01	
075	RCLI	36 46	
076	9	09	
077	X=Y?	16-33	
078	GSBd	23 16 14	
079	CF3	16 22 03	
080	R/S	51	
081	GTOA	22 11	
082	*LBL1	21 01	
083	RCLD	36 14	
084	ENT↑	-21	
085	RCLB	36 12	
086	-	-45	
087	RCLC	36 13	
088	x	-35	
089	8	08	
090	0	00	
091	0	00	
092	0	00	
093	x	-35	
094	RCLi	36 45	
095	÷	-24	
096	PRTX	-14	
097	SPC	16-11	
098	RTN	24	
099	*LBLb	21 16 12	
100	P≠S	16-51	
101	RCL1	36 01	
102	ENT↑	-21	
103	RCL2	36 02	
104	x	-35	
105	RCLB	36 12	
106	÷	-24	
107	PRTX	-14	
108	P≠S	16-51	
109	Σ+	56	
110	RCLI	36 46	
111	1	01	
112	X=Y?	16-33	

REGISTERS

0	1	2	3	4 SAMPLE VOLUME	5 SAMPLE VOLUME	6 SAMPLE VOLUME	7 SAMPLE VOLUME	8 SAMPLE VOLUME	9 SAMPLE VOLUME
S0	S1 mL DICHROMATE	S2 DICHROMATE NORMAL.	S3	S4 SUM x_i	S5	S6	S7	S8	S9 n_x

A	B	C	D	E	I
CODED DATA	DECODED DATA (mL titrant)	x FAS NORLMALITY	x DIGESTION BLANK		COUNTER

Program Listing

KEY ENTRY	KEY CODE	COMMENTS	STEP	KEY ENTRY	KEY CODE	COMMENTS
GSB2	23 02					
SPC	16-11		170			
CF3	16 22 03					
R/S	51					
GTOA	22 11					
*LBL2	21 02					
$\bar{x}$	16 53					
PRTX	-14					
STOC	35 13					
P≠S	16-51					
0	00					
ST04	35 04		180			
ST09	35 09					
P≠S	16-51					
RTN	24					
*LBLc	21 15 13					
RCLB	36 12					
Σ+	56					
RCLI	36 46					
3	03					
X=Y?	16-33					
GSB3	23 03		190			
SPC	16-11					
CF3	16 22 03					
R/S	51					
GTOH	22 11					
*LBL3	21 03					
$\bar{x}$	16 53					
STOD	35 14					
PRTX	-14					
RTN	24					
*LBLd	21 16 14		200			
CLX	-5.					
R/S	51					
PRTX	-14					
ST04	35 04					
R/S	51					
PRTX	-14					
ST05	35 05					
R/S	51					
PRTX	-14					
ST06	35 06		210			
R/S	51					
PRTX	-14					
ST07	35 07					
R/S	51					
PRTX	-14					
ST08	35 08					
R/S	51					
PRTX	-14					
ST09	35 09					
SPC	16-11		220			
3	03					
ST01	35 46					
RTN	24					
R/S	51					

LABELS					FLAGS	SET STATUS		
TA PUT	BDICHROMATE CONSTANTS	C	D	E RESET COUNTER	0	FLAGS	TRIG	DISP
MPLE UMES	b FAS NORMAL	c SUM OF DIG BLANK	d RESET VOL COUNT	e	1	ON OFF 0 ☐ ☐ 1 ☐ ☐ 2 ☐ ☐ 3 ☐ ☐	DEG ☐ GRAD ☐ RAD ☐	FIX ☐ SCI ☐ ENG ☐ n_____
	1 COD CALCULATION	2 $\bar{x}$ FAS NORMALITY	3 $\bar{x}$ BLANK CALCULATION	4	2			
	6	7	8	9	3			

Chapter V

MICROBIAL BIOMASS IN OIL SHALE WASTEWATERS: QUANTITATION AS PROTEIN

C.G. Daughton, B.M. Jones, and R.H. Sakaji

Introduction 2
Microbial Biomass 2
Dry Cell Mass 2
Turbidity 3
Protein 4
Theory 4
Determination of Biomass as Protein 4
Protocol Summary 6
Determination of Biomass as Protein 6
Validation of Method 6
References 8
Figures
1. Lowry Assay Standard Curve for Bovine Serum Albumin 9
2. Correlation of a Dilution Series of Bacteria in Oxy-6 Retort Water with Whole-Cell Protein 9
3. Correlation of Volatile Suspended Solids in Oxy-6 Retort Water with Whole-Cell Protein 10
4. Correlation of Dry Cell Mass in Oxy-6 Retort Water with Whole-Cell Protein 10
5. Correlation of Turbidity (Measured as Absorbance at 660 nm) in Oxy-6 Retort Water with Whole Cell Protein 11
6. Correlation of Turbidity (Measure as Nephelometric Turbidity Units) in Oxy-6 Retort Water with Whole Cell Protein 11
Protocol: Protein Assay 12-14

INTRODUCTION

Microbial Biomass

The quantitation of microbial growth (i.e., time-dependent increase in biomass concentration in conjunction with cell numbers) is necessary for the accurate assessment of biological waste treatment performance and is essential for execution of valid biodegradation experiments (i.e., to correlate substrate disappearance with biological rather than physicochemical activity such as volatilization). The increase in biomass at stationary phases is an indirect measure of organic solute degradation; biomass determinations therefore can be used to validate the actual solute removal values that are determined by other means. Values for solute removal and biomass production can then be used to calculate growth yields.

The quantitation of microbial growth in liquid medium can be accomplished by direct and indirect methods. Direct methods include (i) direct counts (microscopy, electronic particle counting, colony counts), (ii) light scattering (turbidimetry, nephelometry), (iii) most probable number (MPN), (iv) biomass (wet weight, dry weight, suspended solids, volatile suspended solids), (v) nonspecific cellular components (total cellular C, N, P), and (vi) specific cellular constituents (nucleotides, DNA, RNA, protein, muramic acid, lipopolysaccharides). Indirect methods include those which reflect metabolism (manometry, calorimetry, metabolite production, substrate consumption, BOD, chloroform-lysis-incubation).

Few of the direct methods distinguish viable from nonviable cells (e.g., epifluorescence microscopy, ATP, and MPN), and many of these methods are estimators only of cell numbers. Various corrections must therefore be performed to convert these values to biomass (e.g., cell volume and moisture content must be known in conjunction with direct counts). Many indirect methods, in contrast, directly reflect viability, or at least cell function. Methods for determining biomass, cell numbers, viability, and function are discussed extensively by Gerhardt (1981), Hanson and Phillips (1981), Karl (1980), Koch (1981), and Paul and Voroney (1980).

Oil shale process wastewaters have several characteristics that preclude the facile measurement of microbial biomass by the more often used methods such as dry cell mass, turbidity, or protein.

Dry cell mass.

Dry cell mass is usually measured after collection of cells on membrane- or glass-fiber filters or after centrifugation and separation of the cells from the supernatant fluid. Wet- or dry-mass can then be determined gravimetrically or "volatile suspended solids" can be determined by difference. Accurate gravimetric procedures require extensive replication and multiple blanks for obtaining reliable background values (e.g., filter extractables such as wetting agents, humectants, and particulate debris -- see Cooney, 1980 -- can lead to significant losses in filter mass). There are two major problems with the gravimetric approach. (1) The volume of the sample to be filtered must contain at least several milligrams of dry cell mass for accurate gravimetric determination. Since bacterial cells are about 80 percent moisture by weight, this requirement poses the dual problems of consuming excessive amounts of experimental treatment samples for dry mass determinations and of filtering oil shale process wastewaters. These waters are not conducive to filtration; we have found that membranes become clogged before sufficient cell material has been retained by the filter. (2) Separation of cells from the extracellular

fluid also effects removal of nonbiological material such as particulates, colloids, and dissolved compounds which are then measured indiscriminately as biomass. The endogenous levels of abiotic particulates and tarry materials in many oil shale wastewaters also yield high and nonreproducible background levels. Membrane fouling by retort waters results not only in low filtrate yields, but also possibly in removal of solutes from the filtrate (i.e., solubilized compounds) by their partitioning into the tars immobilized on the membrane (Daughton, Jones, and Sakaji, 1981) or by precipitation (e.g., of carbonates; Fox, 1980) on the filter surface. The chemical sorption or precipitation of solutes by electrostatic (Zierdt, 1979) or chemical interactions of the solution with the membrane surface can also effect removal of dissolved solutes. The filtrate, therefore, is not necessarily representative of soluble or dissolved material, and likewise, the retentate cannot be considered solely as representative of suspended matter. A further complication is that these filtration phenomena are partially dependent on the membrane or filter type (e.g., depth filters such as glass-fiber, or screen-type filters such as polycarbonate, mixed cellulose esters, Teflon, nylon, etc.) (see: Chapter II).

Turbidity.

Turbidity, a measure of the light-scattering properties of a mixture, is commonly used as a measure of suspended solids. Cellular material is easily quantitated by measurement of turbidity when the suspended cells are dispersed, but not when they are flocculent. Turbidimetric measurements of nondispersed mixed bacterial cultures can be difficult and inaccurate; flocs, however, can often be disrupted by sonication. The arbitrary units of this approach (e.g., nephelometric turbidity units; NTU) must be standardized against a direct measure such as dry cell mass, because the amount of both scattered and transmitted light is affected by particle shape and size (e.g., bacterial species and growth phase). Even then, abiotic particulates will interfere with turbidity when measured by absorbance or nephelometric techniques. The highly chromophoric character of most oil shale wastewaters prevents direct absorbance measurements. The abiotic particulates of these waters are inherently dark, and bacterial cells grown in these waters sorb and concentrate colored material on their surfaces. This precludes the measurement of turbidity as "optical density" (i.e., by measuring pseudoabsorbance or transmitted light), unless every sample is referenced to its homologous particle-free extracellular fluid. Nephelometry is invalid because much of the scattered light is subsequently absorbed by the chromogenic particulates and fluid. The great advantage of turbidimetric measurements for determining cell concentrations results, however, from its rapid and nondestructive nature; this is especially valuable for small experimental samples that must be conserved for time-course studies.

An effective means of determining turbidity in highly colored waters involves calculating the ratio of transmitted light intensity to the average of two values of scattered light intensity measured at 90° to the incident radiation. This avoids the problems associated with chromophoric solutes and colored suspended solids. Correction curves still need to be calculated, however, because the nephelometric idiosyncrasy of higher sensitivity at higher turbidity (resulting from secondary scattering) will give overestimations of biomass. An instrument that employs this technique is the DRT-100 (HF Instruments, Bolton, Ontario, Canada).

Protein.

Cellular components such as organic carbon or organic nitrogen are nonspecific estimators of biomass. Standard methods for these techniques are time-consuming, and organic carbon is susceptible to bias because of the possibility of storage of large quantities of carbon as intracellular polymers (e.g., polyhydroxybutyrate). Less direct but more specific measures include nucleic acids and protein. Methods for determining protein include UV absorbance, amino acid analysis (reduced ninhydrin reaction), biuret (cupric-peptide chelation), Lowry (aromatic amino acid-phosphomolybdate reduction), and dye-binding (Coomassie Blue-protein binding). Many of these methods are useful only for relatively pure protein solutions. UV absorbance (280 nm) of proteins mainly results from the aromatic amino acids tryptophan and tyrosine; nearly all proteins contain these two amino acids, which are present in a rather constant proportion to other amino acids (Hanson and Phillips, 1981). This approach, however, is prone to many interferences. Total nitrogen (e.g., by Kjeldahl) is very time consuming. The Lowry method, as well as the dye-binding method, has a relatively low minimum detection limit, but it is prone to interferences (e.g., most phenols). A low detection limit, however, does present difficulties in obtaining representative subsamples from nonhomogeneous samples, because the subsample must be initially small or subsequently diluted. Although the biuret method has a higher minimum detection limit which necessitates large sample sizes, it is easy to perform. Since a method suitable for research purposes must have a minimum detection limit sufficiently low to avoid consuming excessive quantities of experimental samples and because sample throughput is critical, only the Lowry and dye-binding assays were considered for the experimental work reported here.

THEORY

Determination of Biomass as Protein

The three major steps in determining cellular protein are (i) cell harvest, (ii) cell digestion (i.e., protein liberation), and (iii) quantitation of whole-cell protein. Cell harvest serves to concentrate the cells when they are present at low concentrations and to separate them from solutes in the extracellular fluid (e.g., free protein, colored substances, and phenols) that may interfere with the subsequent detection step. Cell harvest is usually done by centrifugation (e.g., 10,000 x g, 15 min) followed by collection and washing of the pellet. We have employed a more convenient method that uses filtration, washing, and collection of the retentate. Since the quantitative removal of the retained cells is difficult, it is necessary to digest the cells while they remain on the filter. Basic problems with these steps include representative subsampling of flocculent bacterial cultures, loss of cells during sampling because of sorption to pipette tips and membrane filter holders, cell lysis during washing, and retention by the filter of compounds that interfere with the protein assay. Whole cell protein is solubilized by digestion in alkaline solution; the filter must be able to withstand the digestion and not interfere with the detection of protein.

Protein quantitation can be done by numerous methods, most of which involve formation of a reaction product that absorbs light. Two of the most prevalent methods are the Bradford and Lowry assays. The Bradford assay employs the protein-binding capability of Coomassie Brilliant Blue dye; the absorption maximum of the dye shifts from the red anionic form to the blue form when bound to amino groups of amino acids. The Lowry assay (Lowry et al., 1951) ("reducing phenol method") makes use of the chemical reduction of phosphotungstic-

phosphomolybdic acid (Folin phenol reagent) by phenols as well as many other substances to yield heterophosphomolybdate blue ("molybdenum blue"). Of the amino acids used in biosynthesis, apparently only tyrosine, and to a lesser extent tryptophan, can reduce the reagent. The addition of cupric ions in alkali gives a peptide-cupric biuret reaction, which produces a more reproducible, enhanced color development with the Folin reagent. The biuret reaction results from the chelation of cupric ions by the carbonyl groups of carbamylurea (biuret); cupric ligands are formed similarly by the carbonyl moieties of the peptide amide groupings, but not all peptide linkages react, and the stoichiometry of the ligand is not consistent, being dependent on the type of protein. Nonetheless, proteins give very consistent biuret values, which serves to modulate the more erratic absorbance values obtained with the Folin reaction.

The dye-binding assay suffers from (i) interference by alkali which necessitates neutralization of the digested samples prior to dye addition, (ii) the necessity of subsampling the digestate, and (iii) the particulate nature of the protein-dye complex which causes erratic absorbance readings when a micro-flow-through cell is used in place of cumbersome cuvettes in a spectrophotometer. Although the Lowry assay has an inherently higher minimum detection limit than the dye-binding assay, procedural stipulations (e.g., dilutions are not required) allow the use of much smaller sample sizes during cell harvest. Sample sizes are generally smaller than 1 mL; cultures can therefore be sampled repetitively during time-course studies with minimum volume disruption. A major advantage of using the Lowry assay is that the entire procedure can be performed in a single tube, thereby minimizing dilution errors and glassware usage. For these reasons, the Lowry assay has a lower minimum detection limit in practice for the application described here. A disadvantage is that the color of retort water, in volumes as small as one microliter, can give positive interference; this necessitates thorough rinsing of all extracellular fluid entrained by the filter after cell harvest.

Cell harvest can be achieved with many different filters that have nominal pore diameters of 0.2 to 0.45 µm. Glass-fiber filters and cellulose ester and polycarbonate membranes release silicates, particulates, and phenols during digestion that interfere with the Lowry assay; polycarbonate membranes can be used, however, for the dye-binding assay. Nylon membranes cannot be used for the Lowry assay because of irreversible sorption of the reduced phosphomolybdate complex from solution. The chemical inertness of Teflon membranes has allowed their use in the protocol described here. Their hydrophobicity, however, necessitates their wetting by methanol to allow passage of aqueous sample; they must also be physically restrained under the surface of the digestion solution to ensure contact of the retained cells with the alkali. Inert hydrophilic membranes (polyvinylidene fluoride) that have recently been marketed by Millipore Corp. (Durapore HVLP 025 00) may not have these disadvantages and should be evaluated.

The protein assay described here was developed specifically for the facile and accurate quantitation of microbial biomass in oil shale wastewaters. This method is an adaptation of the modified Lowry method of protein measurement as described by Herbert, Phipps, and Strange (1971) using the Folin phenol reagent. It is important to emphasize that the sensitivity of response will vary with bacterial species because different proteins give widely different absorbances. The method must therefore be standardized against another measure such as dry mass or organic nitrogen for each type of microbial assemblage.

PROTOCOL SUMMARY

Determination of Biomass as Protein

A detailed protocol for whole-cell protein quantitation in suspensions is appended. A Teflon membrane filter (25-mm diameter; 0.45-µm pore diameter) is placed in a glass filtration unit and wetted with 1 mL of methanol. Immediately upon vacuum filtration of the methanol, an appropriate, accurately measured volume of dispersed culture is applied to the filter. The retained cells are rinsed with phosphate buffer and methanol (to remove entrained wastewater and tarry materials, respectively). The membrane is pushed to the bottom of a screw-capped Pyrex culture-tube by a glass rod that rests on the retentate side of the membrane. The glass rod remains inside the tube to hold the membrane under 0.5 mL of aqueous 1 N NaOH. The tubes are sealed with Teflon-lined screw-caps and placed in a fluidized sand bath at 100 °C for 10 minutes; a sand bath has the advantage over a water bath in that the tops of the tubes remain cool, encouraging refluxing and preventing the possible leakage of steam through the cap seals which would lead to irreproducible losses in the volumes of the digestates. To each of the cooled digestates, 2.5 mL of a solution prepared by addition of 25 parts of 5% sodium carbonate to one part of a mixture of 0.25% $CuSO_4 \cdot 5H_2O$ and 0.5% sodium tartrate is added. After mixing, followed by incubation for 10 minutes at ambient temperature, 0.5 mL of 1.0 N Folin-Ciocalteu phenol reagent is added, and the solutions are **immediately** mixed; immediate and thorough mixing is essential because although reduction of the phosphomolybdic/phosphotungstic acid by tyrosine occurs only in sufficiently alkaline solutions, the Folin reagent is also unstable at high pH (Folin and Ciocalteu, 1927). After 30 minutes of incubation at ambient temperature, the absorbance (750 nm) of the samples is read against a reagent blank. Protein values are determined by interpolation from a standard curve prepared from reference standards (e.g., bovine serum albumin) that are developed in parallel with the digested samples.

Validation of Method

A typical standard curve (Fig 1.) was constructed from a dilution series of bovine serum albumin; the coefficient of determination (r^2) was 0.999 for the range from 30 to 150 µg of protein per assay. This corresponds to a limit of quantitation of about 15 µg per assay. The standards for this curve were evaluated on four different days. The applicability of this adapted form of the Lowry assay for quantitation of whole cell protein in oil shale wastewater was evaluated by comparison of results with the relative percent concentration of cells in a solution that was made by resuspending bacteria in filtered Oxy-6 retort water (i.e., abiotic particulates had been eliminated prior to resuspension). Serial dilutions were made of this culture, and protein concentrations were determined and regressed against the relative bacterial concentration (percent) (Fig. 2), volatile suspended solids (VSS) (Fig. 3), dry mass (Fig. 4), turbidity (absorbance at 660 nm) (Fig. 5), and nephelometric turbidity (Fig. 6). Turbidity measurements at A_{660} were zeroed against homologous cell-free filtrates. Nephelometric turbidity was obtained from an HF Instruments DRT-100 turbidimeter. Dry mass determinations were performed using 0.2-µm pore diameter polycarbonate membranes, which were dried at 105 °C for 0.5 h or until the weight was constant. The VSS of each sample was determined by drying the residue of glass-fiber (Whatman GFC) filtration at 103 °C for 1.0 h followed by igniting the sample at 550 °C for 15 minutes; the difference in filter mass was defined as VSS.

The regressions of protein versus relative bacterial concentration, dry mass, VSS, turbidity as absorbance, and nephelometric turbidity, had r^2 values of 0.996, 0.979, 0.978, 0.995, and 0.984, respectively. The excellent correlation of protein with relative bacterial concentration demonstrated that protein accurately reflected biomass concentration. Since protein concentration was shown to be linearly correlated with bacterial concentration, deviations from linearity for the other regressions must have resulted from nonlinearity of the alternate method. Indeed, dry mass, VSS, and nephelometric turbidity appeared to be the least accurate, although they too exhibited excellent linearity. These deviations probably resulted from volatile abiotic particulates, inadequacies of gravimetric analysis, and secondary light scattering. Turbidity as absorbance yielded excellent results, but its use as a routine tool is limited because each sample must be zeroed against its own filtrate necessitating excessive sample consumption and preparation. The slope of the regression line for protein versus dry cell mass (Fig. 4) indicated that the cells used in this study were 53 percent protein on a dry weight basis; this agrees extremely well with published values (Stickland, 1951), although variations should be expected because of compositional differences in species and because of intercomparison of results from different methods normalized to different protein reference standards. This method has been used routinely for following the exponential growth of batch cultures of acclimated bacteria in retort waters. Representative results are presented by Jones et al. (1982), where protein concentration is correlated with substrate removal and with phosphate concentration.

REFERENCES

Cooney, D.O.
"Interference of Contaminants from Membrane Filters in Ultraviolet Spectrophotometry", Anal. Chem. 1980, 52, 1068-71.

Daughton, C.G.; Jones, B.M.; Sakaji, R.H.
"Analytical Problems in Monitoring Retort Water Treatment", in Energy and Environment Division 1980 Annual Report, Lawrence Berkeley Laboratory, 1981, LBL-11989.

Folin, O.; Ciocalteu, V.
"On Tyrosine and Tryptophane Determinations in Proteins", J. Biol. Chem. 1927, 73, 627-50.

Fox, J.P.
"Retort Water Particulates", in Oil Shale Symposium: Sampling, Analysis and Quality Assurance; Gale, C. (Ed.); U.S. EPA: Cincinnati, Ohio, 1980; EPA-600/9-80-022, pp. 226-50.

Gerhardt, P.
"Diluents and Biomass Measurement", in Manual of Methods for General Bacteriology; Gerhardt, P. et al. (Eds.); American Society for Microbiology: Washington, DC, 1981; Chapter 25, pp 504-7.

Hanson, R.S.; Phillips, J.A.
"Chemical Composition", in Manual of Methods for General Bacteriology; Gerhardt, P. et al. (Eds.); American Society for Microbiology: Washington, DC, 1981; Chapter 17, pp 328-64.

Herbert, D.; Phipps, P.J.; Strange, R.E.
"Chemical Analysis of Microbial Cells", in Methods in Microbiology; Norris, J.R. and Ribbons, D.W. (Eds); Academic Press: New York, 1971; Vol. 5B, Chapter III, pp 242-65.

Jones, B.M.; Sakaji, R.H.; Thomas, J.F.; Daughton, C.G.
"Microbial Aspects of Oil Shale Wastewater Treatment", in Energy & Environment Division 1981 Annual Report, Lawrence Berkeley Laboratory, 1982, LBL-13500.

Karl, D.M.
"Cellular Nucleotide Measurements and Applications in Microbial Ecology", Microbiol. Rev. 1980, 44, 739-96.

Koch, A.L.
"Growth Measurement", in Manual of Methods for General Bacteriology; Gerhardt, P. et al. (Eds.), American Society for Microbiology: Washington, DC, 1981, Chapter 11, pp 179-207.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.
"Protein Measurement with the Folin Phenol Reagent", J. Biol. Chem. 1951, 193, 265-75.

Paul, E.A.; Voroney, R.P.
"Nutrient and Energy Flows through Soil Microbial Biomass", in Contemporary Microbial Ecology; Ellwood, D.C. et al. (Eds.), Academic Press: New York, NY, 1980, pp 215-37.

Stickland, L.H.
"The Determination of Small Quantities of Bacteria by Means of the Biuret Reaction", J. Gen. Microbiol. 1951, 5, 698-703.

Zierdt, C.H.
"Adherence of Bacteria, Yeast, Blood Cells, and Latex Spheres to Large-Porosity Membrane Filters", Appl. Environ. Microbiol. 1979, 38, 1166-72.

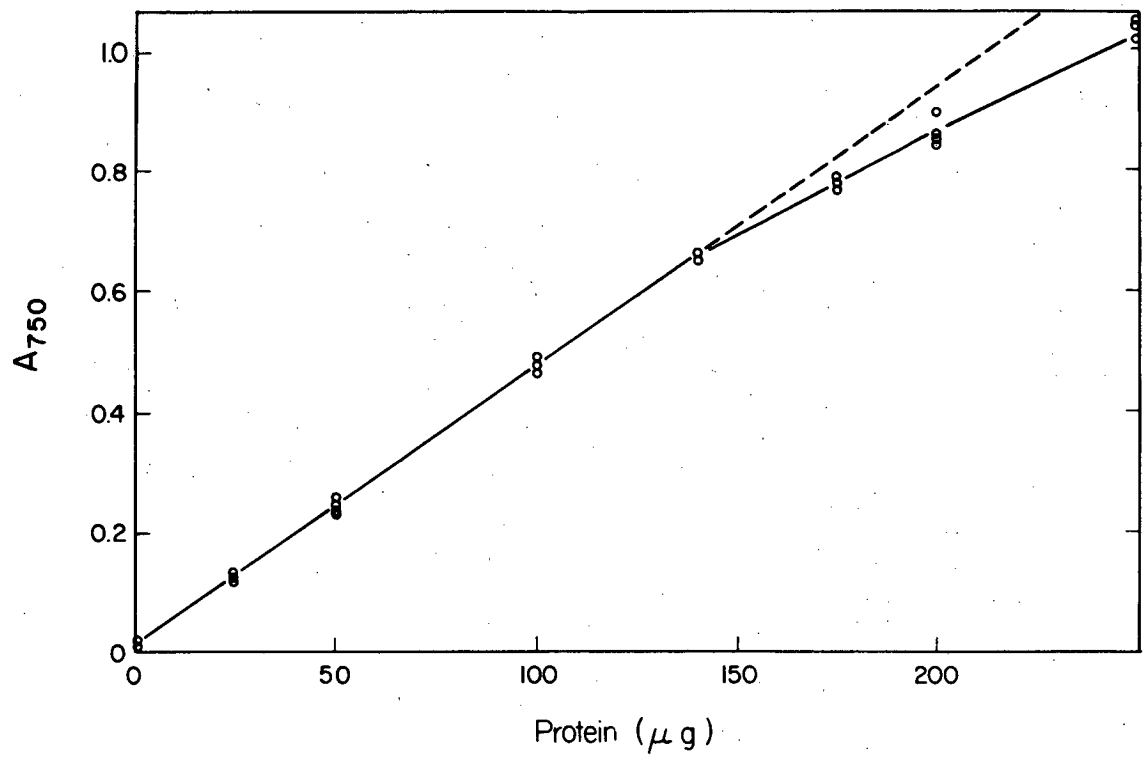


Figure 1. Lowry assay standard curve for bovine serum albumin

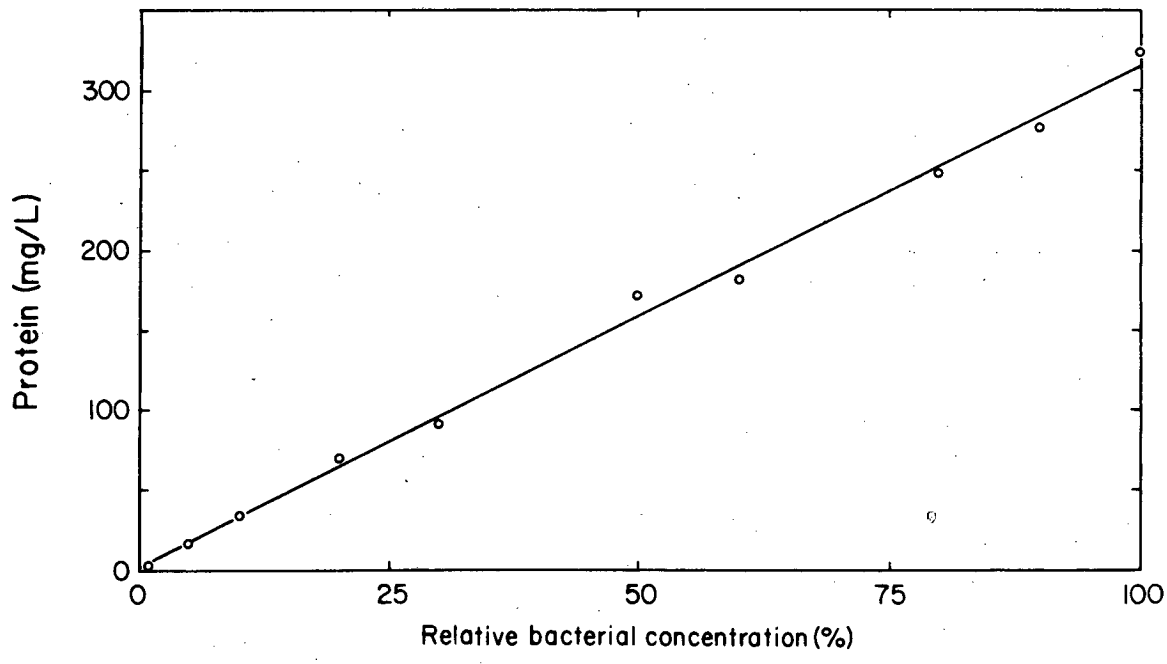


Figure 2. Correlation of a dilution series of bacteria in Oxy-6 retort water with whole-cell protein

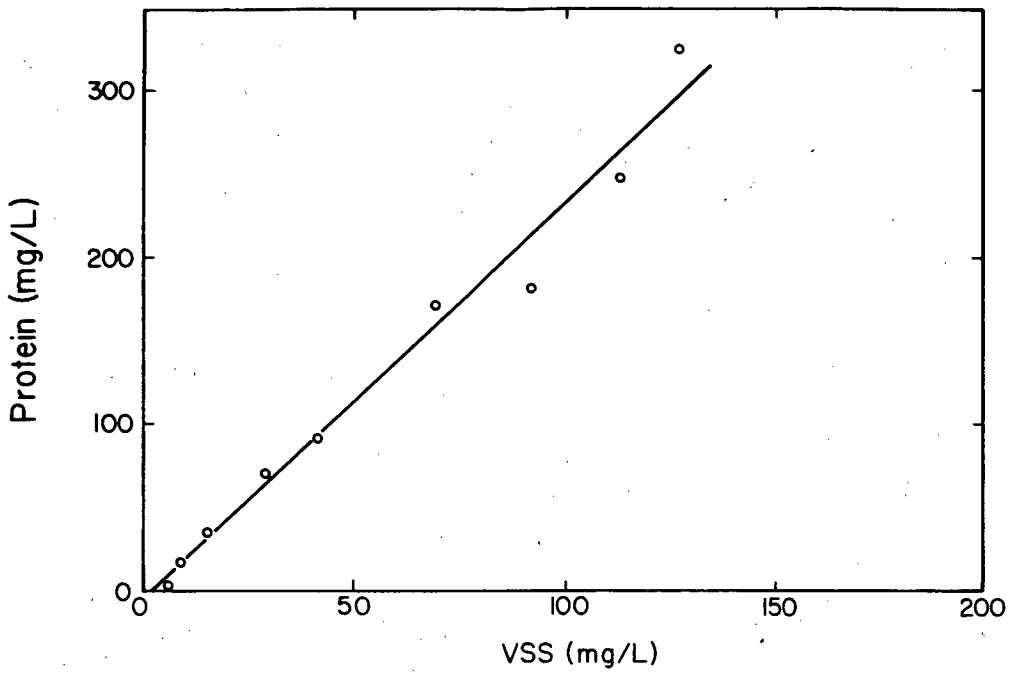


Figure 3. Correlation of volatile suspended solids in Oxy-6 retort water with whole-cell protein

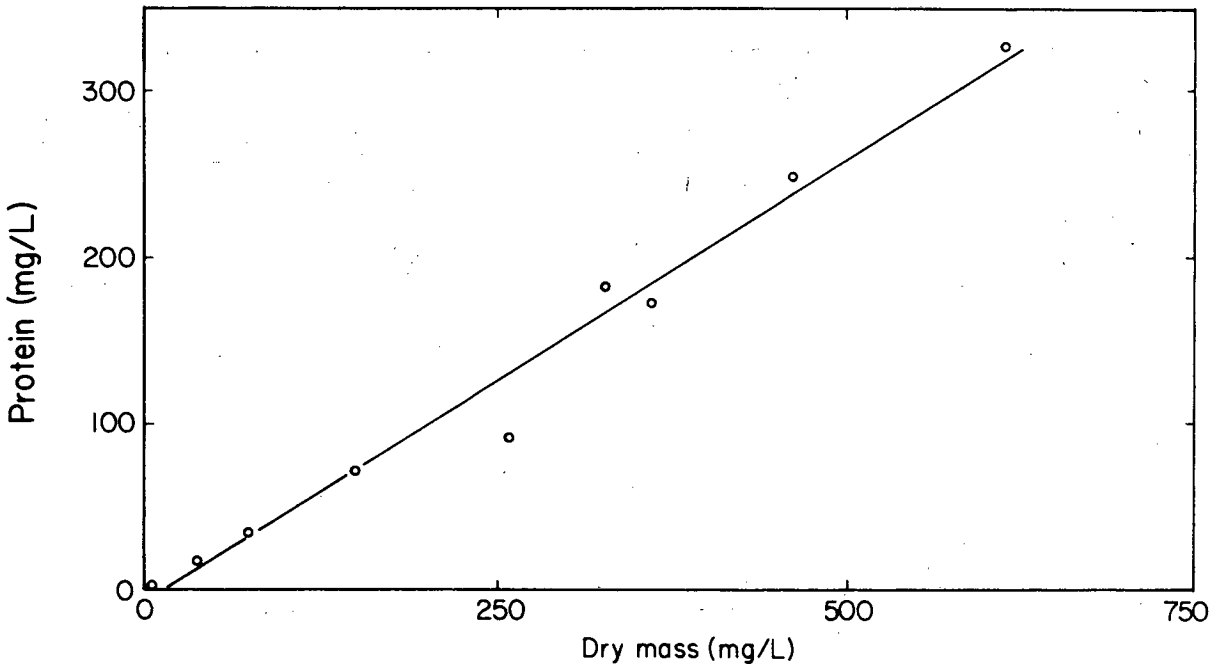


Figure 4. Correlation of dry cell mass in Oxy-6 retort water with whole-cell protein

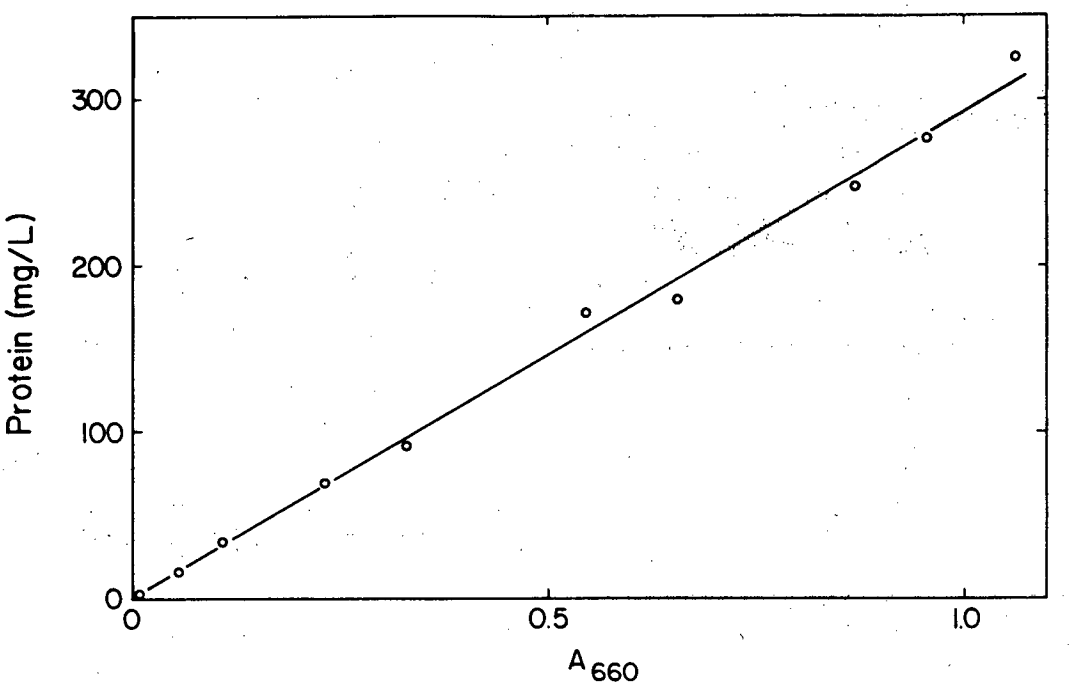


Figure 5. Correlation of turbidity (measured as absorbance at 660 nm) in Oxy-6 retort water with whole-cell protein

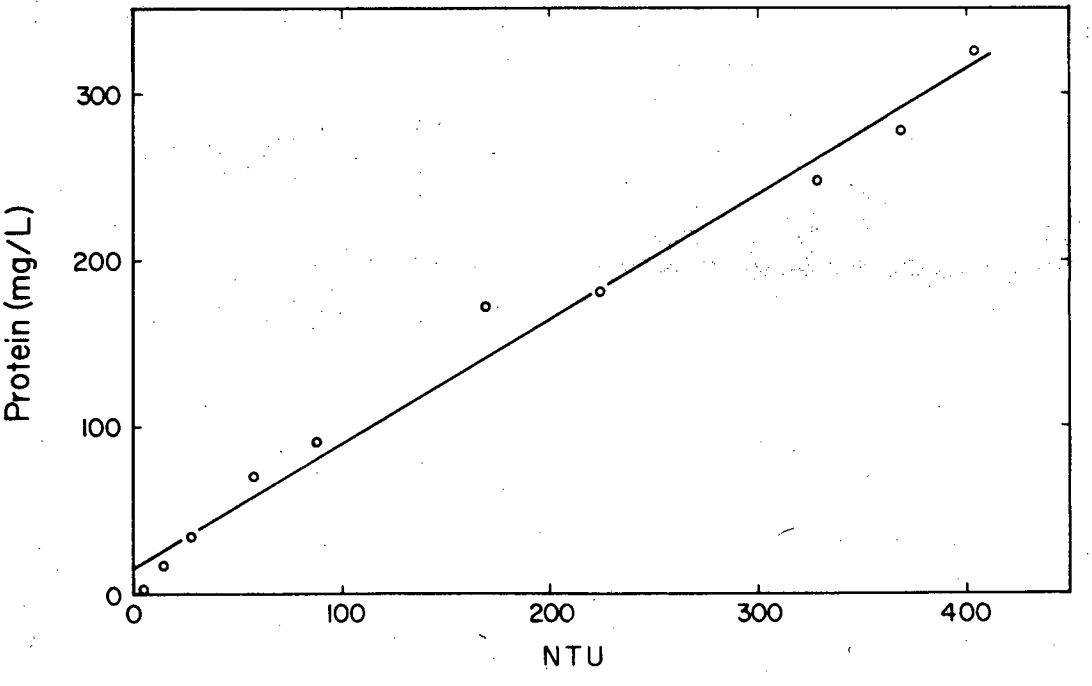


Figure 6. Correlation of turbidity (measured as nephelometric turbidity units) in Oxy-6 retort water with whole-cell protein

PROTOCOL: PROTEIN ASSAY

I. **Apparatus**

A. Glassware (one each per sample)
   1. Culture tubes: Pyrex (16-mm O.D. X 150 mm) with Teflon-lined screw-caps
   2. Glass rods: 3-mm O.D. X 145 mm

B. Filtration Apparatus
   1. Vacuum filtration flask connected to water aspirator or vacuum pump.
   2. Microanalysis filtration unit (e.g., Millipore, catalog # XX1002500 or XX1002530).
   3. Filters: Teflon, 25.0-mm diam., 0.45-µm diam. pore size (e.g., Sartorius, catalog # 11806)
   4. Forceps (2 pairs).

C. Pipettes (calibrated): air displacement (e.g., Gilson, P-1000) and positive displacement (e.g., S.M.I. Micro/Pettors, digital adjust, 20-100 µL)

D. Vortex mixer

E. Heating unit: Tecam fluidized sandbath SBL-1 (Techne, Inc., Princeton, NJ)

F. Sonicator bath (125 watts)

G. Turbidimeter: DRT-100 (HF Instruments Limited, Bolton, Ontario, Canada)

H. Spectrophotometer: (e.g., Bausch & Lomb Spectronic 88 with micro flow-through cell, 1-cm path length; ensure that the EPR O-rings are used)

II. **Reagents** (Note: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.)

A. Stock Protein Standard: bovine serum albumin (BSA) stock solution (2.800 g/L). Slowly dissolve (to prevent foaming) 0.2800 g of BSA in a 100.00-mL volumetric flask with 70 mL of water and bring to volume. Freeze 5-mL portions of stock in small, polyethylene reagent bottles for later use.

B. Lowry Stock Reagents:
   1. 5.0% Na_2CO_3 in water (w/v). Make up 500 mL for stock.
   2. 1.0% $NaKC_4H_4O_6 \cdot 4H_2O$ in water (w/v). Make up 100 mL for stock.
   3. 0.5% $CuSO_4 \cdot 5H_2O$ in water (w/v). Make up 100 mL for stock.
   4. Folin-Ciocalteu phenol reagent, 2 N. (e.g., Sigma Chemical Co.)

C. Digestion Reagent:
   1. 1.0 N aqueous NaOH (make up 100.00 mL for stock solution and store in polyethylene reagent bottle).

D. Filter-Rinsing Reagents:
   1. Phosphate buffer: KH_2PO_4-K_2HPO_4 buffer (pH 7.4; ionic strength = 0.1; buffer capacity = 3.1)
   2. Anhydrous methanol

III. Protocol

A. Preparation of apparatus
   1. Turn on sandbath. Adjust temperature setting and air flow rate to stabilize temperature at 100 °C.
   2. Set up filtration unit.
   3. Turn on spectrophotometer.
   4. Use only culture tubes without chipped rims (to ensure a gas-tight seal). Wash tubes and glass rods in a 35-percent nitric acid bath, and rinse with water. Dry thoroughly. Using forceps, put one glass rod in each test tube. Cap tubes loosely.

B. Preparation of assay solutions
   1. Lowry assay reagent solutions (Prepare fresh on day of assay.)
      a. **Solution A:** (Volume required depends on the number of samples). To twenty-five parts of 5.0% Na_2CO_3, add one part of cupric-tartrate reagent (v/v). Cupric-tartrate reagent is made by combining equal volumes of 0.5% $CuSO_4 \cdot 5H_2O$ and 1.0% $NaKC_4H_4O_6 \cdot 4H_2O$.
      b. **Solution B:** (Volume required depends on the number of samples). Dilute the 2N Folin-Ciocalteu phenol reagent to 1.0 N with water.
   2. Protein standards
      a. Thaw (to ambient temperature) one bottle of BSA stock solution.
      b. Dilute BSA stock solution (2.800 g/L) so that 0.50 mL of a diluted standard contains the following quantity of protein:

         0.140 g: 1.000 mL of stock and dilute to 10.00 mL
         0.125 g: 0.890 mL of stock and dilute to 10.00 mL
         0.100 g: 0.715 mL of stock and dilute to 10.00 mL
         0.070 g: dilute 0.140-g standard with equal vol. water
         0.050 g: dilute 0.100-g standard with equal vol. water
         0.025 g: dilute 0.050-g standard with equal vol. water

   Note: Use positive displacement pipettes whenever possible.

C. Preparation of standard curve
   1. Pipette (positive displacement) 0.500 mL of each BSA standard into a screw-cap culture tube (glass rods not necessary.)
   2. Prepare reagent blank by pipetting 0.500 mL of water into a tube.
   3. To each tube, add 0.500 mL of 1.0 N NaOH.
   4. Assay with digested samples. (Do not heat-treat these standards.)

D. Preparation of samples
   1. Disperse the culture by sonic treatment for 1 minute.
   2. Take turbidity reading. Determine (from NTU) an appropriate amount of culture to filter so that the amount of protein in each sample will yield an absorbance at 750 nm which will fall mid-range on the standard curve (e.g., filter 0.50 to 0.75 mL for a culture of 300 to 600 NTU).
   3. Filter samples. Each culture should be assayed in duplicate.
      a. Place the Teflon membrane on the filtration apparatus.
      b. Turn on the vacuum.
      c. Using positive displacement pipette, sample the predetermined volume of culture.

d. Prewet the Teflon filter with 1 mL of methanol.
e. Dispense the sample immediately after the methanol has been drawn through the membrane. If possible, avoid contact of the sample with the filter-holder funnel. Record the applied sample volume.
f. Wash membrane retentate with 1 mL of phosphate buffer.
g. Following the phosphate buffer wash, rinse the membrane with 1 mL of methanol (to dissolve tarry materials).
h. Place the filter on top of the test tube (retentate side facing up) and push the filter to the bottom of the tube using the glass rod. This can be accomplished by holding the rod with a pair of forceps in one hand while removing the filter from the filter holder with forceps using the other hand. Be sure that the glass rod is on top of the membrane and that the membrane is not folded.
i. If necessary, samples now may be stored in sealed tubes at -20 ^{o}C for assay at a later date.

E. Sample digestion
1. Add 0.500 mL of 1.0 N NaOH to each sample tube.
2. Seal tubes tightly with Teflon-lined screw caps.
3. Place sample tubes in sandbath. Heat at 100 ^{o}C for 10 minutes.
4. Remove sample tubes and cool in waterbath.
5. Add 0.500 mL of water to digested sample.

F. Color development
1. Add 2.50 mL of Solution A to each tube containing sample or standard.
2. Vortex.
3. Incubate at ambient temperature for 10 minutes.
4. Add 0.500 mL Solution B and vortex IMMEDIATELY.
4. Incubate the mixture at ambient temperature for 30 minutes (color is stable for at least one hour).
5. Read the absorbance of the standards and the samples at 750 nm against the reagent blank.

IV. Data Reduction

A. Determine the linear regression equation by "least squares" analysis for the protein standard curve. Calculate the coefficient of determination (r^2).

B. Using the "least squares" equation for the standard curve, interpolate the mass of protein collected on each membrane.
e.g., mass of protein = (m)(A_{750}) + b

C. Calculate the concentration of protein (µg/mL) for each culture.
e.g., concentration of protein (µg/mL) =
mass (µg) of collected protein/filtered sample volume (mL)

Protocol prepared by: K. Yu, B.M. Jones, R.H. Sakaji, and C.G. Daughton.

APPENDIX. Summary of Data Obtained for Oil Shale Wastewaters[1]

	DOC (direct)		TDC			COD		NH_3-N		Oil and Grease	
Wastewater	high-temp	UV-persulfate	high-temp	UV-persulfate	DIC	titrimetric	colorimetric	titrimetric	colorimetric	unfiltered	filtered
Paraho	41 809 (1.4)	42 066 (1.1)	43 415 (0.55)	42 680 (0.66)	209 (1.3)	151 600 (0.79)	141 379 (4.5)	26 385 (1.3)	24 689 (1.0)	na[2]	na
150-Ton	3 147 (0.58)	3 259 (0.46)	4 857 (0.44)	5 060 (0.53)	1 932 (1.8)	11 048 (1.1)	11 662 (2.4)	10 838 (1.3)	10 662 (0.7)	641 (58)	448 (37)
Oxy-6 retort water	2 829 (0.80)	2 942 (0.40)	3 817 (1.2)	3 952 (0.54)	984 (1.0)	8 967 (0.52)	8 990 (0.86)	1 161 (0.5)	1 127 (0.9)	242 (40)	219 (39)
Geokinetics	1 627 (1.1)	1 656 (0.55)	3 674 (1.3)	3 682 (0.45)	1 994 (0.67)	7 191 (0.81)	7 578 (1.5)	1 991 (1.0)	1 905 (0.7)	160 (24)	162 (8)
TV	2 651 (0.23)	2 726 (0.61)	3 370 (0.59)	3 486 (0.97)	824 (0.54)	9 193 (0.47)	9 459 (2.3)	2 401 (0.1)	2 292 (1.2)	276 (20)	175 (18)
Oxy-6 gas condensate	652 (2.6)	641 (0.51)	2 735 (0.63)	2 866 (0.47)	2 213 (0.38)	2 308 (1.0)	2 074 (6.4)	7 157 (0.1)	7 202 (0.7)	86 (48)	71 (35)
S-55	2 213 (0.40)	2 285 (0.34)	2 595 (1.8)	2 633 (0.51)	339 (1.6)	9 414 (0.35)	9 781 (2.4)	4 079 (0.08)	4 047 (1.4)	334 (78)	178 (20)
Omega-9	695 (0.34)	718 (0.44)	2 119 (2.1)	2 174 (0.29)	1 387 (1.3)	3 596 (1.2)	3 729 (1.2)	3 690 (0.4)	3 583 (1.1)	58 (33)	56 (44)
Rio Blanco sour water	206 (1.4)	207 (1.3)	556 (0.25)	548 (0.47)	364 (1.4)	912 (0.33)	925 (0.74)	1 118 (0.3)	1 065 (0.9)	na	na

[1] all boldface values are mg/L; values in parentheses are rsd percentages except for oil and grease, where they are ranges

[2] not available

QUANTITATION OF OIL SHALE WASTEWATER QUALITY

A Manual of Analytical Methods

Designed by Christian G. Daughton
Diagrams by Richard H. Sakaji
Composed at
The Oil Shale Waste Treatment Project
Sanitary Engineering and Environmental Health Research Laboratory,
University of California, Berkeley
in 12 Finance by Programma International Word Processing System
Using an Apple II Plus Computer and Olivetti 221 Printer
Printed and Bound by the Oil Shale Waste Treatment Project

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720