COLLABORATIVE RESEARCH SUPPORT PROGRAM

# POND DYNAMICS / AQUACULTURE

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Corvallis, Oregon [illegible]

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# POND DYNAMICS/AQUACULTURE COLLABORATIVE RESEARCH SUPPORT PROGRAM

## FIFTH WORK PLAN

September 1, 1989
through
August 31, 1991

Pond Dynamics/Aquaculture CRSP
Office of International Research and Development
Oregon State University
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# INTRODUCTION

The Fifth Work Plan of the Pond Dynamics/Aquaculture CRSP was developed by the CRSP Technical Committee at a meeting in Davis, California on May 1 to 4, 1989. This work plan describes activities to be conducted by the CRSP during the period 1 September 1989 to 31 August 1991. This two-year period includes the last year of the current AID Grant and the first year of the proposed continuation grant. Many of the studies described in this work plan are contingent upon the continuation of the program. Additionally, several studies are proposed for which funding is not allocated in the existing grant and the proposed continuation. These studies, which are identified in the text, will be supported by other sources.

The Fifth Work Plan will be implemented in Honduras, Rwanda, Thailand, and the USA. Most field studies will concentrate on the dynamics of freshwater ponds although several experiments in Honduras will be concerned with the dynamics of brackish water ponds. In addition to field activities at the research farms of the cooperating institutions, experiments with cooperating farmers are planned. Analysis and synthesis of the global data resulting from the field studies will be conducted in the United States.

The first three CRSP work plans specified identical experiments at all CRSP sites to provide a baseline for comparisons between sites. The approach for the Fourth Work Plan, which covered the period 1 September 1987 to 31 August 1989, changed in that different, but related, experiments were conducted at the various sites. In this way, many more hypotheses could be tested than if the same experiments were conducted at all sites. The Fifth Work Plan follows the same approach as the Fourth Work Plan. Different, but related, topics will be considered at each site. The particular topics to be studied at each site are based on the research needs of aquaculture in each country and the needs for more information, as identified by the CRSP Technical Committee.

The general goals of this work plan are:

1. To preserve the global nature of the CRSP experiments;
2. To conduct experiments to refine management practices for fertilized ponds;
3. To verify CRSP results with cooperating farmers;
4. To continue adding observations to the global CRSP database; and
5. To provide verified preliminary guidelines for management of fertilized ponds.

# EXPERIMENTS

## THE GLOBAL EXPERIMENT

The Fifth Work Plan will preserve the global nature of the CRSP and will address diverse research needs at each site. First, data will continue to be collected using standardized methods. Second, these data will be entered into the global database, which is maintained at the CRSP Program Management Office in Corvallis, Oregon, USA. The database is used by the Data Analysis and Synthesis Team in their development of generalized models and guidelines. The database also is available to the field stations and other interested researchers throughout the world. Third, related experiments are conducted at the various research stations. For example, studies of maintenance of minimum dissolved oxygen concentrations and the effects of pond size will be conducted in both Thailand and Honduras. Supplemental feeding studies will be conducted in Honduras and Rwanda. On-farm studies are planned for Rwanda and Thailand.

## REQUIRED MEASUREMENTS

This section of the work plan lists the minimum requirements for data collection by the CRSP projects. The accepted methods for data collection are presented in the Summary of Accepted Analytical Methods (Section 3), which starts on page 44. Detailed descriptions of accepted methods are contained in the appendices. Frequencies of data collection as specified in this section are minimum frequencies. Data may be collected more frequently at the discretion of the individual projects.

The following measurements must be taken daily:

- Solar Radiation
- Wind Speed
- Air Temperature (maximum and minimum)
- Rainfall
- Evaporation
- Mortalities
- Pond Depth
- Water Inflow and Overflow

There will be at least three intensive sampling periods for each experiment: (1) during the second week; (2) midway through the experiment; and (3) during the final week. Whole column samples collected at mid-morning should be used unless specified otherwise. The variables to be observed are:

- Total Kjeldahl Nitrogen
- Ammonia Nitrogen
- Total Phosphorous
- Secchi Disk Visibility
- Chlorophyll *a*
- Dark Bottle Respiration
- Total Suspended Solids
- Total Volatile Solids
- Total Alkalinity (3 depths: top, middle, bottom)
- Primary Productivity

Diel studies will be conducted simultaneously with the intensive sampling measurements in order to measure spatial and temporal fluctuations within a pond. Samples for diel studies will be collected at dawn, 1000, 1400, 1600, 1800, and 2300 hours, and at dawn the next day at a minimum of two depths, but preferably at three depths. The exact time of sample collection in the diel studies should be recorded. The three sampling depths will be 25 cm below the water surface, mid-depth, and 25 cm above the pond bottom. The parameters to be measured during the diel studies are:

- Dissolved Oxygen
- Temperature
- pH
- Wind (cumulative between sampling times)
- Solar Radiation (cumulative between sampling times)

Information about the fish and shrimp used in the experiments should be recorded as follows:

- Stocking
  - Total Number
  - Total Biomass
  - Individual Weights (of 10% sample)
  - Individual Lengths (of 10% sample)

- Monthly Sampling
  - Total Number in Sample
  - Total Biomass of Sample
  - Individual Weights
  - Individual Lengths
  - Reproduction Weight

- Harvest
  - Total Number of Stocked Fish Remaining
  - Total Biomass of Stocked Fish
  - Individual Weights (10% sample of stocked fish)
  - Individual Lengths (10% sample of stocked fish)
  - Total Number of Recruits
  - Total Biomass of Recruits

The following pond soil characteristics are to be determined at the beginning and end of each experiment:

- pH
- Phosphorus
- Organic Matter
- Total Nitrogen
- Cation Exchange Capacity
- Metals - Aluminum, Iron, Zinc (only when the pond is first used)
- Lime Requirement
- Exchangeable Hydrogen
- Base Saturation

Pond morphology is to be measured when the ponds are first constructed and whenever pond morphology is altered significantly. Measurements to be taken are:

- Surface Area (at 10 cm depth contours)
- Volume (at 10 cm depth contours)
- Drawing, top view, with scale

The composition of lime, inorganic, and organic fertilizers is to be determined when supplies are delivered and, for organic materials, just before they are totally used up, but not less frequently than once a month. Characteristics to be determined are:

- Percent Dry Matter
- Nitrogen
- Phosphorus
- Chemical Oxygen Demand
- Lime Neutralization Value (for lime only)

The quantities of lime and other amendments must be carefully recorded whenever they are added to the ponds.

Reference ponds are to be established and operated at each station starting the second year of this work plan.

## OPTIONAL MEASUREMENTS

CRSP projects may collect any data, in addition to those data specified under Required Measurements, which they deem appropriate for a particular study. The methods specified in the Summary of Accepted Analytical Methods (Section 3, page 44) or in the appendices (Section 4, page 53) should be used. If a method for a particular parameter is not specified in Section 3, a method from *Standard Methods* (APHA et al., 1985) should be used whenever possible and the Materials and Methods Subcommittee of the CRSP Technical Committee should be informed. If problems are encountered while using the accepted method for a particular application, the Materials and Methods Subcommittee should be contacted. If optional measurements are made and researchers wish to have the data included in the data templates, the Data Base Manager (at the Program Management Office, Oregon State University) should be contacted.

## DATA SUBMISSION

All data should be submitted to the CRSP Database Manager on either Lotus 1-2-3® or Microsoft Excel® worksheets, following the formats and procedures in the most recent CRSP *Instructions for Data Entry*. Data and accompanying text for the CRSP *Data Reports* series should be submitted to the CRSP Program Management Office within six months of harvest of each study. Please contact the Data Base Manager with questions regarding verification of data. The Data Base Manager will print the verified data for publication in *Data Reports* unless other arrangements are made in advance.

## REPORT SUBMISSION

Introduction:

CRSP researchers are aware of the difficulties in compiling the results of the various experiments described in this Work Plan into a single, cohesive *Data Report* for each site. In response to these concerns, the requirements for submitting text to *Data Reports* have changed. The studies detailed in the biennial Work Plans fit the *Research Reports* format better than the *Data Reports* format; consequently, researchers will be required to submit a technical report (text and figures) in the form of a *Research Report* to the Program Management Office (see below). *Data Reports* will serve the following purposes: to protect researchers' data from unauthorized publication and use, to compile all standardized data for use in the Global Experiment and in other analyses, and to document where procedures actually used in experiments have diverged from those described in the Work Plans.

Instructions for Submission of Reports:

1. A technical report for each experiment (each study) is due six months after the experiment ends. Technical reports should be submitted in one of the following formats:

   a) As a *Research Report*, with the understanding that the manuscript will be reviewed, edited, and printed as a short (4-8 pages) report; or
   b) As a *Notice of Publication* under the *Research Reports* series. Notices of Publication are reserved for those articles that have been or will be published in a refereed journal. Authors who wish to use this format for technical reports should submit a copy of a manuscript which is "under review" by a journal, together with a cover letter. It is recommended that authors contact the CRSP Director to discuss whether this is an option.

2. Principal Investigators are also responsible for submitting a *Data Report* for each set of studies. For example, there should be one *Data Report* for Rwanda, one for Thailand, and one for Honduras for the studies described in each biennial Work Plan. The text will be confined to a "Materials and Methods" section, which should be used to describe how the materials or methods used during the studies diverged from those specified in the Work Plan. Text for *Data Reports* is due three months after the end of the latest experiment (study) described in the Work Plan. For example, text for the Rwanda *Data Report* should be submitted three months after Study 4 ends, and should include a "Materials and Methods" section for all studies completed during the course of this Work Plan.

Should difficulties in completing the Work Plan on schedule arise, please advise the Director so that an alternate reporting format can be arranged.

## AFRICA—RWANDA PROJECT

Cooperating Institutions:

National University of Rwanda
Dr. Evariste Karangwa
Mr. Eugene Rurangwa

CIFAD (Oregon State University) - Lead Institution
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[illegible]

Dr. Thomas Popma
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CIFAD (University of Arkansas at Pine Bluff)
Dr. Carole Engle

Pond Systems: Cool freshwater ponds typical of higher elevations.

### INTRODUCTION

The choice of objectives and experimental designs proposed for the Fifth Work Plan of the Rwandan component of the CRSP are based on four general considerations:

1. In Rwanda, as in many high elevation regions of the developing world, potential nutrient inputs for aquaculture are limited. Physical and economic isolation reduces the availability of chemical fertilizers, supplemental feeds, and adequate quantities of animal manures. Consequently, during the Fourth Work Plan of the Rwanda project, grasses and similar vegetation were used as the principal nutrient input for studies on pond dynamics and fish production. Factors evaluated were: a) method of compost preparation; b) benefits of adding chicken manure, urea, or inorganic phosphorus to compost or to compost-enriched ponds; c) weekly loading rates; and d) method of applying compost to ponds (confined vs broadcast). Two important related variables not evaluated during the Fourth Work Plan were fish density and composting regime (constant composting rate throughout the cycle versus an initial high rate to accelerate plankton production followed by proportionally reduced weekly rates).

2. [illegible]

levels significantly lower than those observed elsewhere. Other biological characteristics of tilapia make it an appropriate culture fish for isolated regions with [illegible]

accurately assess the upper elevation limits for profitable tilapia culture. For example, at what temperature does tilapia culture become an inefficient use of resources? In enriched ponds, is reduced growth of tilapia at cool temperatures due more to the reduced productivity of natural food organisms or to the decreased ability of tilapia to efficiently utilize the available food supply? Is appetite, growth, and food conversion efficiency of tilapia more a function of average water temperature or of maximum or minimum daily extremes? There are 3,000 rural fish ponds in Rwanda at altitudes ranging from 1,300 to 2,500 meters. They, along

with available laboratory facilities, present a unique opportunity to answer such temperature-related questions.

3. In any region, enterprise budgets for aquacultural practices and alternative agricultural activities are necessary to evaluate the most appropriate allocation of resources. These data are especially important for aquacultural development programs when cool temperatures and scarce nutrient inputs limit fish yields from ponds. Tilapia farmers in Rwanda are pleased with annual fish yields of 1,000 to 2,000 kg/ha, but an integrated economic analysis could help determine whether fish culture is the best use of the limited resources.

4. In many developing countries, the principal source of food for fish is naturally occurring organisms in enriched ponds. Previous studies suggest that the energy content on these natural foods is insufficient for the optimum utilization of the available protein for fish growth. Supplementary feeding with high-energy feedstuffs, which are less expensive than high-protein feedstuffs, could improve protein utilization efficiency. Data on the relative abundance of digestible protein and energy in organisms cultured under different enrichment regimes is needed for the selection of the best feeds or agricultural by-products for supplementing natural foods.

There are eight studies planned for Rwanda in the Fifth Work Plan (Figure 1). The goal of these studies is to more fully understand the factors affecting pond dynamics and fish growth under climatic conditions considered marginal for tilapia production, using the limited nutrient inputs available to most potential producers in economically depressed regions.

**Figure 1. Schedule for Data Collection in Rwanda during the Fifth Work Plan.**

| Study Title | Site | 1989 | | | | 1990 | | | | | | | | | | | | 1991 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug |
| Study 1. Effects of Fish Density and Nutrient Loading Frequency | Rwasave | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 2. On-Farm Studies - Characterization of Farms | Various | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 3. On-Farm Studies - Effects of Elevation | Various | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 4. On-Farm Studies - Supplemental Feeding | Various | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 5. Effects of Diel Temperature Fluctuations on Growth | Arkansas | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 6. Digestibility of Natural Food Organisms | Rwasave | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 7. Balancing Protein/Energy Ratios of Natural Food | Rwasave | | | | | | | | | | | | | | | | | | | | | | | | |
| Study 8. Economic Analysis of Aquaculture Production Systems | Various | | | | | | | | | | | | | | | | | | | | | | | | |

## RWANDA STUDY 1: EFFECTS OF FISH DENSITY AND NUTRIENT LOADING [illegible]

Objective: To determine the effects of fish [illegible] pond dynamics and fish production in composted ponds in highland Africa.

Significance: This study will provide information on the fish densities needed to produce maximum yields of marketable fish. Also, in Rwanda, plankton blooms generally require several months to develop when a constant rate of fertilization with compost is added. Initial heavy nutrient loading may provoke a more rapid increase in primary production which could lead to greater fish yields.

Experimental Design: Three fish densities x two composting schedules. Three replicates.

Pond facilities: Eighteen ponds at the Rwasave Station, each with a surface area of 700 square meters and a depth of 60-100 cm.

Culture Period: 5 months.

Fish stocking rate: Advanced juveniles of *Oreochromis niloticus* (both sexes) at densities of 0.5, 1, and 2 fish/$m^2$.

Nutrient inputs: Two application regimes with equal total quantities of compost during the production cycle: 1) constant weekly loading at 500 kg/ha (dry weight basis); 2) heavy initial load equivalent to 30% of total (3000 kg/ha), followed by proportionally reduced weekly loads (370 kg/ha).

Water management: Replace evaporation and seepage losses weekly.

Sampling schedules: As per standard protocol.

Null Hypotheses:
1. Higher fish densities have no effect on total fish yield, average final weight or pond dynamics.
2. Initial heavy loading of compost will not accelerate plankton blooms or increase fish production.

Statistical methods: Two-way ANOVA.

Schedule: Data collection, 11/89 - 4/90; technical report, 9/90.

## RWANDA STUDY 2: ON-FARM STUDIES—CHARACTERIZATION OF FARMS

Objective: To characterize rural fish ponds at four elevation levels between 1,200 and 2,300 m ASLs on the basis of soil and water chemistry and willingness of the owners to cooperate.

Significance: In order for more detailed on-farm studies to be conducted, baseline information on farms must be collected.

Pond facilities: In coordination with the National Fisheries Extension Service of Rwanda, 50 to 100 private rural fish ponds, with surface areas of 200 to 800 $m^2$ and average depths of approximately 1 m, will be examined.

Fish stocking rate: Species cultured, densities, and size will be documented.

Nutrient inputs: Type of nutrient and rate of application will be documented.

Water management: Currently used practices will be documented.

Sampling schedule: Once per pond.
Physical parameters:
- Soil: texture and temperature at 60-cm depth in unsaturated soils.
- Water Source: temperature, volume available (L/s).
- Pond water: temperature.
- Pond morphology: surface area, depth, shade.
- Elevation (m ASL).
- Climate (monthly averages): air temperature (max-min), evaporation, rainfall, and solar radiation.

Chemical parameters:
- Water source and pond water: all chemical parameters listed under standard protocol for intensive sampling.

Biological parameters (from last two production cycles):
- Fish sizes and numbers.
- Yield.

Operational & sociological parameters:
- Pond management practices.
- Extension agent's opinion of farmer's competency and dedication.
- Farmer's expressed willingness to participate in studies.

Data analyses: Compilation and cross-tabulation.

Schedule: Coordination with Fishery Extension Service to begin in 8/89. Data collection, 3/90 - 6/90; technical report, 8/90.

## RWANDA STUDY 3: ON-FARM STUDIES—THE EFFECTS OF ELEVATION

Objective: To quantify the effects of elevation on pond dynamics and fish growth in ponds enriched with a standardized compost regime.

Significance: This study will lead to a better understanding of the elevation at which the culture of *O. niloticus* becomes inappropriate.

Experimental design: Five elevations. Replicated five times.

Pond facilities: Five ponds at each elevation range: 1300-1400 m (ASL), 1500-1600 m, 1700 m (Rwasave Station), 1800-2000 m, and 2100-2300 m.

Culture Period: At least 5 months, continuing until fish reach an average weight of 150 g.

Fish stocking rate: Juvenile *O. niloticus* (both sexes) at 1 fish/$m^2$.

Nutrient inputs: Fresh green grass of a genus common to all sites at 500 kg/ha/wk (dry weight basis) plus 8 kg/ha/wk N as urea and TSP to give total N:P ratio of 4:1. Lime may be added at specific sites to standardize conditions.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: As per standard protocol except as noted.
Physical parameters:
- Max-min water temperature, water depth, color, and Secchi disk visibility: weekly.
- Temperature of unsaturated soil at a depth of 60 cm: three times.
- Continuous recording of water temperature over weekly periods in at least one pond per elevation range.

Chemical parameters:
- No diurnal, primary productivity, respiration, or soil measurements.

Biological parameters:
- Fish weight: three times.
- Stomach samples: 15 fish/pond are to be collected during month 5 at 1500-1700 hours. Analyze for dry matter, volatile matter, and Kjeldahl-N.

Null Hypotheses:
1. Elevation has no effect on fish yield.
2. There is no relationship between tilapia growth and the productivity of natural food organisms.

Statistical methods: One-way ANOVA and linear regression.

Schedule: Data collection, rural ponds, 7/90-5/91; Rwasave, 7/90-1/91. Technical report, 8/91.

## RWANDA STUDY 4: ON-FARM STUDIES—SUPPLEMENTAL FEEDING

Objective: To determine to what extent the addition of supplemental feedstuffs to enriched (composted) ponds changes the effect of elevation on pond dynamics and fish growth.

Significance: This study will provide an indication of the benefits of supplemental feed at higher elevations.

Experimental design: Five elevations. Five replicates.

Pond facilities: Five ponds at each elevation range: 1300-1400 m (ASL), 1500-1600 m, 1700 m (Rwasave Station), 1800-2000 m, and 2100-2300 m.

Culture Period: At least 5 months, continuing until fish reach an average weight of 100 g.

Fish stocking rate: Juvenile *O. niloticus* (both sexes) at 1 fish/$m^2$.

Nutrient inputs: Fresh grass plus N + P, as in Study 3, plus a rice bran or commercial poultry ration at 2% of average body weight daily.

Water management: Replace evaporation and seepage weekly.

Sampling schedule: As per standard protocol except as noted.
Physical parameters:
- Max-min water temperature, water depth, color, and Secchi disk visibility: weekly.
- Temperature of unsaturated soil at depth of 60 cm: three times.
- Continuous recording of water temperature over weekly periods in at least one pond per elevation range.

Chemical parameters:
- No diurnal, primary productivity, respiration, or soil measurements.

Biological parameters:
- Fish weight: three times.
- Stomach samples: 15 fish/pond are to be collected during month 5 at 1500-1700 hours. Analyze for dry matter, volatile matter, and Kjeldahl N.

Null Hypotheses:
1. Elevation has no effect on the growth of tilapia.
2. Supplemental feed will not improve fish growth.

Statistical methods: One-way ANOVA, two-way ANOVA (combining data from Study 3), linear and multiple regression.

Schedule: The stocking of ponds for this study will begin 5/91 and will be completed during the next work plan.

## RWANDA STUDY 5: EFFECTS OF DIEL TEMPERATURE FLUCTUATION ON GROWTH

Objective: To evaluate, under laboratory conditions, the effect of temperature on the feed intake, growth, and feed conversion efficiency of tilapia.

Significance: This study will help determine if maximum water temperature is more critical than average temperatures. This could have great impact on pond management and determination of appropriate regimes for supplemental feeding practices for high elevation fish ponds.

Experimental design: Four constant temperatures and two cyclic temperature regimes. Three replicates.

Pond Facilities: Eighteen aquaria at the University of Arkansas-Pine Bluff aquatic laboratory.

Culture period: Four weeks plus an acclimation period of two weeks.

Fish stocking rate: Juvenile *O. niloticus* at a standing stock of 10 g/L.

Nutrient inputs: Floating catfish feed of appropriate size. Satiation feeding for 15 minutes, 2 times daily. Feed consumption per meal is to be determined by removing uneaten feed, and drying and weighing it.

Water management: Constant aeration and water flow sufficient to exchange water two times a day. Water inflow is suspended during feeding. Water temperatures are to be either constant or fluctuate in a diel pattern to simulate natural temperature fluctuations. The constant water temperature regimes will be 16°C, 20°C, 24°C, and 28°C. The cyclic regimes will be 16 to 24°C with a daily average of 20°C and 20 to 28°C with daily average of 24°C.

Sampling schedule:
Physical parameters: water temperature: at least 3 times daily.
Chemical parameters: dissolved oxygen, pH, ammonia: initially, daily.
Biological parameters:
- Fish mortality and weight of carcass: daily.
- Total fish weight and number: biweekly.

This study will investigate:
1. Whether maximum water temperature is a better indicator of the temperature limitation of tilapia growth than average temperature.
2. Whether appetite of tilapia is directly correlated with temperature.
3. Maximum water temperature and average temperature are equivalent indicators of the temperature limitations for tilapia growth.

Statistical methods: ANOVA and linear regression.

Schedule: Data collection, 11/90 - 3/91, depending on supplementary funding. Technical report, 8/91.

## RWANDA STUDY 6: DIGESTIBILITY OF NATURAL FOOD ORGANISMS

Objective: To determine the digestibility of the protein and energy in natural food organisms ingested by tilapia in ponds that are enriched by selected inorganic and organic fertilizers.

Significance: Information on the relative abundance of crude protein and digestible energy in the natural foods consumed by tilapia in enriched ponds would be useful for the selection of supplemental feeds.

Experimental design: Six nutrient input regimes. Two replicates.

Pond facilities: 12 ponds at the Rwasave Station, each with a surface area of 700 $m^2$ and average depth of approximately 1 m. The 'edible leaves' treatment will use floating cages placed in ponds with low natural productivity.

Culture period: Eight weeks.

Fish stocking rate: 25 kg per pond (350 kg/ha) of advanced juvenile and young adult male *O. niloticus* .

Nutrient inputs: Six nutrient input regimes as follows: 1) Chemical fertilizer at a recommended rate; 2) Chicken manure at 500 kg/ha/wk; 3) Swine manure at 500 kg/ha/wk; 4) Fresh grass and N + P at recommended rates; and 5) Fresh edible leaves of two plant species.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Chemical parameters:
- Feces and stomach contents: ash-free dry matter, Kjeldahl nitrogen, gross energy by bomb calorimetry, and Chromogen "indicator" by spectrophotometry after acetone extraction.
- Fish carcasses: dry matter, crude protein, and lipids.

Biological parameters:
- Feces and stomach content samples from 80 fish from each pond after weeks six and eight. For each pond and each sampling date, combine individual samples to form two composite samples of feces and two of stomach contents.
- Determine relative abundance of food groups by microscopic examination from subsamples of the fresh composite samples
- Net fish yield after 8 weeks.

Null Hypothesis: The energy/protein ratio of the natural food ingested by tilapia is not different from 11 kcal of digestible energy per gram of digestible crude protein.

Statistical Methods: *t* test.

Schedule: Data collection, 7/90 - 11/90; technical report, 2/91.

## RWANDA STUDY 7: BALANCING PROTEIN/ENERGY RATIOS OF NATURAL FOODS

Objective: To test the conclusions from the digestibility studies regarding the protein-energy balance of natural foods in enriched ponds by comparing the growth of tilapia in manured ponds receiving different levels of supplemental dietary digestible energy.

Significance: If this study substantiates the benefits of supplemental dietary energy, the energy-rich commercial feeds formulated for warm-blooded animals (these are generally available in developing countries whereas specifically formulated fish feeds are not), may have considerable potential as supplementary feeds.

Experimental design: Six control ponds plus three levels of supplemental energy (0.75, 1.5, and 3.0 kcal DE/$m^2$/d). Four replicates.

Pond facilities: Eighteen ponds at the Rwasave Station, each with a surface area of 700 $m^2$ and an average depth of approximately 1 m.

Culture period: Five months.

Fish stocking rate: Juvenile *O. niloticus* (both sexes) at 1 fish/$m^2$.

Nutrient inputs: All ponds will be fertilized with the enrichment regime from Study 6 that is demonstrated to be the most energy deficient. The source of supplemental energy should have a very low protein content, such as cassava meal which has been treated to improve palatability.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: As per standard protocol.

Null Hypothesis: Adding supplemental digestible energy has no effect on fish growth.

Statistical methods: One-way ANOVA.

Schedule: Data collection, 1/91 - 7/91; technical report, 10/91.

## RWANDA STUDY 8: ECONOMIC ANALYSIS OF AQUACULTURAL PRODUCTION TECHNOLOGIES

Objective: To estimate costs and benefits for different aquacultural production technologies practiced in Rwanda.

Significance: Fish culture is a relatively new production enterprise in Rwanda. Many fish ponds are located at elevations often considered outside the feasible range for profitable tilapia culture, yet many farmers continue to raise fish. This study should provide indications of how fish culture compares to traditional crops from an economic perspective. It will further indicate some of the economic trade-offs between fish culture and traditional agricultural enterprises in Rwanda.

Data collection: Secondary data will be collected in Rwanda from previous surveys (including the Enquete Nacional Agricole and current World Bank and GTZ projects) on the levels of resources (land, labor, capital, fertilizer materials) commonly available on Rwandan farms. Data on fish yields and costs will be obtained from farm records collected by the Rwandan National Fish Culture Project based in Kigembe. A follow-up survey of fish farmers will document other crops being produced, land utilization, labor availability, and constraints to production.

Hypothesis: Fish culture yields are as beneficial to Rwandan farmers as other agricultural enterprises in terms of animal protein production for home consumption and supplemental income.

Analytical Methods: Enterprise budget analysis will be used to estimate costs and returns of the aquaculture production technologies that have been and are being developed for Rwanda. Net returns from fish culture will be compared to net returns from bean production and other crops for which sufficient information is available. The analysis of net returns will include consideration of a farmer's need for stable yields and risk-reducing strategies.

Schedule: Data collection and economic analysis, 7/89 - 2/90; technical report, 4/90.

Remarks: This study and analysis will be implemented by the University of Arkansas at Pine Bluff and is funded by a subcontract with Oregon State University. This economic analysis is complemented by another AID-funded study on the sociological impact of fish culture in Rwanda.

## CENTRAL AMERICA—HONDURAS PROJECT

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Pond Systems: Tropical freshwater and brackish water ponds.

### INTRODUCTION

The proposed research activities in Honduras during the Fifth Work Plan focus on the examination and refinement of management procedures that can potentially increase fish yields. Management procedures that will be examined are stocking density, supplemental feeding, temperature effects on fry production and sex-reversal, and supplemental aeration.

Most of the research activities will be conducted at the freshwater station at El Carao. The recent installation of automated data logging and control systems at the El Carao station will enable much more thorough monitoring of spatial and temporal fluctuations in water quality than previously possible.

Six studies are planned for the Fifth Work Plan (Figure 2). The goal of these studies is to increase in fish yields through improved management practices.

**Figure 2. Schedule for Data Collection in Honduras during the Fifth Work Plan.**

| Study Title | Site | 1989 | | | | 1990 | | | | | | | | | | | | 1991 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug |
| Study 1. Supplemental Feed/Manure Combinations | El Carao | ********** | | | | | | | | | | | | | | | | | | | | | | | |
| Study 2. Tilapia - Tambaqui Polyculture Studies | El Carao | | | | | | ********** | | | | | | | | | | | | | | | | | | |
| Study 3. Temperature Effects on Fry Production | El Carao | ********** | | | | | | | | | | | | | | | | | | | | | | | |
| Study 4. Factors Affecting Tilapia Sex Reversal | El Carao | ********** | | | | | | | | | | | | | | | | | | | | | | | |
| Study 5. Maintenance of Minimum Dissolved Oxygen Concentrations | El Carao | | | | | | | | | | | ********** | | | | | | | | | | | | | |
| Study 6. Supplemental Feeding - Effects of Fish Density | El Carao | | | | | | | | | | | | | | | | | ********** | | | | | | | |

## HONDURAS STUDY 1: SUPPLEMENTAL FEED/MANURE COMBINATIONS

Objective: To determine the effects of various combinations of feeding and fertilization with chicken manure on fish production and water quality.

Significance: Data from this study will provide information which will contribute to the effort to determine the optimum input levels of chicken litter and supplemental feeds.

Experimental design: Four treatments. Three replicates.
- Chicken litter applied at 1000 kg total solids/ha/wk.
- Chicken litter during the first month at 1000 kg/ha/wk, followed by feed only at 3% body weight/day.
- Chicken litter during the first 2 months at 1000 kg/ha/wk followed by feed only at 3% body weight/day.
- Chicken litter during the first 3 months at 1000 kg/wk/wk followed by feed only at 3% body weight/day.

Pond facilities: El Carao, twelve 1000 $m^2$ ponds, 0.75 m deep.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 1 fish/$m^2$ and guapote tigre (*Cichlasoma managuense*) at 0.05 fish/$m^2$.

Nutrient inputs: Chicken litter at 1000 kg/ha/wk and a commercial feed at 3% body weight/d depending on the treatment.

Water management: Replace evaporation and seepage losses after 5 cm loss.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Diel measurements: monthly.

Chemical parameters:
- Ammonia-N, total phosphorus, soluble reactive phosphorus, Kjeldahl-N, $NO_3$-N, suspended solids, and volatile solids: weekly.
- Diel measurements: monthly.

Biological parameters:
- Zooplankton and chlorophyll *a*: 2 ponds/treatment/wk.

Data loggers:
- Monitor all ponds at 30-minute intervals using column sampler.
- Monitor pH, temperature, and dissolved oxygen.

Null Hypothesis: Comparable yields will be obtained using either chicken litter or a combination of chicken litter followed by feed.

Statistical methods: ANOVA.

Schedule: Data collection, 7/89 - 12/89, technical report, 6/90.

HONDURAS STUDY 2: TILAPIA- TAMBAQUI (*Colossoma macropomum*) POLYCULTURE STUDIES

Objectives: 1) To determine growth and yield of tambaqui in polyculture with tilapia under various combinations of chicken litter and supplemental feed; 2) To determine the effect of supplementing chicken litter with nitrogen applications as urea on primary productivity and fish yield; 3) To quantify the development of benthic respiration in ponds fertilized with chicken litter, and chicken litter plus N supplement; and 4) To measure the effects of pond renovation on pond seepage.

Significance: Polyculture with tambaqui could increase fish yield and enterprise income. Also nitrogen supplementation of chicken litter may significantly increase fish yield.

Experimental design: Four treatments. Three replicates.
- Chicken litter at 750 kg/ha/wk.
- Chicken litter at 750 kg/ha/wk plus nitrogen at 10 kg/ha/wk.
- Chicken litter at 1000 kg/ha/wk for 2 months followed by supplemental feed (20-25% protein at 3% body weight per day.
- Chicken litter at 500 kg/ha/wk plus supplemental feed (20-25% protein) at 1.5% body weight per day.

Pond facilities: El Carao, twelve newly renovated ponds, 0.1-ha, 1.0 m deep.

Culture period: 135 days.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 10,000 fish/ha, 500 guapote tigre (*Cichlasoma managuense*) /ha, and 5,000 tambaqui (*Colossoma macropomum*) /ha.

Nutrient inputs: Chicken litter at 500, 750, or 1000 kg/ha/wk; nitrogen at 10 kg/ha/wk.

Water management: Replace evaporation and seepage after 5 cm loss.

Sampling schedules: Standard protocols except as noted; measurements in ponds from chicken litter only and chicken litter plus nitrogen treatments only.
Physical parameters:
- Diel measurements: every 3 weeks at 4 h intervals in 2 ponds from each treatment.

Chemical parameters:
- Ammonia-N, total solids and total volatile solids: 2x/wk.
- Kjeldahl-N, total phosphorus and soluble reactive phosphorus: 1x/wk.
- Diel measurements: every 3 weeks at 4 h intervals.

Biological parameters:
- Chlorophyll *a*, Secchi disk visibility: 2x/wk.
- Zooplankton: 1x/wk.
- In-situ benthic respiration and dark-bottle respiration: every 3 weeks in 2 ponds from each treatment.
- Diel measurements: every 3 weeks at 4 h intervals in 2 ponds from each treatment.

Null Hypotheses: Tambaqui yield will be similar for all management strategies. Nitrogen supplementation of chicken litter will not increase primary productivity or fish yield, or affect benthic respiration. Pond seepage will be unaffected by pond renovation.

Statistical methods: ANOVA, multiple regression.

Schedule: Data collection, 2/90 - 6/90, technical report, 12/90.

## HONDURAS STUDY 3: TEMPERATURE EFFECTS ON TILAPIA FRY PRODUCTION

Objective: To determine the effects of temperature on the production of tilapia fry that are less than 15 mm in total length.

Significance: Fry production during winter months is reduced. This study will provide information for developing management practices that can provide a year-round supply of 15-mm fry for sex-reversal.

Experimental design: Two sets of ponds stocked simultaneously, harvested either 17 or 20 days later. Restocking 10 days after each harvest.

Pond facilities: El Carao, four ponds, 500 $m^2$, 0.75 m deep.

Culture period: 18 months.

Fish stocking rate: Tilapia (*O. niloticus*)breeders at 0.75 fish/$m^2$; 2 females/1 male.

Nutrient inputs: Chicken litter at 1000 kg/ha upon filling the pond. Commercial pelleted feed for broodstock at 3% body weight/d, 5 d/wk.

Water management: Drain at either 17 or 20 days, refill within 1 to 3 days.

Sampling schedules:
Physical parameters:
- Air and water temperatures (maximum and minimum): 6 d/wk.

Biological parameters:
- Harvest ponds at either 17 or 20 days after stocking.
- Note number and size distribution of fry collected at harvest.

Null Hypothesis: The time period required for fry production is the same year-round.

Statistical methods: Paired *t* test, regression analysis.

Schedule: Data collection 1/89 - 4/90, technical report, 10/90.

## HONDURAS STUDY 4: FACTORS AFFECTING TILAPIA SEX-REVERSAL

Objectives: To evaluate the effects of treatment duration, fry density, and temperature on the efficacy of sex-reversal.

Significance: Availability of sex-reversed tilapia on a year-round basis could lead to substantially increased fish yields.

Experimental design: 3 x 2 factorial: stocking density x length of treatment period.

Pond facilities: El Carao, six 1 $m^2$ hapa nets for treatment and twelve 2 $m^2$ hapa nets for grow-out to 10-15 g in a 2000 $m^2$ pond.

Culture period: One year, with 21-day and 28-day treatment cycles. New experimental cycles initiated every six weeks.

Fish stocking rate: 2000, 4000 and 6000 *O. niloticus* fry/$m^2$ during treatment; 150 fish/$m^2$ during grow-out.

Nutrient inputs: Treated feed (60 mg testosterone/kg feed) at 20% body weight/d decreasing to 10% body weight/d during treatment. Commercial feed at 5 to 10% body weight/d during grow-out.

Water management: Replace evaporation and seepage losses after 5 cm loss.

Sampling schedules:
Physical parameters:
- Air and water temperatures (maximum and minimum): 6 d/wk.

Biological parameters:
- Size and count tilapia before and after treatment.
- Size, count, determine the sex ratio of tilapia after treatment.
- Chlorophyll *a* in treatment pond: weekly.

Null Hypotheses:
1. Temperature has no effect on success of sex-reversal.
2. Treatment duration has no effect on the success of sex-reversal.

Statistical methods: Chi-square, ANOVA, regression.

Schedule: Data collection 3/89 - 4/90, technical report, 10/90.

## HONDURAS STUDY 5: MAINTENANCE OF MINIMUM DISSOLVED OXYGEN CONCENTRATIONS

Objectives: To quantify the effects of aeration on tilapia growth, primary productivity, and water quality in heavily manured systems.

Significance: In heavily manured systems, the positive effects of adding additional nutrients can be offset by the increased oxygen demand of the manure. Aeration should minimize negative effects and increase yields.

Experimental design: Three treatments. Three replicates.
- No aeration.
- Minimum allowable dissolved oxygen = 10% saturation.
- Minimum allowable dissolved oxygen = 50% saturation.

Pond facilities: El Carao, nine ponds, 1000 $m^2$, 0.75 m deep. Aerators controlled by data logger will be installed in six ponds. Aerators will be turned on whenever bottom dissolved oxygen concentrations drop below the allowable minimum.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$ and guapote tigre (*Cichlasoma managuense*) at 0.05 fish/$m^2$.

Nutrient inputs: Best chicken litter/feed combination from Honduras Study 1.

Water management: Replace evaporation and seepage losses after 5 cm loss.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Diel measurements: 4 times per wk.

Chemical parameters:
- Ammonia-N, total phosphorus, soluble reactive phosphorus, Kjeldahl-N, $NO_3$-N, suspended solids and volatile solids: 2 ponds/treatment/wk.
- Diel measurements: 4 times per wk.

Biological parameters:
- Zooplankton and chlorophyll *a*: 2 ponds/treatment, twice weekly.

Data loggers:
- Monitor all ponds at 1-hr intervals using column sampler.
- Monitor pH, temperature, and dissolved oxygen.

Null Hypotheses:
1. Aeration will not increase fish growth, survival, or primary productivity.
2. Minimum dissolved oxygen levels have no correlation with fish yields.

Statistical methods: ANOVA, regression.

Schedule: Data collection, 7/90 - 12/90; technical report, 6/91.

## HONDURAS STUDY 6: SUPPLEMENTAL FEEDING—EFFECTS OF FISH DENSITY

Objective: To determine the effects of fish density on yield and water quality in ponds receiving the best nutrient input regime identified during earlier experiments.

Significance: More efficient use of nutrient inputs and primary productivity may be possible with higher stocking rates. However, higher stocking densities often yield smaller fish. Manipulation of stocking densities may allow fish farmers to direct their production to markets with different size preferences.

Experimental design: Four stocking densities. Three replicates.

Pond facilities: El Carao, twelve 1000 $m^2$ ponds, 0.75 m deep

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 0.5 fish/$m^2$, 1 fish/$m^2$, 2 fish/$m^2$, and 3 fish/$m^2$ with guapote tigre (*Cichlasoma managuense*) at 0.05 fish/$m^2$

Nutrient inputs: Best input regime identified from earlier studies.

Water management: Replace evaporation and seepage losses after 5 cm loss.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Diel measurements: twice weekly.

Chemical parameters:
- Ammonia-N, total phosphorus, soluble reactive phosphorus, Kjeldahl-N, $NO_3$-N, suspended solids, and volatile solids: 2 ponds/treatment/wk.
- Diel measurements: twice weekly.

Biological parameters:
- Zooplankton and chlorophyll *a*: 2 ponds/treatment/wk.

Data loggers:
- Monitor all ponds at 1-hr intervals using column sampler.
- Monitor pH, temperature, and dissolved oxygen.

Null Hypotheses:
1. Density will have no affect on fish yield.
2. Density will have no effect on fish size.
3. Density will have no effect on primary productivity.

Statistical methods: ANOVA.

Schedule: Data collection, 1/91 - 6/91, technical report, 12/91.

## ASIA—THAILAND PROJECT

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Pond System: Freshwater tropical ponds typical of lower elevations.

Experiments conducted under the first four CRSP work plans have addressed the relationships of fertilizer input to yield of fish--primarily tilapia--and shrimp under standardized conditions. These relationships have been tentatively quantified. In addition, some work on nutrient balancing, pond depth, and stocking density has been conducted. During the Fifth Work Plan, work on the management of fertilized ponds used for tilapia monoculture will be completed; work will start on polyculture and supplemental feeding, and the conclusions from the standardized experiments will be subjected to field verification at a variety of sites in Thailand.

The continuing research on fish farm management and its effects on the dynamics of tilapia ponds will examine the effects of pond size, stocking density, nutrient balancing, polyculture, supplemental feeding, circulation, and aeration. Additionally, a bioassay system will be developed for the practical determination of limiting nutrients in fish ponds. Eleven studies are planned with two or more studies being conducted simultaneously (Figure 3).

The two goals of the Thailand Project during the Fifth Work Plan are:

1. To improve our understanding of the effects of management practices on the dynamics of fertilized tilapia ponds in tropical climates, and
2. To develop fertilization guidelines for tilapia ponds in Thailand.

**Figure 3. Schedule for Data Collection in Thailand during the Fifth Work Plan.**

| Study Title | Site | 1989 | | | | 1990 | | | | | | | | | | | | 1991 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug |
| Study 1. Effects of Density and Fertilization on Tilapia Reproduction | Bangsai | *** | | | | | | | | | | | | | | | | | | | | | | | |
| Study 2. Nutrient Cycling - Organic Carbon | Bangsai | | ********** | ********** | ********** | | | | | | | | | | | | | | | | | | | | |
| Study 3. Tilapia/Clarias Polyculture | Bangsai | | | | | | | | | ********** | ********** | ********** | | | | | | | | | | | | | |
| Study 4. Nutrient Cycling - Nitrogen and Phosphorus | Bangsai | | | | | | | | | | | | | ********** | ********** | ********** | ********** | | | | | | | | |
| Study 5. Effects of Circulation in Deep Ponds - Wet Season | AIT | ********** | ********** | ********** | | | | | | | | | | | | | | | | | | | | | |
| Study 6. Effects of Circulation in Deep Ponds - Dry Season | AIT | | | | | ********** | ********** | ********** | ********** | ********** | | | | | | | | | | | | | | | |
| Study 7. Nutrient Cycling - Use of Bioassays | AIT | | | | | ********** | ********** | ********** | ********** | ********** | | | | | | | | | | | | | | | |
| Study 8. Supplemental Feeding in Fertilized Ponds | AIT | | | | | | | | | | ********** | ********** | ********** | ********** | | | | | | | | | | | |
| Study 9. Effects of Pond Size | AIT | | | | | | | | | | | | | | | | ********** | ********** | ********** | ********** | ********** | ********** | | | |
| Study 10. Maintenance of Minimum Dissolved Oxygen Concentrations | AIT | | | | | | | | | | | | | | | | ********** | ********** | ********** | ********** | ********** | ********** | | | |
| Study 11. On-Farm Studies - Verification of CRSP Results | Various | | | | | | | | | | | | | ********** | ********** | ********** | ********** | ********** | ********** | ********** | ********** | ********** | ********** | ********** | ********** |

## THAILAND STUDY 1: EFFECTS OF DENSITY AND FERTILIZATION ON TILAPIA REPRODUCTION

Objective: To increase our understanding of how stocking density and resource availability affect the age of first maturation and extent of reproduction of tilapia.

Significance: By better understanding the factors that affect tilapia reproduction, management of environmental factors may be used to delay maturation and minimize unwanted reproduction.

Experimental design: 2 x 2 factorial with two stocking densities and two fertilization rates.

Pond facilities: Bangsai, Sixteen 220 $m^2$ ponds, 1 m deep (at stadia).

Culture period: Twenty weeks.

Fish stocking rate: *O. niloticus* fingerlings (both sexes). Eight ponds at 1 fish/$m^2$ and eight ponds at 3.14 fish/$m^2$.

Nutrient inputs: Eight ponds at 50 kg chicken manure/ha/wk and eight at 250 kg chicken manure/ha/wk. All ponds supplemented with urea to bring total nitrogen:phosphorus ratio to 5:1.

Water management: Replacement of evaporation and seepage losses weekly.

Sampling schedule: Standard protocol except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.
- Diel measurements: every 6 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.
- Diel measurements: every 6 wks.
- Proximate analysis of chicken manure: weekly.

Biological parameters:
- Fish sampling for growth: every 3 wks. Remove 5% of population at each sampling for gonad samples. Evaluate state of maturity.
- Zooplankton sampling: every 3 wks.
- Intensive measurements: every 2 wks.
- Light-dark bottle productivity: every 2 wks.

Null Hypotheses: Stocking density and fertilization rate do not influence the timing of maturation, first reproduction, or final fish size.

Statistical methods: Two-way ANOVA with pairwise comparisons between treatments, correlation and regression to describe within-treatment variation.

Schedule: Data collection, 4 - 9/89; technical report, 3/90

## THAILAND STUDY 2: NUTRIENT BALANCE—ORGANIC CARBON

Objective: To quantify the relationships between growth, yield, and the rates associated with processes linking manure and the carbon cycle.

Significance: This study will lead to a greater understanding of the requirement for organic carbon in fertilized ponds. This understanding could lead to improved fertilization guidelines which more efficiently utilize manure and minimize low oxygen concentrations associated with high manure loads.

Experimental design: Five manure levels. Three replicates.

Pond facilities: Bangsai, fifteen 220 $m^2$ ponds, 1 m deep (at stadia).

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: Chicken manure will be added at five levels: 25 kg/ha/wk; 50 kg/ha/wk; 100 kg/ha/wk; 200 kg/ha/wk; and 400 kg/ha/wk. The manure will be supplemented with urea and TSP to bring the loading rates of total nitrogen and total phosphorus to a 5:1 ratio.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Null Hypotheses:
1. Fish yield will be the same regardless of the loading rates used.
2. Average dissolved oxygen concentrations will not be affected by manure loading rates.

Statistical methods: ANOVA, regression.

Schedule: Data collection, 10/89 - 3/90; technical report, 8/90.

## THAILAND STUDY 3: TILAPIA AND *CLARIAS* POLYCULTURE

Objective: To collect baseline information on simple polyculture systems and refine hypotheses regarding pond dynamics in these systems.

Significance: Development of polyculture systems appropriate to local conditions could lead to substantially increased yields.

Experimental design: Randomized block, three treatments. Four replicates.
- *Clarias* in cages in ponds, tilapia at large in the pond, supplemental feed given to the *Clarias* only.
- *Clarias* and tilapia at large in ponds with supplemental feed for the *Clarias* only.
- *Clarias* and tilapia at large in fertilized ponds without any supplemental feed.

Pond facilities: Bangsai, twelve 220 $m^2$ ponds, 1 m deep (at stadia). Four 2m x 2m x 1m cages will be placed in the each pond in the treatment using cages.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$. *Clarias macrocephalus* fingerlings will be stocked in cages at 25 fish/$m^3$ and 50 fish/$m^3$. In each pond with cages, two cages will be stocked at the high density and two cages at the low density.

Nutrient inputs: In the fertilized ponds, 250 kg/ha/wk of chicken manure supplemented with urea and TSP to bring total nitrogen input to 35 kg/ha/wk and total phosphorus input to 7 kg/ha/wk. A commercial *Clarias* feed will be used as the supplemental feed. The feeding rate will start at 10% body weight per day and decrease to 3% body weight per day by the end of the study.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Null Hypotheses:
1. Culture of tilapia and *Clarias* together will not increase total yield.
2. Stocking density in cages will not affect the growth of *Clarias*.

Statistical methods: ANOVA.

Schedule: Data collection, 4/90 - 9/90; technical report, 2/91.

## THAILAND STUDY 4: NUTRIENT BALANCE—NITROGEN & PHOSPHORUS

Objective: To quantify the relationships between growth, yield, and the rates associated with processes linking manure and the cycling of nitrogen and phosphorus.

Significance: This study will lead to greater understanding of the requirement for nitrogen in fertilized ponds. This understanding could lead to improved fertilization guidelines which more efficiently utilize manure and enhance fish production.

Experimental design: Four total nitrogen levels. Three replicates.

Pond facilities: Bangsai, twelve 220 $m^2$ ponds, 1 m deep (at stadia).

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: Chicken manure will be added at the optimum level identified in Study 2. The manure will be supplemented with urea to bring the total nitrogen loading rates to 35 kg/ha/wk, 70 kg/ha/wk, 105 kg/ha/wk and 140 kg/ha/wk. TSP will be added to maintain the ratio of total nitrogen to total phosphorus at 5:1.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Null Hypotheses:
1. Fish yields are not affected by nitrogen loading rates.
2. Ammonia-N levels are not affected by light limitation.
3. Ammonia-N levels are not affected by nitrogen loading rates.

Statistical methods: ANOVA, regression.

Schedule: Data collection, 10/90 - 3/91; technical report, 8/91.

## THAILAND STUDY 5: EFFECTS OF CIRCULATION IN DEEP PONDS—WET SEASON

Objectives: To determine if the increased fish yield resulting from proportional increases in pond depth, fish numbers, and nutrient inputs observed in a dry season experiment will occur during the rainy season. To determine the effects of circulation within deep ponds on fish yield, water quality, and system efficiency during the wet season.

Significance: The management techniques under study have the potential for substantially increasing yields per unit area.

Experimental design: Five treatments (0.9 m deep without fish; 0.9 m with fish; 1.6 m without fish; 1.6 m with fish; and 1.6 m with fish and circulation). Three replicates. Circulators will be operated continuously.

Pond facilities: AIT, CRSP ponds 1-9 (1.6 m deep at stadia, 474 $m^3$) and CRSP ponds 11–16 (0.9 m deep, 298 $m^3$). All ponds are 382 $m^2$.

Culture period: Four months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2.56 fish/$m^3$ (equivalent to 2 fish/$m^2$ in the 0.9 m deep ponds). The 1.6 m deep ponds will receive 1216 fish/pond and the 0.9 m ponds will receive 764 fish/pond.

Nutrient inputs: All ponds will be fertilized with chicken manure at 32 g/$m^3$/wk supplemented with urea and triple super phosphate to bring total nitrogen input to 4.5 g/$m^3$/wk and total phosphorus input to 0.90 g/$m^3$/wk. These rates are equivalent to 250 kg/ha/wk of manure, 35 kg/ha/wk of N and 7 kg/ha/wk of P in the 0.9 m ponds.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Data loggers:
- Monitor pH, temperature, and dissolved oxygen.
- Two depths, 8 ponds/wk. At least 2 ponds/wk for deep treatments.
- Sampling periods will last 48 h with samples at 30-min intervals.

Null Hypotheses:
1. Circulation will not affect fish yields or water quality.
2. Proportional increases in pond depth, fish number, and nutrients will not affect yield.

Statistical methods: ANOVA, linear regression.

Schedule: Data collection, 8/89 - 11/89; technical report, 6/90.

## THAILAND STUDY 6: EFFECTS OF CIRCULATION IN DEEP PONDS—DRY SEASON

Objective: To determine the effects of circulation in deep ponds on fish yield, water quality, and system efficiency during the dry season.

Significance: Circulation has the potential for increasing fish yield.

Experimental design: Three treatments (0.9 m deep, 1.6 m deep, and 1.6 m deep with circulation). Three replicates.

Pond facilities: AIT, CRSP Ponds 1 - 6 (1.6 m deep at stadia, 474 $m^3$) and ponds 11-12 (0.9 m deep, 298 $m^3$). All ponds are 382 $m^2$. Circulators will be operated continuously.

Culture period: Five months.

Stocking rate: Sex reversed *O. niloticus* fingerlings at 2.56 fish/$m^2$ (equivalent to 2 fish/$m^2$ in 0.9 m deep ponds). The 1.6 m deep ponds will receive 1216 fish/pond and the 0.9 m ponds will receive 764 fish/pond.

Nutrient inputs: All ponds will be fertilized with chicken manure at 32 g/$m^3$/wk supplemented with urea and triple superphosphate to bring total nitrogen input to 4.5 g/$m^3$/wk and total phosphorus input to 0.90 g/$m^3$/wk. These rates are equivalent to 250 kg/ha/wk of manure, 35 kg/ha/wk of nitrogen and 7 kg/ha/wk of phosphorus in the 0.9 m ponds.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Data loggers:
- Monitor pH, temperature, and dissolved oxygen.
- Two depths, 8 ponds/wk.
- Sampling periods will last 48 h with samples at 30-min intervals.

Null Hypothesis: Circulation will not affect fish yields or water quality.

Statistical methods: ANOVA.

Schedule: Data collection, 1/90 - 6/90; technical report 9/90.

## THAILAND STUDY 7: NUTRIENT CYCLING—USE OF BIOASSAYS

Objective: To evaluate nutrient bioassays as indicators of limiting nutrients in fish ponds.

Significance: Development of a simple and accurate bioassay technique for fertilization that could be used by farmers to improve utilization efficiencies of nutrient inputs resulting in higher fish yields.

Experimental design: Five incremental levels of nitrogen and five levels of phosphorus will be added to water samples from 10 ponds fertilized at five different N:P ratios.

Pond facilities: AIT, Ponds 11-20 (1.0 m deep at stadia, 394 $m^2$/pond).

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: All ponds to be fertilized with chicken manure at 100 kg/ha/wk with sufficient urea added for a total nitrogen input of 0.6 g/$m^2$/day. Total phosphorus input will be regulated with TSP so duplicate ponds will be fertilized at N:P ratios of 3:1, 5:1, 7:1, 9:1 and 11:1.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.
- Daily bioassay for one week per month by adding incremental amounts of nitrogen and phosphorus to water samples from each pond. After 3 days of incubation at ambient temperature and light, compare algal growth visually and with a turbidity meter. Turbidity will be correlated with chlorophyll *a* concentrations to provide estimates of algal productivity.

Null Hypotheses:
1. Daily bioassay results will not correlate with temporal changes in nutrient limitation.
2. Fish yields and primary productivities will not be affected by different N:P input ratios.

Statistical methods: ANCOVA and correlation analysis.

Schedule: Data collection, 1/90 - 6/90, technical report, 1/91.

## THAILAND STUDY 8: SUPPLEMENTAL FEEDING IN FERTILIZED PONDS

Objectives:
1. To assess the contribution of natural and supplemental foods to growth.
2. To evaluate the economic feasibility of supplemental feeding.

Significance: Supplemental feeding may lead to larger harvest size and greater yields and marketability.

Experimental design: Three treatments. Three replicates.
- Chicken manure with nitrogen and phosphorus supplements.
- Feed only.
- Chicken manure, nitrogen, and phosphorus supplements, and feed.

Pond facilities: AIT, CRSP ponds 11-19 (1 m deep at stadia, 394 $m^2$/pond).

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: Fertilization rate of 250 kg/ha/wk of chicken manure, supplemented with urea and TSP to bring total nitrogen input to 35 kg/ha/wk and total phosphorus input to 7 kg/ha/wk. Satiation feeding with commercial feed.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Null Hypothesis: Supplemental feed will not affect fish growth or yield.

Statistical methods: ANOVA.

Schedule: Data collection, 7/90 - 12/90, technical report, 6/91.

THAILAND STUDY 9: EFFECTS OF POND SIZE

Objective: To determine the relationships of pond size to fish yield, management practices, and system efficiency.

Significance: [illegible]

the dynamics of larger ponds. Also, economies of scale can be evaluated.

Experimental design: [illegible]

Pond facilities: AIT; three 200 $m^2$ ponds; three 394 $m^2$ ponds selected from CRSP ponds 11-20; three 800 $m^2$ ponds and three 1600 $m^2$ ponds. All ponds 1 m deep (at stadia). The three 394 $m^2$ ponds also will be used as the unaerated treatment in Study 10.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: 250 kg/ha/wk of chicken manure, supplemented with urea and TSP to bring total nitrogen input to 35 kg/ha/wk and total phosphorus input to 7 kg/ha/wk.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus: every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Null Hypothesis: Pond size does not affect yield or efficiency per unit area.

Statistical methods: ANOVA.

Schedule: Data collection, 1/91 - 6/91; technical report 8/91.

## THAILAND STUDY 10: MAINTENANCE OF MINIMUM DISSOLVED OXYGEN CONCENTRATIONS

Objectives: To quantify the effects of aeration on tilapia growth, primary productivity, and water quality in fertilized systems.

Significance: In heavily fertilized systems, the positive effects of adding additional nutrients can be offset by the increased oxygen demand of the plankton. Aeration should minimize these adverse effects and increase yields.

Experimental design: Three treatments. Three replicates.
- No aeration.
- Minimum allowable dissolved oxygen = 10% saturation.
- Minimum allowable dissolved oxygen = 50% saturation.

Pond facilities: AIT, CRSP ponds 11 - 18 (1.0 m deep at stadia, 394 $m^2$ each). The three ponds in the unaerated treatment will be used as the 394 $m^2$ treatment in Study 9. Aerators controlled by data logger will be installed in six ponds. Aerators will be turned on whenever bottom dissolved oxygen concentrations drop below the specified minimum.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: 250 kg/ha/wk of chicken manure, supplemented with urea and TSP to bring total nitrogen input to 35 kg/ha/wk and total phosphorus input to 7 kg/ha/wk.

Water management: Replace evaporation and seepage losses weekly.

Sampling schedule: Standard protocols except as noted.
Physical parameters:
- Intensive measurements: every 2 wks.

Chemical parameters:
- Intensive measurements: every 2 wks.
- $NO_2$-N, $NO_3$-N, and soluble reactive phosphorus every 2 wks.

Biological parameters:
- Intensive measurements: every 2 wks.

Data loggers:
- Monitor all aerated ponds and two unaerated ponds at 30-min intervals at 2 depths.
- Monitor pH, temperature, and dissolved oxygen.

Null Hypotheses:
1. Aeration will not increase fish growth, survival, or primary productivity.
2. Minimum dissolved oxygen levels have no correlation with fish yields.

Statistical methods: ANOVA, regression.

Schedule: Data collection, 1/91 - 6/91; technical report, 8/91.

## THAILAND STUDY 11: ON-FARM STUDIES— VERIFICATION OF CRSP RESULTS

Objective: To determine the applicability of results obtained under controlled conditions to field conditions.

Significance: Before results from CRSP studies can be disseminated to the public, these results must be tested under field conditions to ensure that farmers will have similar results.

Experimental design: Twenty farms will use a specified stocking and fertilization scheme based on previous studies which were conducted at research stations. The on-farm results will be compared against results from the earlier CRSP studies.

Pond facilities: Twenty private farms selected with the assistance of the Royal Thai Department of Fisheries.

Culture period: Five months.

Fish stocking rate: Sex-reversed *O. niloticus* fingerlings at 2 fish/$m^2$.

Nutrient inputs: Recommended rate from earlier CRSP studies.

Water management: Standard farm practice.

Sampling schedule:
Physical parameters:
- Pond morphology: at start of study.
- Water temperature and Secchi disk: every two wks.

Chemical parameters:
- Dissolved oxygen, pH, alkalinity, and ammonia-N: every 2 wks.
- Proximate analysis of inputs: 3 times during the study.

Biological parameters:
- Fish measurements: beginning and end of experiment.

Operational parameters:
- Source of nutrients and rate of nutrient addition: every two wks.

Null Hypothesis: The same inputs will produce the same field yield in both farmers' ponds and at research stations.

Statistical methods: ANOVA and multiple regression.

Schedule: Data collection, 9/90 - 6/91; technical report, 8/91.

Note: This study is contingent upon the availability of additional funds.

# DATA ANALYSIS AND SYNTHESIS TEAM

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The Pond Dynamics/Aquaculture CRSP is different from most other aquaculture research efforts because the field experiments are conducted at several geographic locations using standardized sampling protocols. This has made possible the collection and compilation of a comprehensive set of data from similar experiments at a variety of sites into a single computerized database. The database is one of the important products of the CRSP as it is a unique source of information for the study of aquaculture ponds.

Analysis of the data collected in the CRSP is carried out at two levels. The first level of analysis is carried out by the principal investigators and field researchers responsible for each of the field experiments. The second level of analysis is carried out by the Data Analysis and Synthesis Team (DAST). At the second level, data from the various sites are analyzed for ecological relationships and global trends by statistical analysis. Computer models are developed and calibrated using CRSP data. The results from the statistical analyses and models may be used for the development of management practices, to identify possible areas for future field research, and to propose production guidelines that incorporate CRSP findings.

Four studies are proposed by the DAST for The Fifth Work Plan (Figure 4). The goals of these studies are:

1. To determine the numerical relationships between variables and the critical rate processes required for predicting responses of pond production systems;

2. To develop computer models that can be used to examine paradigms of pond dynamics, to propose management actions for field testing, and to identify topics deserving further research; and

3. To develop methods and tools for disseminating pond management guidelines based on CRSP research findings.

**Figure 4. Schedule of Activities and Reports of the Data Analysis and Synthesis Team during the Fifth Work Plan.**

| Study Title | 1989 | | | | 1990 | | | | | | | | | | | 1991 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug |
| Study 1. Data Analysis | | | | | | | | | | | | | | | | | | | | | | | | |
| - Influence of Climate on Growth and Yield | * | * | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | |
| - Fish Growth and Density | * | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | | |
| - Anabolism and Catabolism Coefficients | * | * | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | |
| - Fish Growth, Environment and Nutrients | * | * | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | |
| Study 2. Ecosystem Model | | | | | | | | | | | | | | | | | | | | | | | | |
| - Initial report | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | | | |
| - Subsequent reports | | | | | | | | | | | | | * | * | * | | | | * | * | * | | | * |
| Study 3. Dissolved Oxygen Model | | | | | | | | | | | | | | | | | | | | | | | | |
| - Phytoplankton Condition | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | | | |
| - Dissolved Oxygen Management, initial report | | | | | | | | * | * | * | * | * | * | | | | | | | | | | | |
| - Subsequent reports | | | | | | | | | | | | | | | | * | * | * | | | | * | * | * |
| Study 4. Fertilization Guidelines | | | | | | | | | | | | | | | | | | | | | | | | |
| - Functional Classification of Ponds | * | * | * | * | * | * | * | * | * | * | * | | | | | | | | | | | | | |
| - Quantitative Relationships of Fish Yield | | | | | * | * | * | * | * | * | * | * | * | | | | | | | | | | | |
| - The Expert System | | | | | | | | * | * | * | * | * | * | | | | | | | | | | | |
| - Fertilization Manual, first edition | | | | | | | | * | * | * | * | * | * | * | * | * | * | * | | | | | | |
| - Verification of Manual Guidelines | | | | | | | | | | | | | * | * | * | * | * | * | * | * | * | * | * | * |

## DAST STUDY 1: DATA ANALYSIS

During the Fifth Work Plan, statistical analyses and numerical modeling will continue to be used to test ecological and growth relationships and determine rate processes important in understanding pond limnology, fish growth, and production. In addition, the value of coefficients for use in a production model similar in structure to the Ursin model will be estimated. This model integrates variables important to fish growth and evaluates the extent of effects of variables such as size and density on growth.

Objectives:

1. To understand the influence of global elements (e.g., climate) on growth and yield in warmwater fish ponds;
2. To estimate the numerical relationships between fish growth, environmental conditions and nutrient inputs;
3. To quantify the relationship between fish growth and stocking density and other factors that lead to reduced fish growth; and
4. To determine the anabolism and catabolism coefficients of the Ursin model.

Methods: A range of statistical techniques will be used to attain the specified objectives. These techniques include analysis of variance, regression, multiple regression, correlation analyses, logistic analyses, and principle component analysis. Mainframe statistical packages such as SAS and MIDAS will be used in addition to statistical packages on microcomputers. The primary source of data for these analyses will be data collected during the first three work plans although data from the Fourth Work Plan will be included as they become available.

Schedule for Completion of Analyses:

- Influence of global factors on fish yield - 9/90
- Relationships of fish growth, environment, & nutrients - 8/90
- Relationship of fish growth and density - 9/90
- Anabolism & catabolism coefficients - 9/90

## DAST STUDY 2: ECOSYSTEM MODEL

An ecosystem model has been developed and tested against data collected during the first three work plans. The model is used to simulate state variables (dissolved oxygen, ammonia, nitrate, nitrite, inorganic phosphorus, phytoplankton, fish, etc.) over a diel cycle. Data available for testing the model have been limited due to the frequency of observations and schedules used in the first three work plans. Changes implemented during the Fourth Work Plan have resulted in more complete data sets which can be used for model verification and identification of areas requiring additional research.

Objectives:

1. To identify general topics requiring further field research;
2. To develop specific hypotheses for possible field testing; and
3. To develop pond management guidelines based on model results.

Methods: Results from the existing model will be compared to data collected during the Fourth Work Plan. Discrepancies between the model simulations and the data will be used to identify general areas of the model where the proposed functional relationships (equations) do not produce results that match field measurements. These discrepancies will be used as an indicator of areas requiring additional research. The model will also be used to test pond response to hypothetical situations and investigate possible management actions. Results of these simulations will be translated into hypotheses for possible field testing. Lastly, the model will [illegible]

develop general guidelines for pond management.

Schedule: Model development [illegible]

takes place over time. The first report of this study will be completed in 3/90 with subsequent reports due every six months thereafter.

## DAST STUDY 3: DISSOLVED OXYGEN MODEL

Results obtained from the models of diel dissolved oxygen have indicated the need for an indicator of phytoplankton condition. In current CRSP dissolved oxygen models, the response of phytoplankton to nutrient concentrations, light intensity and temperature are based on production functions developed from limnological studies. The CRSP models have served to identify errors in some of the assumptions behind these production functions. An indicator of phytoplankton "condition" will be developed to improve the accuracy of simulation of primary production in aquaculture ponds.

Objectives:

1. To develop techniques for field evaluation of phytoplankton "condition"; and
2. To develop techniques for oxygen management.

Methods: Using dissolved oxygen models, phytoplankton growth parameters will be estimated such that dissolved oxygen production rates match measured oxygen concentrations. Night measurements of dissolved oxygen will be used to obtain estimates of whole pond respiration. Using measurements of light intensity, chlorophyll *a*, and estimated phytoplankton growth rates, an efficiency value will be calculated that describes the relative efficiency of light utilization by algae. Changes in this efficiency value will serve as indicators of shifts in algal condition.

Work will continue on studying the possible changes in dissolved oxygen concentration as a result of management actions such as water level control and fertilization. These simulations will be used to propose oxygen management techniques.

Schedule: First phytoplankton condition report - 3/90. First report on oxygen management - 6/90. Subsequent oxygen management reports at six-month intervals.

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Fish/Shrimp Length, Individual | From a representative 10% subsample of the grab sample, determine total length of each individual and express as mean length per individual. | | | cm |
| Fish/Shrimp Production | Fish and shrimp stocks will be weighed as a group and counted at stocking and harvest. Tilapia will be sexed individually. Compute gross and net production. | | | kg and # |
| Fish/Shrimp Weight, Group | At 30-day intervals throughout each experimental cycle, collect grab sample equivalents to 10% of initial stock from each pond and weigh as a group. Indicate number of individuals in sample. Note observations on reproduction and fish health. | | | kg/#, individual |
| Fish/Shrimp Weight, Individual | From a representative 10% subsample of the grab sample, determine weight of each individual and express as weight per individual. | | | g |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Chlorophyll a (corrected and uncorrected) | Collect one sample per pond by pooling three 90 cm column samples. Take samples with diel studies. | | Acetone extraction and Spectrophotometric Determination, from "Standard Methods" (APHA et al., 1985) | mg/m3 |
| Dark Bottle Respiration - see Respiration, Dark Bottle | | | | |
| Depth, Pond | Install staff gauge in each pond and read to nearest 0.5 cm at same time each day, before restoring to specified depth. | No type specified. | | m |
| Dissolved Oxygen - see Oxygen, Dissolved | | | | |
| Evaporation and Inflow | Surface Inflow/Outflow and Evaporation should be determined using procedures described in Appendix F of CRSP Work Plan III, 1985, (Wood, J.W. 1974. Diseases of Pacific Salmon: Their Prevention and Treatment. pp 71-77) or comparable approaches. | | | mm/d (evaporation); m3/d (infiltration) |
| Feed Composition | See Analytical Methods Report | | | various |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Air Temperature - see Temperature, Air | | | | |
| Alkalinity | Near center of each pond, take readings at 25 cm below the water surface, midwater and 25 cm above the bottom. Keep samples cool in refrigeration unit or ice chest, and analyze within 24 hours. | Hach Digital Titrator Test Kit/Alkalinity (optional). | Low or Standard Alkalinity method (as appropriate) (from "Standard Methods," APHA et al., 1985), or Hach Test Kit | mg CaCO3/L |
| Ammonia | Collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours. | Kontes or comparable Kjeldahl nitrogen apparatus. See Nitrogen, Kjeldahl Apparatus | Nesslerization Method (Michigan State University Limnological Research Laboratory, 1984). | mg/L |
| Benthos Composition | Collect at least three cores of mud per pond. Process samples through a No. 30 sieve, sort organisms and fix in 10% formalin or a 70% ethanol solution. Identitfy at the order level or lower. Count number of organisms per unit volume or area. | | | various |
| Chemical Oxygen Demand - see Oxygen, Chemical Demand. | | | | |

## DAST STUDY 4: FERTILIZATION GUIDELINES

One of the goals of the CRSP is to translate research findings into guidelines for managing aquaculture ponds under different environmental and production regimes. Consistent with this goal, the CRSP intends to prepare a manual of pond fertilization for optimizing yields and increasing the reliability and efficiency of pond systems. This manual will be prepared in two forms: a printed handbook and a computer application. The manual will address three audiences: aquacultural producers, development planners, and aquaculture scientists. The manual will contain an ecological classification of ponds, functional relationships between ecological variables and fertilization practices, and recommended fertilization practices for each classification.

Objectives:

1. To complete the development of a functional system for classifying ponds according to ecological variables and fertilization practices that have the highest probability of improving fish yields;
2. To develop quantitative relationships that describe the appropriate levels and frequencies of fertilization for each pond category;
3. To integrate the classification model and functional relationships into an expert system;
4. To compile the organized information into a manual of farm fertilization guidelines (only for internal project use at this stage); and
5. To verify the guidelines contained in the manual.

Methods: Pond environments will be ordered into a hierarchical classification according to fertilization practices corresponding to each combination of climate and chemical and physical characteristics of the source water and pond soils. Quantitative relationships from DAST Study 1 which relate fish yields and nutrient inputs will be used with deterministic models (DAST Studies 2 and 3) to calculate required nutrient additions. A database on nutrient availability of different fertilizers will be compiled from the agricultural literature. Laboratory analyses of fertilizers will be conducted when adequate information on nutrient availability is not available from the literature.

The existing shell of the expert system program for pond classification that was completed in the Fourth Work Plan, will be modified by incorporating the new fertilization guidelines. Versions of the program will be written to run on either IBM-compatible or Apple Macintosh personal computers.

Based on the completed expert system, an initial version of a manual for pond fertilization will be compiled. The first version of the manual will then be verified using field data collected during the Fourth Work Plan.

Schedule:
- Functional classification system - 1/90
- Quantitative relationships estimated - 4/90
- Expert system operational - 6/90
- First edition of manual - 8/90
- Verification complete - 8/91

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Flow, Water | See Evaporation and Inflow | | | |
| Morphometric Characteristics: Maximum Length, Maximum Width, Area, Depth, Volume | At project initiation and whenever pond facilities are altered, prepare contour maps of ponds at 10 cm intervals. Note inflow and outflow locations. | | | m, m2, m3, (as appropriate) |
| Nitrogen Total Kjeldahl | For each pond, pool three 90 cm column samples. Composite samples should be refrigerated and analyzed within 24 hours. | Kontes or comparable Kjeldahl Nitrogen apparatus | Semi-Micro-Kjeldahl Method (Michigan State University Limnological Research Laboratory, 1984) | mg/L |
| Oxygen, Chemical Demand | Please refer to the appendices from Work Plans III & IV, attached. (methods from: "Standard Methods for the Examination of Water and Wastewater," APHA et al., 1985). | | | |
| Oxygen, Dissolved | Near center of each pond at 25 cm below water surface, mid-water and 25 cm above the bottom. Take readings as part of diel study at seven different times beginning with pre-dawn. | Yellow Springs Instrument Model 57 Dissolved Oxygen Meter. Calibrate meter each time using the Winkler Method or HACH Digital Titrator Kit/Dissolved Oxygen. | Winkler or Iodometric method (from "Standard Methods," APHA et al., 1985) | mg/L |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| pH, Water | Near center of each pond, take readings at 25 cm below the water surface, mid-water, and 25 cm above the bottom. If a probe is used, calibrate using a precision thermometer. Calibrate meter with standard buffers at pH 7 and pH 10. | pH Meter with Combination Electrode comparable to Orion 2000 Series with Ross Model 81-55 Electrode. | | pH units |
| Phosphorus, Total | Collect one sample (by pooling three 90 cm samples) from each pond. Samples should be refrigerated and analyzed within 24 hours. | | Persulfate Digestion and Ascorbic Acid/Colorimetric Method, from "Standard Methods" (APHA et al., 1985) | mg/L |
| Phytoplankton Composition | Monthly and when changes in the community are observed, collect samples using a Van Dorn or Kemmerer bottle. Use a compound microscope and references to identify to appropriate taxonomic level and count or estimate bio-volume. | | | various |
| Primary Productivity | Whole pond method preferred. Light-dark bottle at three depths acceptable. | | | |
| Pond Depth - see Depth, Pond | | | | |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Pond Soil Characteristics - see Soil Characteristics | | | | |
| Pond Temperature - see Temperature, Water | | | | |
| Precipitation - see Rainfall | | | | |
| Rainfall | Install three rain gauges at study site; read and empty at 24-hour intervals, or more frequently to prevent gauge overflow; report average of three readings. | No type specified. Recommended gauge from Grassroot Co., Wisconsin. | | cm/d |
| Respiration, Dark Bottle | Collect one sample (by pooling three 90 cm column samples) from each pond. Incubate for four hours hours or as appropriate to prevent oxygen depletion, in dark bottles suspended at mid-depth in ponds. | | Oxygen method, adapted from "Standard Methods" (APHA et al., 1985). | mg C/m3/d |
| Salinity | Near center of each pond, collect a 500-ml sample at 25 cm below the water surface, mid-water, and 25 cm above the bottom. Mix the samples and analyze. | Use a temperature-compensated refractometer or a salinity meter. | | ppt |
| Secchi Disk Visibility - See Visibility, Secchi Disk | | | | |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Seepage | Determine seepage from a 24-hour water balance, preferably when there is no rainfall, inflow, or outflow: Seepage = Evap x 0.10 - (Final Depth - Initial Depth), where Evap is in mm/d and the depth measurements are in cm and and taken 24 hours apart. | | | cm/d |
| Soil, Characteristics: pH, Phosphorus, Organic Matter, Total Nitrogen, Cation Exchange Capacity, Metals (Aluminum, Iron, Zinc ) , Lime Requirement, Exchangeable H, Base Saturation | At the end of an experiment and before beginning another, collect twelve 15 cm core samples from each pond, combine and dry as described in Appendix D of Work Plan III (attached). Take a subsample for each pond and analyze using a qualified local or U.S. laboratory. | | | As appropriate |
| Solar Radiation | Install Solar Monitor and Quantum Sensor and read the cummulative radiation each day and at end of each time interval during diel study. | | | E/m2<br>E/m2/d |
| Solids, Total Suspended | See Appendices | | In: "Standard Methods" (APHA et al., 1985) | mg/L |

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Solids, Volatile Suspended | See Appendices | | In: "Standard Methods" (APHA et al., 1985) | mg/L |
| Temperature, Air | Install three maximum-minimum thermometers in the shade near ponds; read at 24-hour intervals and report average maximum and average minimum. | Maximum-minimum thermometer comparable to Taylor Model 5460. | | Max °C; Min °C |
| Temperature, Water | Near center of each pond, take readings at 25 cm below the water surface, midwater, and 25 cm above the bottom. Take readings as part of diel study at 7 different times. If probe is used, calibrate using a precision thermometer. | YSI Model 57 Dissolved Oxygen Meter with Temperature Indicator. | | °C |

Total Kjeldahl Nitrogen - see Nitrogen, Total Kjeldahl

Total Phosphorus - see Phosphorus, Total

Total Volatile Solids - see Solids, Volatile Suspended

## SUMMARY OF ACCEPTED ANALYTICAL METHODS

| Parameter | Procedure | Instrumentation | Analytical Method | Reporting Unit |
|---|---|---|---|---|
| Wind Speed | Install totalizing anemometer, read at 24 hour intervals (between 0800 and 0900 hours), and calculate average hourly wind speed. | Totalizing anemometer comparable to WEATHERtronics Model 2510. The instrument should be located in the pond complex 2m above the level of the pond banks. | | km/h |
| Visibility, Secchi Disk | At two locations in each pond, calculate Secchi Disk Visibility using procedure described by Lind (1974). | | | cm |
| Zooplankton Composition | Monthly and when changes in the community are observed, collect at least three 90 cm column samples per pond or use trap or zooplankton net, as appropriate. Use a microscope to identify at the order level and count number of organisms per unit volume. | | | No./m3 |

# APPENDICES

Appendix D from Work Plan III

Appendix F from Work Plan III

Appendix from Work Plan IV

APPENDIX D from Work Plan III

PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

# APPENDIX D

## PROCEDURE FOR POND SOIL SAMPLING AND ANALYSIS

Pond mud will be analyzed for particle size distribution and concentration of a number of constituents. Samples should be taken from each pond prior to initiating each of the two experiments during the third cycle. Analyses may be carried out by qualified laboratories within host countries or the U.S.

Sufficient time should be allowed for analysis by laboratories such that results will be received in time to apply lime if necessary. About two weeks should be allowed between application of lime and the first fertilizer application (see Appendix A).

### Soil Sampling

1. Begin at the shallow end of each of the twelve empty ponds, opposite the drain.

2. Proceed in an 'S' shape toward the deep end of each pond, collecting 12 core samples of the top 15 cm of bottom material.

3. For each pond, combine the 12 subsamples and mix the composite thoroughly. Note that wet mud mixes more easily than dry mud.

4. Spread each mud sample in a thin layer on a plastic sheet to dry.

### Analysis

Researchers should identify a qualified laboratory or laboratories in the U.S. or host country, capable of carrying out the particle size distribution analysis and the 22 tests identified on Table D-1. The amount of each sample required and handling and labeling procedures should be determined in each case. Soil samples entering the U.S. must be accompanied by USDA form and shipping label. These may be obtained (in advance) from the laboratory accomplishing the analyses.

TABLE D-1
SOIL ANALYSIS

| Analysis |
| --- |
| Determination of clay, silt, and sand fractions (by pipette method and including removal of organic matter) |
| Sample preparation (grinding, handling, storage) |
| pH |
| Phosphorus |
| Extractable Bases (Ca, Mg, K and Na) |
| Organic Matter |
| Total Nitrogen |
| Nitrate Nitrogen ($NO_3$-N) and Ammonium Nitrogen ($NH_4$-N) |
| Cation Exchange Capacity |
| Soluble Salts |
| Heavy Metals (Zn, Mn and Cu) |
| Sulfate-Sulfur ($SO_4$-S) |
| SMP Lime Requirment |
| Free $CaCO_3$ or $CaCO_3$ Equivalent[1] |
| Exchangeable H |
| Exchangeable Na |
| Aluminum |
| Iron |
| TOTAL per sample cost, excluding analyses for aluminum and iron. |

[1] If it is known that soil pH is below 7, then no free $CaCO_3$ will be detected and this analysis is unnecessary.

# APPENDIX F from Work Plan III

# MATERIALS AND METHODS REFERENCE

# APPENDIX F

## MATERIALS AND METHODS REFERENCE

As previously discussed in Chapter 2, the third cycle of CRSP pond dynamics experiments involve a number of daily, weekly, monthly and occasional measurements. The measurement of some parameters require the use of specific instrumentation, procedures or analytical methods. To the extent possible, catalog references and standard methods for selected procedures and analyses have been reproduced for use by researchers at host country locations. Researchers should read these references carefully to confirm that the described materials and methods are applicable given site specific conditions. After reviewing these materials, any planned modifications should be reported to the Program Management Office before implementation.

This appendix is presented in three sections: Instruments, Procedures, and Analytical Methods. The contents of each section are:


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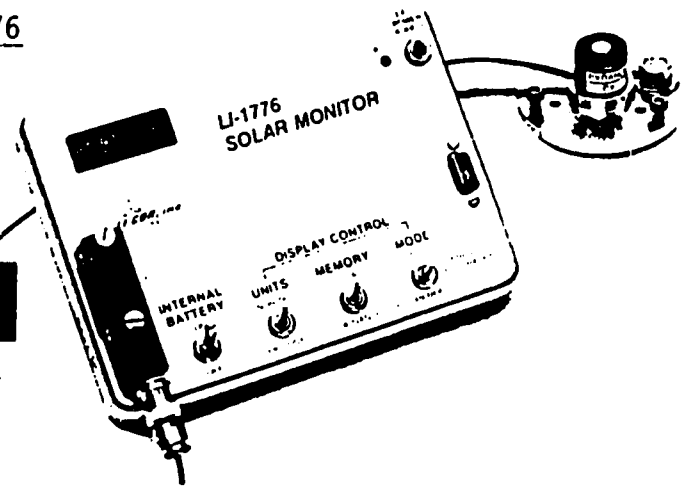
| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| SOLAR MONITOR WITH QUANTUM SENSOR | E $m^{-2}$ $day^{-1}$ |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| LI-COR<br>4421 Superior Street/P.O. Box 4425<br>Lincoln, Nebraska 68504 (402) 467-3576 | monitor: $750<br>sensor: $260 |

DESCRIPTIVE INFORMATION

Solar monitor model LI-1776




## LI-1776

# Solar Monitor

*Microprocessor Controlled Integrator/Meter*

- Integrated and instantaneous measurements
- Memory storage of integrated values for unattended monitoring
- Direct readout in engineering units
- Compatible with all LI-COR sensors (with Calconnectors)
- Module for mV signal sensors
- Weatherproof enclosure for environmental monitoring*
- Cassette tape or RS-232C output*
- Analog output of instantaneous values

### Totalized Measurements

The LI-1776 Solar Monitor averages the effects due to changing atmospheric conditions, solar elevation, etc. Meter readings, point measurements and strip chart plots are difficult to interpret in such situations. Instead of an erratic strip chart plot the LI-1776 displays an integrated value. Dividing this total by the integration time provides the average value.

### Typical Applications

When used with the LI-200SB Pyranometer, the LI-1776 provides a sophisticated system for solar energy assessment and site evaluation, performance evaluation, meteorological studies, and irrigation scheduling. For various applications in plant science, a quantum sensor can be used to measure photosynthetically active radiation (PAR). A photometric sensor is used for illuminance measurements.

### COMPUTER COMPATABILITY

Expanded datalogging capability is provided with any one of these variations of the LI-1776 Solar Monitor: LI-1776/02A, LI-1776/02M or LI-1776/07.

- Nine user-selectable integration periods (7.5, 15, 30 minutes, 1, 2, 4, 8, 12, and 24 hours)
- Real time operation (set in 7.5 minute intervals)
- Compatible with any RS-232C peripheral (computer, terminal, modem, printer, etc.)

Data is transferred in 7 bit plus parity ASCII characters through the LI-1776 Output. For either the LI-1776/02A or LI-1776/02M, the 1600-05 Cassette Tape Reader and a tape recorder are needed to transfer data to a computer system.

#### LI-1776/02A

Integrated data that is stored in the memory can be transferred directly onto cassette tape. Data is transferred *automatically* at the end of each integration period. This is especially useful for long-term monitoring, where up to 900 integration values can be recorded on one side of a 60 minute tape without user attention.

#### LI-1776/02M

With the LI-1776/02M, the user initiates the transfer of data onto cassette tape. All of the values in memory are transferred onto tape each time a transfer is initiated.

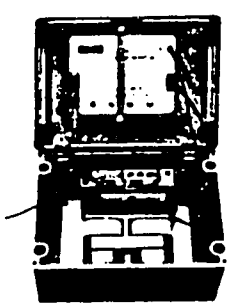
#### LI-1776/07

Through the use of a standard RS-232C serial interface, the LI-1776/07 can be connected *directly* to a terminal, computer or modem for data transfer. The LI-1776/07 can function as either the DCE (Data Communication Equipment) or the DTE (Data Terminal Equipment). The factory-set Baud rate is 300 (600 or 1200 optional).

#### 1600-05 Cassette Tape Reader

Integrated data stored in the memory of an LI-1776/02A or LI-1776/02M can be stored on cassette tape using the 1600-04 Cassette Tape Recorder. Battery life has been increased by internal modifications to allow extended operation in the field.

The 1600-05 Cassette Tape Reader is used to interface the tape-recorded data to a computer system (see page 6). The 1600-05 is compatible with any computer system that utilizes the EIA (Electronic Institute of America) RS-232C interface. Tape recorded data can also be interfaced using either a Campbell Scientific or Omnidata International cassette tape reader.



#### 1776-06 Weatherproof Case

For field operation, this fiberglass case provides a weatherproof enclosure for the LI-1776 and a cassette tape recorder (optional). Included with the 1776-06 is a 12 volt battery pack and external battery leads.

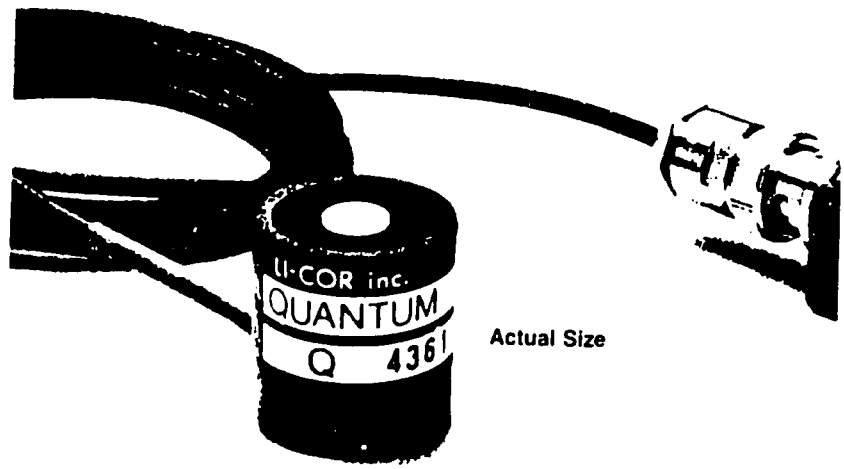
Battery Life: 2 months at 25 C. Size: 29.9 x 20.8 x 18.0 cm deep (11.8 x 8.2 x 7.1). Weight: 4.8 kg (10.6 lbs).

SOLAR MONITOR WITH QUANTUM SENSOR (continued)

## Quantum sensor model LI-190SB

*Measure Photosynthetically Active Radiation (PAR): 400 to 700 nm waveband*

Plants use the 400 to 700 nm waveband of the light spectrum for photosynthesis (3,9). A simple integral relationship exists between the number of molecules changed photochemically and the number of photons absorbed within the requisite waveband regardless of photon energy (12). The preferred measurement for PAR is *Photosynthetic Photon Flux Density* (PPFD) (9,14). This is the number of photons in the 400 to 700 nm waveband incident per unit time on a unit surface.




Actual Size

LI-190SB [illegible]

Plant scientists, meteorologists, horticulturists, ecological survey groups and other environmental scientists are using this sensor to measure PPFD in the atmosphere, growth chamber and greenhouse.

Accurate measurements are obtained under ***natural and artificial light conditions*** because of the computer-tailored spectral response of the LI-190SB. This sensor, which has been developed from earlier work (1), was pioneered by LI-COR and has become a standard for PPFD measurement in most photosynthesis related studies.

The LI-190SB is also used in oceanography, limnology, and marine science as a reference sensor for comparison to underwater PPFD measured by the LI-192SB Underwater Quantum Sensor.

*Units currently in use are einsteins, moles, photons and quanta (6, 9, 14) $1\ \mu E\ s^{-1}\ m^{-2} \approx 1\ \mu mol\ s^{-1}\ m^{-2} \approx 6.02 \cdot 10^{17}$ photons $s^{-1}\ m^{-2} \approx 6.02 \cdot 10^{17}$ quanta $s^{-1}\ m^{-2}$

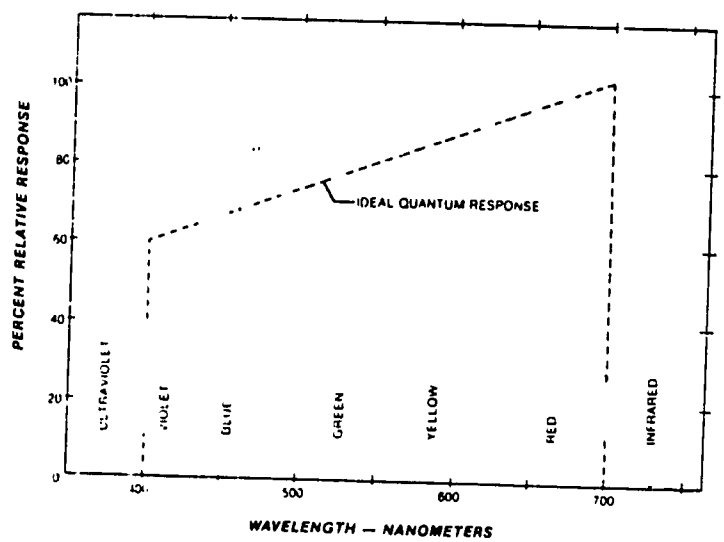


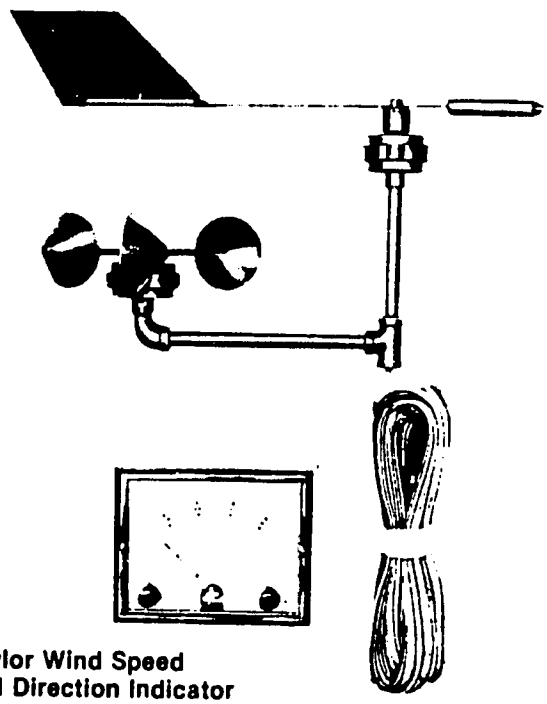

**Figure 1. Typical spectral response of LI-COR Quantum Sensors vs. Wavelength and the Ideal Quantum Response (equal response to all photons in the 400-700 nm waveband). An interference filter (for sharp near infrared cutoff at 700 nm), and colored glass filters, tailor the silicon photodiode response to the desired quantum response.**

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| WIND SPEED AND DIRECTION METER* | km/hr and direction |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| Ben Meadows Company<br>3589 Broad Street<br>P.O. Box 80549<br>Atlanta(Chamblee), GA 30366 (800) 241-6401 | $345 |

DESCRIPTIVE INFORMATION

Instantaneous TAYLOR Wind Speed and Direction Indicator



**Taylor Wind Speed and Direction Indicator**

Includes cup and vane assembly. Self-contained—no outside power source needed. Outdoor parts are corrosion-resistant. Mahogany-finished indoor indicating unit is 6½" wide x 5" high. Furnished with 60 ft. of lead-in wire and instructions.

**No. 110930** Shipping Weight 9 lb. . . . . **$345.00**

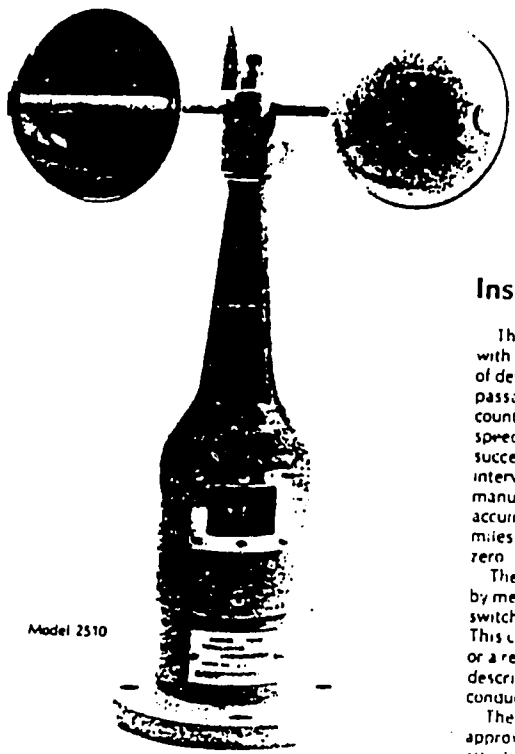
*Note: The totalizing anemometer described on the following page is the preferred instrument for CRSP research.

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| TOTALIZING ANEMOMETER | km/elapsed time<br>calculate km/hr |

| SUGGESTED SUPPLIER | | COST (MAY 1984) |
|---|---|---|
| WEATHERtronics<br>A Division of Qualimetrics, Inc.<br>P.O. Box 41039<br>Sacramento, CA 95841 | (916) 481-7750 | $370 |

DESCRIPTIVE INFORMATION

## Model 2511 Totalizing Anemometer



Model 2510

### Features:

- 6-digit mechanical counter
- Electrical contact output
- Metric or English models
- Predrilled mounting base

### Instrument Description:

The Model 2510 totalizing anemometer is equipped with a counter to provide a simple, yet precise, method of determining average wind speed and total air passage. An internal gear train converts cup rotation to counter input (917 revolutions per mile). Average wind speed can be calculated from the difference between successive counter readings divided by the time interval between readings. The 6-digit counter is not manually resettable. The anemometer can typically accumulate wind run for a year or longer (up to 99,999.9 miles or kilometers) before automatically resetting to zero.

The 2510 also provides an electrical contact output, by means of a magnet-activated reed switch. The switch furnishes one closure per 0.1 mile or kilometer. This contact can be used to advance an event recorder or a remote digital counter. (These instruments are described in detail on pages 160-162 and 166.) Two-conductor cable makes the necessary connections.

The 4-inch-diameter brass cups have a threshold of approximately 1 mph. The cups are beaded and are attached to the hub by sturdy arms. Turning radius is 6 inches. Self-lubricating stainless steel bearings support the anemometer shaft.

The flanged base of the instrument is predrilled, permitting mounting on a wooden, metal, or concrete support. In an evaporation station the anemometer is typically mounted on the platform supporting the evaporation pan. A mast adapter is available for mounting to a 1¼-inch O.D. pipe.

### Specifications:

| | |
|---|---|
| Type | 3-cup anemometer |
| Cup size | 4" diameter |
| Cup material | Brass |
| Cup design | Conical and beaded |
| Counter | Six digit-mechanical |
| Electrical contact | Reed switch |
| Contact current rating | 0.4 A at 24 VDC resistive load |
| Resolution (counter & contact) | 0.1 mile or 0.1 kilometer |
| Cup constant | 917 rev./mi. (570 rev./km) |
| Threshold | 1-2 mph |
| Body | Cast aluminum |
| Flange | Four 3/8" dia. predrilled holes |
| Max. speed | 100 mph (160 km/hr) |
| Size | 12" dia. x 16" H (305 x 400 mm) |
| Weight/shipping | 5 lbs./9 lbs. (2.3 kg./4 kg.) |

### Ordering Information:

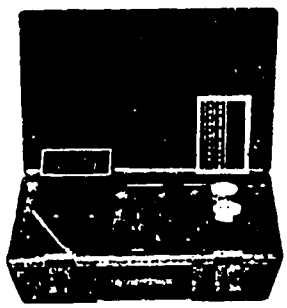
| Model | |
|---|---|
| 2510 | Totalizing Anemometer with 6 digit counter registering each 0.1 mile of wind, switch closure for remote recording included |
| 2511 | Totalizing Anemometer with 6 digit counter registering each 0.1 kilometer of wind, switch closure for remote recording included |
| 25101 | Mounting Adapter for mounting on 1¼" O.D. pipe |
| 600502 | Two-conductor, 20 AWG, Shielded Cable |

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| pH METER WITH COMBINATION ELECTRODE | pH units |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| Orion Research<br>840 Memorial Drive<br>Cambridge, MA 02139 (800) 225-1480 | Model 201: $285<br>Model 211: $295<br>Model 221: $395<br>Ross Electr: $175 |

DESCRIPTIVE INFORMATION

## 200 Series pH meters



All models in the ORION 200 Series come in a foam-lined carrying case supplied with everything you need to make measurements in the field or lab: rinse water and buffer containers, an electrode support attachment, an unbreakable electrode that never needs refilling; and, a line adaptor for meter use without battery drainage.

Model 201 includes everything you need to make routine pH measurements, especially outside the laboratory. This meter reads over the entire pH range in 0.05 increments and displays results in large, high intensity LEDs.

Model 211 has features usually associated with more expensive meters - readability to 0.01 pH, slope adjustment and recorder output. And a large LCD display makes the instrument ideal for reading in sunlight.

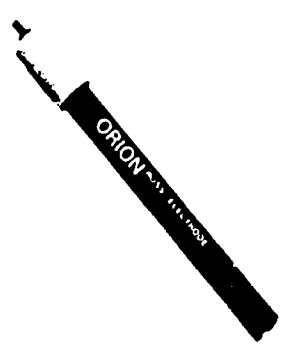
Model 221 measures pH to 0.01 *and* temperature inside or outside the laboratory. The meter is supplied with a precision temperature probe so you can measure sample temperature accurately. At the lab bench Model 221 becomes an economical method for measuring biochemical oxygen demand when used with the ORION 97-08 dissolved oxygen electrode.

| | Model 201 | Model 211 | Model 221 |
|---|---|---|---|
| pH range/resolution | 0 - 14/ ± 0.025 | 0 - 14/ ± 0.00[illegible] | 0 - 14/ ± 0.005 |
| temperature compensation | manual, 0 to 100°C | manual, 0 to 100°C | manual, 0 to 100°C |
| display | 7.5 mm high LED | 1 cm high LCD | 1 cm high LCD |
| battery complement | six size AA 1.5 volt batteries | six size AA 1.6 volt batt | one size D 9 volt battery |
| battery life | 3000 ten second measurements | 3000 ten second measures | 3000 ten second measurements |
| temperature range | --- | --- | -5°C to 100°C, ± 0.1°C or 1% |
| size and weight | 14 x 9 x 4.5 cm, 0.4 kg | 14 x 9 x 4.5 cm, 0.4 kg | 14 x 9 x 4.5 cm, 0.4 kg |
| line adaptor | 115 or 230 V AC, ± 20%, 50/60 Hz | 115 or 230 V AC, ± 20%, 50/60 Hz | 115 or 230 V AC, ± 20%, 50/60 Hz |
| recorder output | | ± 100 mV, fixed | |

## Ross Model 81-55 combination electrode

Superior to conventional ectrode that is supplied with the Orion 200 series meters.

| | | | |
|---|---|---|---|
| Model | 81-55/81-56 | Temperature Range | 0-100°C |
| Description | Ross combination with epoxy body, bulb guard. | Internal Reference | Ross |
| | | Junction | Glass Fiber |
| Recommended Use | Use for ruggedness and durability in field pH measurements | Dimensions | Length: 120 mm<br>Diameter: 12 mm<br>Cap Diameter: 16 mm |
| pH Range | 0-14 | Connector & 100 cm cable | U.S. Standard/BNC |
| Drift | Less than 0.002 pH per day | ORION Cat. No. | 815500/815600 |




63

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| DISSOLVED OXYGEN METER -WITH TEMPERATURE INDICATOR | mg/l , °C |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| Scientific Division<br>Yellow Springs Instrument Co., Inc.<br>Yellow Springs, Ohio 45387 (513) 767-7241 | meter: $795<br>probe: $157<br>cable: $ 94 |

DESCRIPTIVE INFORMATION

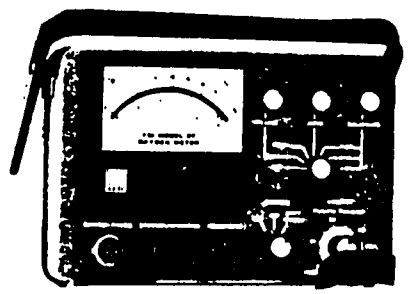
## YSI Model 57 Dissolved Oxygen Meter

The YSI Model 57, an exceptionally rugged D.O. meter, is designed for extra hard duty in the field. It features shock resistant, water resistant construction, metal meter bezel and an ABS molded case. Accidentally knock the instrument overboard and it will float until you retrieve it.

This is also an extremely sensitive instrument, measuring dissolved oxygen in three different reading ranges of 0-5, 0-10 and 0-20, mg/l. with accuracy to ±0.1 mg/l. A -5° to +45°C scale indicates temperature. Membrane coefficient and changes in water temperature are compensated for automatically. Calibration is by the fast air calibration technique, which can be completed in seconds. Two flashlight batteries operate the Model 57 for up to 1,000 hours, and an internal rechargeable battery pack is available to operate the submersible stirrer. There's also a recorder output for making permanent records.

Corrections for ocean salinity can be made by direct-dialing the salinity concentration, 0 to 40 PPT, to cover the full range from fresh water to sea water. For frequent salt water measurements the YSI 33 S-C-T Meter (salinity, conductivity, temperature) is an ideal companion instrument for determining salinity.

These and other features provide maximum convenience and reliability for on-the-spot measurements just about anywhere under the toughest working conditions.



**OXYGEN MEASUREMENT**
RANGES 0-5 0-10 and 0-20 mg/l (0-2.5, 0-5 and 0-10 mg/l with YSI 5776 High Sensitivity Membrane)

ACCURACY ±1% full scale at calibration temperature or ±0.1 mg/l, whichever is greater

READABILITY 0.025 mg/l on 0-5 scale; 0.05 mg/l on 0-10 scale, 0.1 mg/l on 0-20 scale

**TEMPERATURE MEASUREMENT**
RANGE -5 to +45 C

ACCURACY ±0.5 C

READABILITY 0.25 C

**COMPENSATION**
Automatic temperature compensation accurate to ±1% of D.O. readings made within ±5°C of calibration temperature, and accurate to ±3% of D.O. readings from -5° to +45°C. Manual, direct dial salinity compensation from fresh water to sea water, 0 to 40 PPT accurate to ±2.5% of compensated reading

**AMBIENT RANGE**
Instrument and probe operating range is -5 to +45 C

**SYSTEM RESPONSE TIME**
Typical response time for temperature and D.O. readings is 90% in 10 seconds at constant temperature of 30 C

**RECORDER OUTPUT**
0 to 114.130mV. Recorder should have 50,000 ohms minimum input impedance

**POWER SUPPLY**
Two disposable C size carbon zinc batteries provide approximately 1,000 hours instrument operation. Optional internal rechargeable battery pack YSI 5721 operates the submersible stirrer (see accessories)

**DIMENSIONS**
21.6 x 28 x 9.5cm 1.9 kg (8.5 x 11 x 3.75 inches, 4.2 lbs)

## D.O. Field Probe with 3 Meter Cable

YSI 5739 Dissolved Oxygen Probe (without cable)
- YSI 5740-10 Probe Cable — 3 meters (10')
- YSI 5740-25 Probe Cable — 7.5 meters (25')
- YSI 5740-50 Probe Cable — 15 meters (50')
- YSI 5740-100 Probe Cable — 30.5 meters (100')
- YSI 5740-150 Probe Cable — 46 meters (150')
- YSI 5740-200 Probe Cable — 61 meters (200')

(For longer cables, order YSI 5740-X and specify length to 250'. All cables 25' or longer supplied with storage reel.)

Note: BOD Bottle Probes are also available at a cost of $353 each.

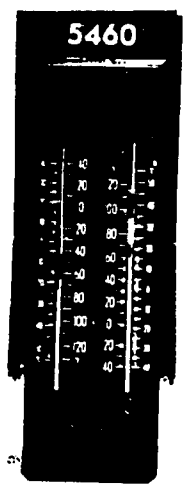
64'

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| MAXIMUM-MINIMUM THERMOMETER (AIR TEMPERATURE) | $^{o}C$ |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| Taylor Scientific Consumer Instruments<br>Division of Sybron Corp.<br>Glenn Bridge Road (704) 684-8111<br>Arden, North Carolina 28704 | $22.98 |

DESCRIPTIVE INFORMATION

Model 5460 Maximum-Minimum Thermometer

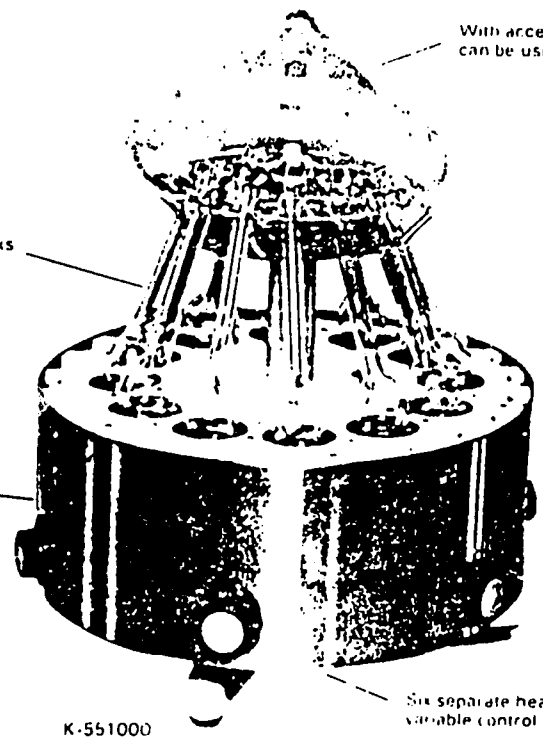



Thermometer registers high and low readings from last setting. Pushbutton reset. F° & C° scales. 8¾" x 2¾". Blister carded, 4 per carton. Weight: 6 ozs.

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| KJELDAHL NITROGEN APPARATUS | mg/l |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| Kontes<br>Spruce Street<br>Vineland, New Jersey 08360 (609) 692-8500 | $2,081.80<br>See itemization in this section |

DESCRIPTIVE INFORMATION




**K-551000 ROTARY KJELDAHL DIGESTION APPARATUS**

Design of this apparatus positions all flasks an equal distance from adjacent flasks to assure uniform heat distribution. More uniform digestions are accomplished since cold-end flasks are eliminated as found in older straight line units. The entire apparatus can be rotated 320° on the base to allow easy access to every flask.

An accessory glass fume hood fits over the digestion apparatus, allowing it to be used on the laboratory bench. A connection to vacuum is made at the base. Heat generated by the apparatus prevents condensation. This accelerates exhaustion of $SO_2$ and $SO_3$ gases through the lower opening.

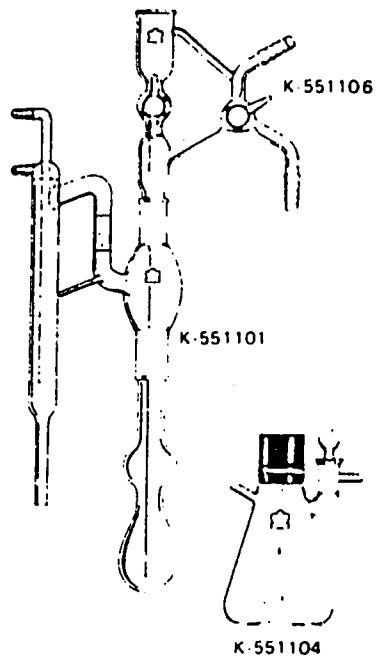
Heat control is maintained by six variable output switches each controlling two adjacent heating units. For a wider range of temperature selection an accessory variable voltage transformer may be used.

Power Requirement: 115 VAC 50/60 Hz, 1320 watts
Dimensions: 18" (430 mm)
Height: 12" (304 mm)
Weight: 25 lbs (11.5 Kg)

Accessories

| | |
|---|---|
| K-551001-0025 | Fume Hood, 25 mm dia. holes to accept K-551500 30 and 100 ml flasks |
| K-551001-0030 | Fume Hood, 30 mm dia. holes to accept K-551600 30 and 100 ml flasks |
| K-551002-0000 | Replacement Adapter Rings to accommodate 30 ml flasks |
| K-552100-0000 | Variable Voltage Transformer 115 vac 50/60 Hz, 20 amps (Not illustrated) |

# KJELDAHL NITROGEN APPARATUS (Continued)




## K-551100 KJELDAHL DISTILLATION APPARATUS

Design Features Include

- Entrance tube for complete transfer to the distillation flask. No chance to introduce sample into the sidearm
- Platinum gauze assures maximum distillation rates with negligible blank
- Hopkins Condenser directly attached to distillation head. Removes possibility of atmospheric condensation and possible contamination
- Pressurized steam entry into sample improves distilling efficiency
- Velocity of ammonia emerging from condenser is maximized prior to entrapment in a boric acid solution
- Sample can be based on the actual weight or the amount of distillable ammonia after digestion. Can be used for ultra-micro, micro or semi-micro determinations

Assembly

Insert digestion tube with sample into the distillation head and connect steam generator to unit with flexible tubing. The steam generator consists of a two-liter flask with a stopcock vent and a 600-watt immersion heating coil. It will not be damaged if it runs dry. Heat control can be maintained by using an accessory variable voltage transformer (See K-552100). Approximate overall height is 485 mm.

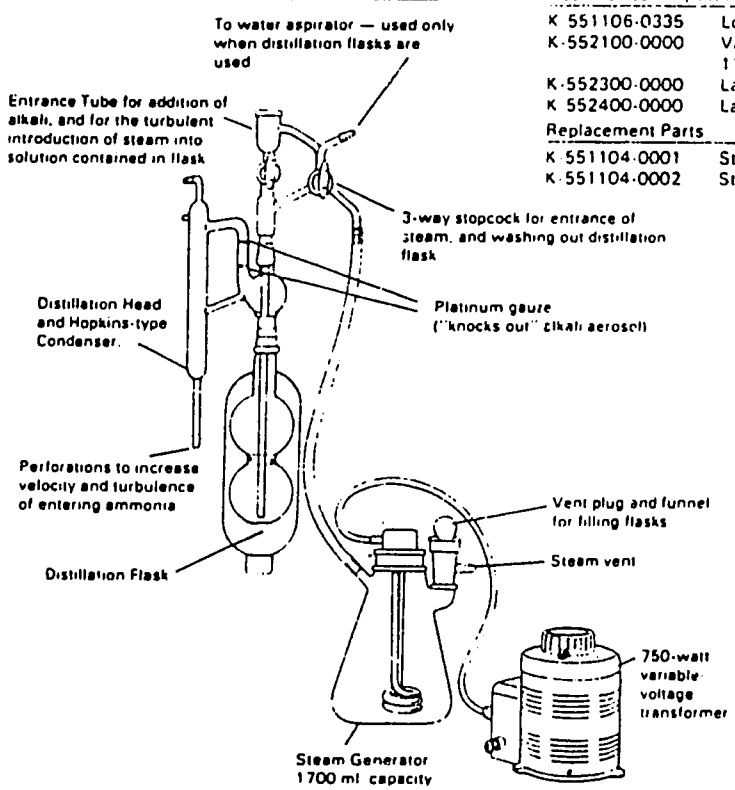
See K-551600 30 ml and 100 ml or K-551700 100 ml flasks for use with short entry tube. If K-551700 300 ml is needed, use accessory long entry tube.

Power Requirement: 115 VAC 50/60 Hz, 600 watts

| Parts | |
|---|---|
| K-551101-0000 | Distillation Head and Condenser |
| K-551104-0000 | Steam Generator |
| K-551106-0285 | Short Entrance Tube 285 mm Teflon* |

| Accessories (Not Illustrated) | |
|---|---|
| K-551106-0335 | Long Entrance Tube 335 mm Teflon |
| K-552100-0000 | Variable Voltage Transformer 115 vac 50/60 Hz, 20 amps |
| K-552300-0000 | Lab Stand |
| K-552400-0000 | Lab Clamp (2 required) |

| Replacement Parts | |
|---|---|
| K-551104-0001 | Steam Generator Flask 2-liter |
| K-551104-0002 | Steam Generator Immersion Heater 600 watt |



KJELDAHL NITROGEN APPARATUS (Continued)

K-551600

**K-551600 KJELDAHL DIGESTION/DISTILLATION FLASK**

Dual purpose flask which can be used in both the K-551000 Rotary Digestion Apparatus and K-551100 Distillation Apparatus. ₮ 24/40 joint allows connection to the distillation assembly following the digestion process, thus saving a transfer.

Flask design includes an expansion bulb as a guard against bumping and foaming during digestion. Also allows a more vigorous introduction of steam during distillation.

| Capacity, ml | Overall Height, mm |
|---|---|
| 30 | 210 |
| 100 | 210 |

| Quantity | Catalog No. | Description | Price |
|---|---|---|---|
| 1 | K551000-0000 | Digestion apparatus, Kjeldahl, rotary, 12-place, 115 V, 50/60 Hz, 1325 Watts | $1,249.30 |
| 1 | K551001-0030 | Fume hood | 138.80 |
| 1* | K551100-0000 | Distillation apparatus, Kjeldahl | 529.30 |
| 12* | K551600-0100 | Flask, Kjeldahl, 100 ml, @ 13.70 | 164.40 |
| | | Total | $2,081.80 |

* Number of flasks and distillation apparatus needed is dependent on number of samples to be collected and manpower available to clean glassware, etc.

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| DIGITAL TITRATOR TEST KIT/DISSOLVED OXYGEN | mg/l |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| HACH Company<br>P.O. Box 389<br>Loveland, CO 80539 (800) 525-5940 | $139 |

DESCRIPTIVE INFORMATION

Model OX-DT with Digital Titrator

**DISSOLVED OXYGEN TEST KIT**
**Model OX-DT with Digital Titrator**

This kit uses the Winkler dissolved oxygen method with azide modification and a 200-mL sample size. All sample treatment reagents (alkaline iodide-azide, manganous sulfate and sulfamic acid) are packaged in unit-dose powder pillows for convenience. Titrations are performed with Hach's Digital Titrator and a prestandardized PAO titration cartridge. A glass-stoppered sample bottle is included.

**Range:** 0-10 mg/L as DO
**Smallest Increment:** 0.01 mg/L
**Sample:** 300 mL
**Analysis Method:** Titrimetric
**Number of Tests:** 50 (average)
**Case:** 41 × 20 × 18 cm (16 × 8 × 7")
**Shipping Weight:** 4.1 kg (9 lbs)
**Order Cat. No. 20631-00 ..... $139.00**

Note: If digital titrator is purchased for dissolved oxygen determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters, including alkalinity and total hardness.

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| DIGITAL TITRATOR TEST KIT/ALKALINITY | mg/l |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| HACH Company<br>P.O. Box 389<br>Loveland, CO 80539 (800) 525-5940 | $110 |

DESCRIPTIVE INFORMATION

High and Low Range
Model AL-DT with Digital Titrator

**ALKALINITY TEST KIT**
**High and Low Range**
**Model AL-DT with Digital Titrator**

Model AL-DT is a higher-accuracy version of our Model AL-AP Test Kit. This kit contains the precise Digital Titrator, two prestandardized sulfuric acid titration cartridges and premeasured indicator powder pillows. Hach's Model AL-DT allows measurement in two ranges, 0-100 and 0-1000 mg/L alkalinity as calcium carbonate, with results read directly in mg/L. Both phenolphthalein (P) and total (M) alkalinity are determined.

**Ranges:** 0-100 and 0-1000 mg/L as $CaCO_3$
**Smallest Increment:** 0.1 and 1 mg/L
**Sample:** 100 mL
**Analysis Method:** Titrimetric
**Number of Tests:** 200 (average) (100 phenolphthalein and 100 total)
**Case:** 23 × 18 × 13 cm (9 × 7 × 5")
**Shipping Weight:** 2.3 kg (5 lb)
**Order Cat. No. 20637-00 ..... $110.00**

Note: If digital titrator is purchased for alkalinity determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters. including dissolved oxygen and total hardness.

| INSTRUMENT | UNIT OF MEASURE |
|---|---|
| DIGITAL TITRATOR TEST KIT/TOTAL HARDNESS | mg/l |

| SUGGESTED SUPPLIER | COST (MAY 1984) |
|---|---|
| HACH Company<br>P.O. Box 389<br>Loveland, CO 80539 (800) 525-5940 | $123 |

DESCRIPTIVE INFORMATION

High and Low Range
Model HAC-DT with Digital Titrator

**MAGNESIUM, CALCIUM AND TOTAL HARDNESS TEST KIT**
**High and Low Range**
**Model HAC-DT with Digital Titrator**

Measure total hardness, calcium and magnesium (by difference) in two ranges with this kit. Tests are performed using Hach's Digital Titrator and two different EDTA titration cartridges. Determine total hardness using ManVer 2 Hardness Indicator Powder Pillows; the color change is from red to blue. Repeat the same titration using CalVer® 2 Calcium Indicator Powder Pillows to measure calcium content. All reagents and apparatus needed are included in the kit carrying case.

**Ranges:** 0-100 and 0-1000 total or calcium hardness as $CaCO_3$
**Smallest Increment:** 0.2 and 2 mg/L
**Sample:** 50 mL
**Analysis Method:** Titrimetric
**Number of Tests:** 200 (average) (100 total and 100 calcium)
**Case:** 23 × 18 × 13 cm (9 × 7 × 5")
**Shipping Weight:** 2.3 kg (5 lb)
**Order Cat. No. 20639-00 ..... $123.00**

Note: If digital titrator is purchased for total hardness determinations, investigators need only purchase appropriate HACH reagents and titration cartridges to determine levels of 18 other parameters, including dissolved oxygen and alkalinity.

PROCEDURE

POND MAPPING

REFERENCE

Lind, O.T. 1974. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis, pp. 5-16.

# CHAPTER ONE PHYSICAL LIMNOLOGY

## MAPPING

### Mapping a pond or small lake by plane table method

The mapping of lakes is usually beyond the capability of the limnologist, and he must rely on maps made by well-equipped survey teams. Often aerial photographs with known scale will suffice. However, small ponds (generally less than 10 acres) may be accurately mapped with a minimum of equipment. Whenever possible, mapping in winter on sound ice is preferable. Welch (1948) includes an extensive section on map methods from simple to complex.

*Apparatus*

Plane table and tripod
Alidade (a crude alidade may be made by setting pins in opposite ends of wooden ruler for "sights")
Compass
Ruler, graduated in tenths of inches
Map paper
Hard lead pencils
Round-headed map pins
100-foot steel measuring tape
2 steel stakes, approximately 2 feet long





Wooden stakes, approximately 3 feet long (25 to 100 depending on size of pond)
Sledgehammer
Stadia rod or other painted pole at least 6 feet long
Metal-core clothesline rope, of sufficient length to reach across pond, marked in 5-foot increments
Small boat or canoe
Depth sounding line or electronic depth sounder

*Procedure*

1. Select the longest relatively straight section of lakeshore for establishing a base line.

2. Drive steel stake near shore for one end of base line. Most of the pond should be visible from this point. Repeat for other end of base line at a point at least 100 feet from first stake and approximately the same distance from shore as the first. Make the base line as long as possible (Fig. 1).

3. Drive a wooden stake at water's edge at every major change in shoreline configuration. Maximum distance between any two wooden stakes should probably not exceed 50 feet. Drive wooden stakes just deep enough to stay in place for mapping. Steel stakes on base line are permanent and should be driven so only 4 to 6 inches protrude. Tie a cloth "flag" to top of every fifth stake to facilitate counting.

4. Attach map paper to plane table. Set up tripod directly over one metal stake at end of base line, and plumb table center to the stake. Level table and align one edge with true north-south line. Lock table in place. Draw true north arrow in corner of map; also indicate magnetic north (Fig. 2).

5. Judge shape of pond and length of base line, and determine appropriate scale. Record scale on map near north-south arrow.

6. Determine position on map that represents end of base line where table is set up. Mark this on map by firmly setting map pin in table. The pin must not move.

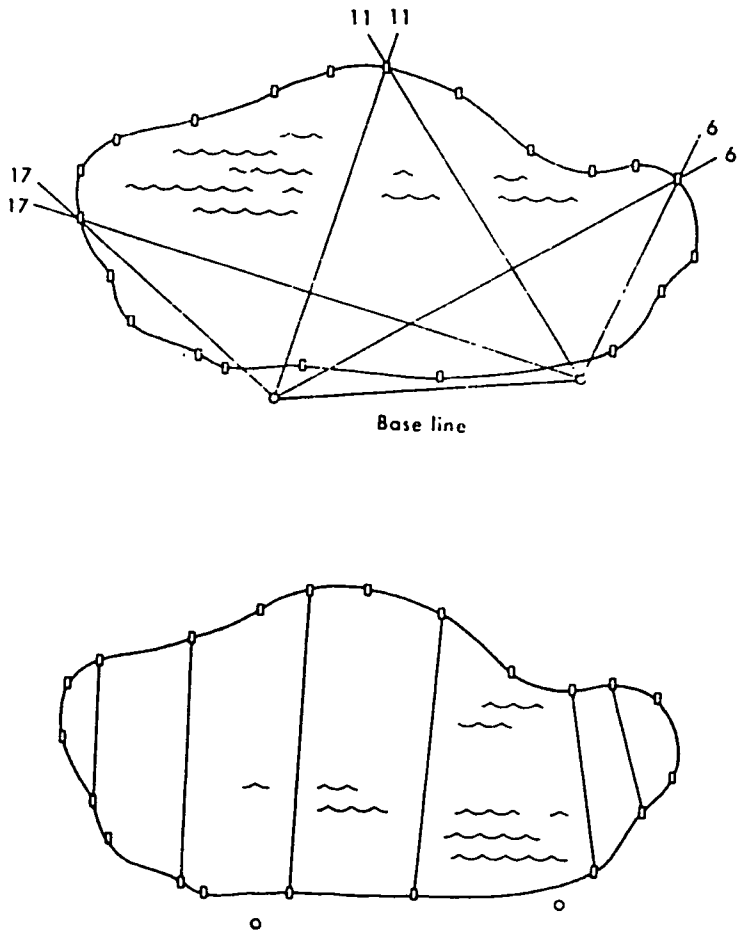



FIG. 1. Plane table map *(top)* showing position of a numbered shoreline stake and the base line. Distances for three shoreline points as determined by triangulation from the ends of the base line are shown. In a small lake or pond, bottom contours may be determined by sounding along a calibrated rope stretched between approximately opposite shoreline stakes *(bottom)*.



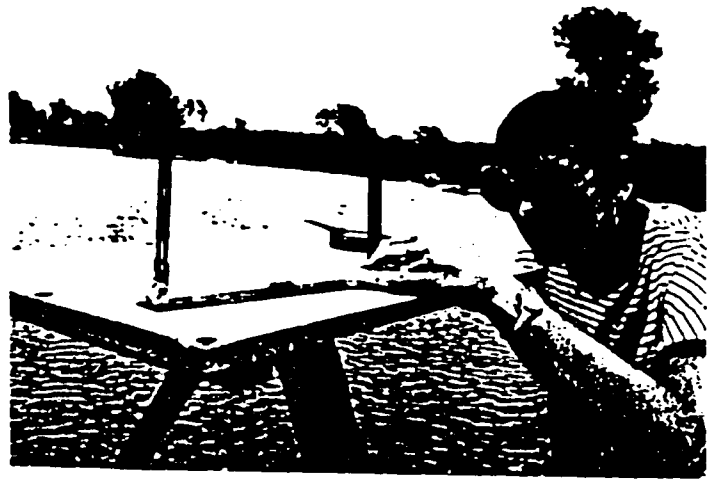


FIG. 2. Student using an open-sight alidade and a plane table to map a small pond. A compass is used to ascertain north orientation of the map. The edge of the alidade at the end nearest the student is always placed against a map pin firmly set in the table. This pin is the map position corresponding to the base line stake over which the table is centered.

Check again to be certain that all parts of pond will fall on map according to scale selected and position of pin.

7. Have flagman set stadia rod vertically on steel rod at other end of base line. Place zero mark on alidade against pin, and sight through alidade down base line to line up with stadia rod (Fig. 2). Draw base line on map along edge of alidade for the appropriate length as determined by scale. *Be careful not to bump the plane table at any time.*

8. Now in a similar manner, progressively sight alidade around the pond on each wooden stake (flagman holds stadia rod just behind stake), and draw line along edge of alidade. Number each line consecutively. The

fifth line and every multiple of 5 should be sighted on a stake with flag. Any deviation indicates a missed stake. After each flagged wooden stake has been sighted and the line drawn, resight on other end of base line. If this sighting does not coincide with original line, the table has been moved and another series must be taken.

9. After a complete circuit has been made, move and reset table over stake at other end of base line. Set pin in map at this end of base line and align table by sighting back down to other end of base line. Repeat step 8.

10. The point where corresponding numerical lines intersect is the position of each wooden stake marking the shoreline. Connect these points with a smooth line. By doing this in the field, you are able to include minor changes in shoreline. Also indicate position of obvious features in the water (logs, weed beds, and others)

11. It is more difficult to map bottom contours for the purpose of morphometric calculations. The problem is the accurate plotting on the map of the position of the sounding team. On smaller ponds, mapping bottom contours may be done as follows: starting at one end of the pond, stretch the calibrated rope between any two known wooden stake locations. Represent this by a light line on the map.

12a. The sounding team in the boat moves along this line, taking soundings at appropriate intervals from one shore to the other (intervals vary depending on amount of change in bottom contours). With the use of the scale, a point is placed at the proper position on the line, and the measured depth written in at that point (Fig. 1).

12b. An alternate procedure, more suitable for larger bodies of water but less precise so far as exact position on the lake is concerned, is the use of timed echo soundings. Echo sounders are becoming increasingly available, especially as "fish finders." The boat's pilot makes a line of sight between two stakes on opposite banks and sets the boat at a constant slow speed. It is



important that he make every effort to maintain a straight line and constant speed between the two points. A second person calls out regular time intervals—for example, every 30 seconds—and a third person reads and records the depth shown on the echo sounder at that time. The total time taken to transect the lake is recorded. Thus the total time for distance is known, and time for any timed increment can be calculated. Although this procedure is less precise, it has a compensating advantage in that it allows many more soundings to be taken, which more accurately gives the shape of the lake basin and allows for the plotting of more closely spaced bottom contours. This increases the accuracy of the lake volume calculation.

13. Repeat the chosen sounding procedure for a parallel series of lines across the pond.

14. Determine degree of bottom development to be shown (1-m intervals are often used, but intervals may be more or less). Draw in contour lines by connecting the appropriate points and the lines. For shape between points, parallel the shoreline.

15. Label map and include date and names of map crew. Roll up map (do not fold).

16. Return map to laboratory, retrace shoreline and contours in permanent ink. Clean up other working lines with soft eraser.

## Direct measure modification of plane table method

The direct measure modification of the plane table method is suited for ponds of regular outline and open water. It is simple, and depth soundings may be taken simultaneously if the work party is of sufficient size.

### *Apparatus*

Same as for plane table method plus the following

Good quality 18- to 24-inch ruler graduated in tenths of inches

Steel-core clothesline rope, of sufficient length to

reach across pond's longest dimension, marked in 5-foot increments

If approximate size of pond is known before going to the field, a previously prepared table of scale conversions of feet to inches will facilitate time spent in the field

A small boat or canoe may be necessary if obstructions are present

*Procedure*

1. Select a base point that is relatively open of vegetation and from which all parts of the pond may be seen. Drive iron stake and center drawing board over this stake. All work will be done from this location.
2. Prepare board for drawing as in plane table procedure.
3. Set out shoreline stakes as in plane table procedure.
4. Select point on paper to correspond with plane-table setup over iron stake, and set map pin.
5. Use alidade and take sightings on first stake to left. Draw light line along alidade from base point out to near edge of paper.
6. Place zero end of calibrated line on iron stake, and measure distance to the sighted stake to the nearest foot. Convert this distance to scale in inches; using ruler, measure out from base point the corresponding distance on the line, and mark point. Thus location and distance of first stake have been determined.
7. Repeat step 6 for all remaining stakes.
8. Connect the points, filling in detail from observation.
9. If the map party is of sufficient size, a boat sounding team may work alongside the measuring rope, taking soundings and distance between base point and stake. Depths called out may be marked on map at proper distance from base point (see following section, Morphometry).



## MORPHOMETRY

Most limnological phenomena and productivity are directly related to the morphological features of the water basin. Therefore, certain morphometric features are of interest to a limnologist beginning a study of any water. Morphometric measurements are based on good hydrographic maps, and in general, the larger the map, the more reliable the morphometric data that may be obtained from them.

### Area by polar planimeter

Whenever instrumentation is available, the polar planimeter method is preferred. The polar planimeter is a delicate instrument, and care must be taken whenever it is used. Read over the directions supplied by the manufacturer for the instrument in use.

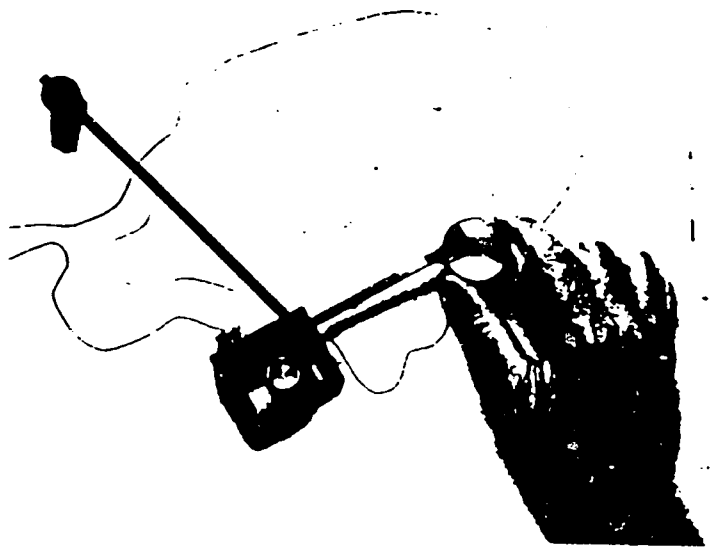


FIG. 3. Compensating polar planimeter used to determine areas. Here the planimeter is tracing the shoreline to determine the total area of a pond drawn by the plane table method.

1. Prepare map by placing it on hard, smooth surface. Tape it in place.
2. Most maps will be too large to be covered by one cycle of the planimeter. These maps must be ruled off into halves or smaller segments and the areas of each summed for the total.
3. Check the calibration of your instrument on the map paper being used. Use calibration device supplied by manufacturer, or carefully rule off a known area and trace with planimeter 3 times.
4. Proceed with actual planimetry by tracing outline of lake or bottom contours following carefully the manufacturer's directions.

### Area by cut and weight

1. Lightly trace map outline with bottom contours onto a good grade of paper.
2. From an area outside traced area, cut out a square of known area (a 9-inch square is usable) and weigh this piece of paper. Calculate weight per square inch.
3. Cut out outline of entire lake and weigh. Calculate area by dividing weight of entire lake by weight of 1 square inch.
4. Repeat step 3 for each successive bottom contour.

### Volume by calculation

If a lake basin is considered as a cone, then the volume may be calculated by the appropriate equation (cone volume = $1.047\ r^2h$). However, because the slopes of lake bottoms are rarely regular, a better approximation for volume may be obtained by calculating and then summing the volumes of conical segments (frustra), with upper and lower surfaces delimited by the areas of sequential depth contours. The calculation is then as given by Welch (1948):

$$\text{Lake volume} = \sum \text{frustrum volumes}$$

where



$$\text{frustrum volume} = \frac{h}{3}(a_1 + a_2 + \sqrt{a_1 a_2})$$

$h$ = depth of frustrum
$a_1$ = area of frustrum surface
$a_2$ = area of frustrum bottom

### Volume by planimetry

Lake volume may also be determined by planimetric integration.

1. Using linear graph paper, plot the area at a given depth against that depth. Make the plot with the horizontal axis for area at the top and the vertical axis for depth at the left of the page. This places the 0—0 ordinate in the upper left corner.
2. Integrate the area beneath the curve by using a polar planimeter or by counting squares. Include those squares that are more than half within, and disregard those that are more than half outside the line. If equal di-

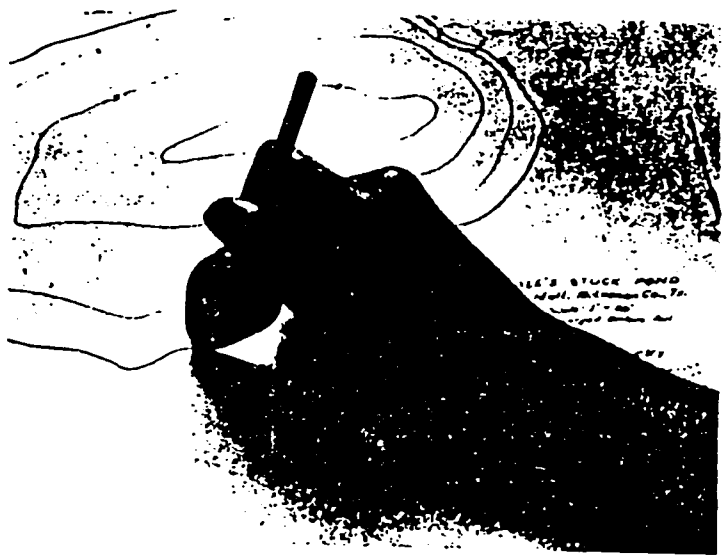


**Fig. 4.** Cartometer in use measuring the shoreline length of a plane table map.

mensions for each square of the grid are used (that is, each grid has dimensions of 1 $m^2$ in one direction versus 1 m in the other direction), that grid represents 1 $m^3$ volume. Summing the number of cubic meter volume grids under the curve will give the approximate total volume of the lake.

### Shoreline length: cartometer method

The map measure (cartometer) is a convenient method for measuring lengths of shorelines (Fig. 4). It is also a delicate instrument and deserves care in handling.

1. Set dial by turning wheel to zero line. Draw a line of known length on the map paper, and trace three times with the instrument to check its accuracy of calibration.
2. Set instrument to zero line, and carefully trace the shoreline of the lake. Watch carefully to see if the dial revolves more than one time. Record number of inches or centimeters, and convert to feet or meters per scale.
3. Repeat for each of the submerged contours.

### Shoreline development

Shoreline development is an index of the regularity of the shoreline. For a lake that is a perfect circle, the shoreline development is 1. As the value departs from unity, irregularity is indicated. This value is calculated as follows:

$$\text{Shoreline development (SLD)} = \frac{S}{2\sqrt{a\pi}}$$

where

S = length of shoreline
a = area of lake

### Maximum length and orientation of main axis

These two factors are usually the same portion of the lake, but because of unusual irregularities, they may not be so. Maximum length is the longest straight line that may be drawn without intersecting any mainland.



Not all lakes will have a segment that may be considered to represent a maximum length. The orientation is expressed as opposing points on a 16-point compass: for example, SSE-NNW.

### Maximum depth

The maximum depth is the deepest spot in the lake.

### Mean depth

The mean depth is an important value, since it is used in certain other calculations, such as heat budgets. It is calculated as follows:

$$\text{Mean depth in meters} = \frac{\text{volume in } m^3}{\text{surface area in } m^2}$$

PROCEDURE

# DETERMINING SECCHI DISK VISIBILITY

REFERENCE

Lind, O.T. 1974. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis, pp. 22-23.



## VISIBILITY

Visibility is a measure of the depth to which one may see into the water. Obviously this is variable with the day conditions and the eyesight of the observer. The Secchi disk (Fig. 6) is a simple device used to estimate this depth. It consists of a weighted circular plate, 20 cm in diameter, with the surface painted with opposing black and white quarters. It is attached to a calibrated line by a ring at the center so that when held by the line, it hangs horizontally. To determine the Secchi disk visibility, slowly lower the disk into the water until it disappears, and note this depth. Lower the disk a few more feet, then slowly raise it until it reappears, and note this depth. The average of these two readings is taken for the final Secchi disk visibility depth.

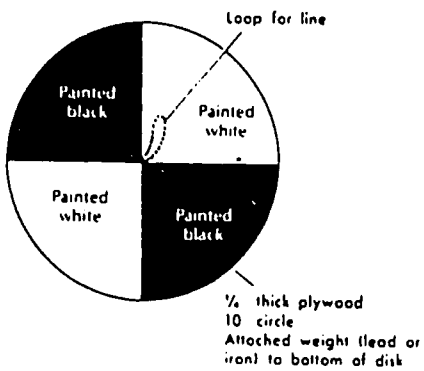



FIG. 6. The Secchi disk may be easily made from a 20-cm diameter metal or weighted wooden disk. Opposite quarters are painted gloss white and gloss black as shown. It is important that the calibrated line be attached so that the disk hangs horizontally in the water (From Vivian, V. E.: Sourcebook for environmental education, St. Louis, 1973, The C. V. Mosby Co.)



The Secchi disk visibility is useful as a means of comparing the visibility of different waters, especially when measured by the same observer. Since clearness of the day, position of the sun, roughness of the water, and the observer all are significant considerations, they should be recorded along with the visibility depth data. Most important is for an observer to establish a standard set of operating conditions for himself; for example, always take readings while standing, with or without glasses or sunglasses, on the lee side of the boat with the sun to the observer's back, sometime between 9:00 AM and 3:00 PM.

Since one of the more frequently used optical relations in water studies is the photic depth, or depth of 1% surface illumination, some limnologists find it convenient to "calibrate their eye" to estimate photic depth by using only the simple Secchi disk. This is done by accurately determining the true photic depth by use of a submarine photometer and at the same time taking a series of Secchi disk readings to obtain an average. Dividing true photic depth by Secchi disk visibility depth will obtain a factor by which Secchi disk visibility depth is multiplied. This factor is used in the future to estimate photic depth when a submarine photometer is unavailable. If this procedure is used over a range of different water conditions, one general factor may be developed and a fair approximation of photic depth obtained.

PROCEDURE

GLASSWARE WASHING

REFERENCE

Michigan State University Limnological Research Laboratory, 1983. Unpublished memo on glassware washing procedures. East Lansing, MI. 2 pp.

$PO_4$ Glassware:

1) Rinse 3 times with distilled $H_2O$
2) Soak in 1:1 HCl ($PO_4$ ONLY) bath for at least 5 hours
3) Rinse 3 times with distilled $H_2O$, once with deionized
4) Dry (protect from dust) and cap with aluminum foil

NOTE: It may be necessary to periodically wash this glassware with HOT 1:1 HCl

$NH_3$-N and Kjeldahl-N Glassware:

1) Soak in Liquinox-$H_2O$ solution, rinse with tap $H_2O$, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled $H_2O$
3) Soak and store in 0.1N HCl until next use, rinse 5 times with deionized $H_2O$ before use

NOTE: Soak glassware stored dry in the 0.1N HCl for 15 minutes before use

$NO_2$-N, $NO_2$-$NO_3$-N Glassware:

1) Soak in Liquinox-$H_2O$ solution, rinse with tap $H_2O$, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled $H_2O$
3) Soak in the 1 N HCl bath for 30 minutes, rinse 3 times with deionized $H_2O$
4) Dry and cap with aluminum foil

Nalge Polypropylene Sample Bottles and Other Plastic Labware:

1) Rinse 3 times with distilled $H_2O$
2) Soak in 1 N $HNO_3$ bath for 15 minutes and rinse 3 times with distilled $H_2O$
3) Soak in 1 N HCl bath for 15 minutes and rinse 3 times with deionized $H_2O$
4) Dry and replace cap or cap with aluminum foil for storage

GLASSWARE WASHING (continued)

General Analytical Glassware (including other glassware not listed above):

1) Soak in Liquinox-$H_2O$ solution, rinse with tap $H_2O$, drip dry
2) Soak in Chromic-Sulfuric Acid cleaning solution (Chromerge) for 15 minutes, rinse 3 times with distilled $H_2O$
3) Soak in 1 N $HNO_3$ bath for 15 minutes, rinse 3 times with distilled $H_2O$
4) Soak in 1 N HCl bath for 15 minutes, rinse 3 times with deionized $H_2O$
5) Dry (protect from dust) and cap with aluminum foil

PROCEDURE

## MEASURING HYDROLOGIC CHARACTERISTICS

REFERENCE

Wood, J. W., 1974. Diseases of Pacific salmon: their prevention and treatment. State of Washington, Department of Fisheries, Olympia, WA. pp. 71-77.

EVAPORATION: Each project team should purchase from a commercial source or have constructed evaporation pans (3) to be floated and tethered on the surface of the ponds. It is expected that this will provide the best estimate of the evaporation rate at the pond surface. The pans should be of clear or white plexiglas, lexan (polycarbonate) or polypropylene. The dimensions (ID) should be approximately 70 (L) x 70 (W) x 25 (D) cm with vertical sides. A volume of water should be added to each pan and the change in water depth monitored over time. From the change in depth of water in the pans, pan area and the rainfall measurements at the site, the evaporation rate per unit area can be calculated. The pans must not overflow or dry out between measurement intervals.

INFLOW:

There are several ways to calculate the inflow into a pond. The following represent several methods of determining the rate of water inflow:

**Direct Measurement** – This may be done by simply placing a container of known volume under the spout and determining the time it takes to fill the container.

Example: A 50-gallon drum is filled in 25 seconds. 50 gals/25 secs x 60 secs = 120 gpm

**Measurement from Table "Flow from Pipes"** – This table (Table 2) was prepared by our engineers for 3

For efficient use of this table, you should make up a wooden square, much like a carpenters square, with one leg (the Y leg) being either 9 or 16 inches long; the inside edge of the other leg (the X leg) should be marked off in 1 or ½ inch increments and be long enough to measure any water flow that you encounter at your hatchery.

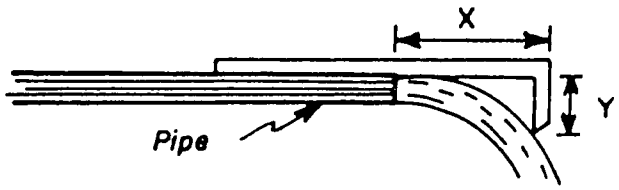
In use, the X leg of the square is placed on top of the pipe in a position where the end of the Y leg just touches the top of the extruding "jet" of water. Next, refer to the table under the correct column listing the diameter of pipe and length of Y that you used; go down the column until you find the X [illegible] to the one that you measured, then read across to the appropriate gpm column to determine flow in gpm.

and the length of the Y leg of the square is 16 inches. When the end of the Y leg just touches the discharging water, the X leg extends out 24 inches. Referring to the appropriate column, it may be seen that the flow is between 240 and 250 gpm.

## MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

**Table - 2**

**FLOW FROM PIPES**




Formula – $G = \frac{2.56\ D^2\ X}{\sqrt{Y}}$ $X = \frac{G\sqrt{Y}}{2\ 56\ D^2}$

D = DIA. OF PIPE IN INCHES

G = GALLONS PER MINUTE (gpm)

| *4" Pipe Y = 16"* | | *3" Pipe Y = 16"* | | *4" Pipe Y = 9"* | | *3" Pipe Y = 9"* | |
|---|---|---|---|---|---|---|---|
| *X"* | *gpm* | *X"* | *gpm* | *X"* | *gpm* | *X"* | *gpm* |
| 4.88 | 50 | 8.68 | 50 | 3.66 | 50 | 6.51 | 50 |
| 5.86 | 60 | 10.42 | 60 | 4.39 | 60 | 7.81 | 60 |
| 6.84 | 70 | 12.15 | 70 | 5.13 | 70 | 9.11 | 70 |
| 7.81 | 80 | 13.89 | 80 | 5.86 | 80 | 10.42 | 80 |
| 8.79 | 90 | 15.62 | 90 | 6.59 | 90 | 11.72 | 90 |
| 9.77 | 100 | 17.36 | 100 | 7.32 | 100 | 13.02 | 100 |
| 10.74 | 110 | 19.10 | 110 | 8.06 | 110 | 14.32 | 110 |
| 11.72 | 120 | 20.83 | 120 | 8.79 | 120 | 15.23 | 120 |
| 12.70 | 130 | 22.57 | 130 | 9.52 | 130 | 16.93 | 130 |
| 13.67 | 140 | 24.30 | 140 | 10.25 | 140 | 18.23 | 140 |
| 14.65 | 150 | 26.04 | 150 | 10.99 | 150 | 19.53 | 150 |
| 15.62 | 160 | 27.78 | 160 | 11.72 | 160 | 20.83 | 160 |
| 16.60 | 170 | 29.51 | 170 | 12.45 | 170 | 22.13 | 170 |
| 17.58 | 180 | 31.25 | 180 | 13.18 | 180 | 23.44 | 180 |
| 18.55 | 190 | 32.98 | 190 | 13.91 | 190 | 24.74 | 190 |
| 19.53 | 200 | 34.72 | 200 | 14.65 | 200 | 26.04 | 200 |
| 20.51 | 210 | 36.46 | 210 | 15.38 | 210 | 27.34 | 210 |
| 21.48 | 220 | 38.19 | 220 | 16.11 | 220 | 28.64 | 220 |
| 22.46 | 230 | 39.93 | 230 | 16.85 | 230 | 29.95 | 230 |
| 23.43 | 240 | 41.66 | 240 | 17.58 | 240 | 31.25 | 240 |
| 24.41 | 250 | 43.40 | 250 | 18.31 | 250 | 32.55 | 250 |
| 25.39 | 260 | 45.14 | 260 | 19.04 | 260 | 33.85 | 260 |
| 26.37 | 270 | 46.87 | 270 | 19.77 | 270 | 35.15 | 270 |
| 27.34 | 280 | 48.61 | 280 | 20.51 | 280 | 36.46 | 280 |
| 28.32 | 290 | 50.34 | 290 | 21.24 | 290 | 37.76 | 290 |
| 29.30 | 300 | 52.08 | 300 | 21.97 | 300 | 39.06 | 300 |
| 1" x = 10.2 gpm | | 1" x = 5.89 gpm | | 1" x = 13.7 gpm | | 1" x = 7.7 gpm | |

X in every case equals inches and parts of inches.

## MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

**Pipe Larger or Smaller than 3 or 4 Inches** – The table is made up for only 3 inch and 4 inch diameter pipes but the formula at the top of the page may be used for calculating the discharge from any diameter pipe or for X measurements in excess of those listed in the table.

**Example:** A pipe is 6 inches in diameter and running full of water. A square with a Y leg 16 inches long just touches the top of the discharging water when the X leg extends out 30 inches.

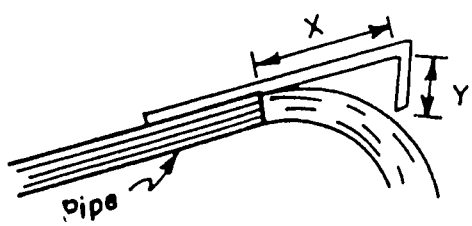
D = 6 inches
X = 30 inches
Y = 16 inches
G = Flow in gpm

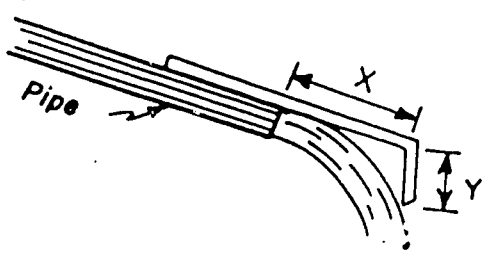
$$G = \frac{2.56\,D^2\,X}{\sqrt{Y}} = \frac{(2.56)\,(6 \times 6)\,(30)}{\sqrt{16}} = \frac{(2.56)\,(36)\,(30)}{4}$$

G = 691 gpm

**Note:** A 6 inch pipe running full will deliver 4 times the flow of a 3 inch pipe running full at the same X and Y.

**Pipes not Level** – The "Flow from Pipes" table (Table 2) may be used even if the pipe is not horizontal (level). To be used correctly, however, you cannot use a square; the X leg is placed on top of the pipe but the Y leg must extend directly down to the top of the water jet as in the diagrams below. The X reading is determined and the formula or table is used in the same manner as for a level discharge pipe.




**Pipes not Full** – The table (Table 2) and formula are only applicable for direct use if the pipe is running full of water. They may still be used with a pipe running partly full if a factor is used to compensate for the reduced flow resulting from the pipe only being partially filled. The following table, entitled "Effective Area Factor" table (Table 3), is used for any size pipe to find the effective area factor (EAF) after determining the ratio of the freeboard (F) to the diameter (D) of the pipe. Freeboard, in this case, is the measurement in inches of that portion of the diameter of the pipe that is not full of water. Determine X and Y in the usual manner, find the theoretical flow (as if the pipe was running full) from the "Flow from Pipes" table or formula; then multiply the theoretical flow by the effective area factor (EAF) to find the actual flow.

**Example:** A pipe is 4 inches in diameter and running with only 3 inches of water, Y is 16 inches and X is 24 inches. The free board (F) is 1 inch (4 inches minus 3 inches); therefore, the ratio of F to D (F/D) is 0.25. Looking at the "Effective Area Factor" table (Table 3) you can see that the EAF for an F/D of 0.25 is 9.805. In the "Flow from Pipes" table (Table 2), the flow for X = 24 inches and Y = 16 inches would be about 246 gpm if the pipe was full.

246 gpm X 0.805 = 198 gpm

Table 3
"Effective Area Factor" Table. For use in the calculation of flows thru pipes running partially full of water.

| *Ratio F/D* | *EAF* | *Ratio F/D* | *EAF* | *Ratio F/D* | *EAF* |
|---|---|---|---|---|---|
| 0.00 | 1.000 | 0.23 | 0.826 | 0.37 | 0.664 |
| 0.10 | 0.948 | 0.24 | 0.816 | 0.38 | 0.651 |
| 0.11 | 0.939 | 0.25 | 0.805 | 0.39 | 0.639 |
| 0.12 | 0.931 | 0.26 | 0.793 | 0.40 | 0.627 |
| 0.13 | 0.922 | 0.27 | 0.782 | 0.41 | 0.614 |
| 0.14 | 0.914 | 0.28 | 0.770 | 0.42 | 0.602 |
| 0.15 | 0.905 | 0.29 | 0.759 | 0.43 | 0.589 |
| 0.16 | 0.896 | 0.30 | 0.747 | 0.44 | 0.577 |
| 0.17 | 0.886 | 0.31 | 0.735 | 0.45 | 0.564 |
| 0.18 | 0.877 | 0.32 | 0.723 | 0.46 | 0.551 |
| 0.19 | 0.867 | 0.33 | 0.712 | 0.47 | 0.538 |
| 0.20 | 0.858 | 0.34 | 0.700 | 0.48 | 0.526 |
| 0.21 | 0.847 | 0.35 | 0.688 | 0.49 | 0.513 |
| 0.22 | 0.837 | 0.36 | 0.676 | 0.50 | 0.500 |

Note: For 6 inch pipes, the following factors may be used instead of Table 3 for obtaining flows from the 3 inch pipe table:

Full 6" pipe – 4 times 3" pipe full
5/6 Full 6" pipe – 3.5 times 3" pipe full
3/4 Full 6" pipe – 3.4 times 3" pipe full
2/3 Full 6" pipe – 2.8 times 3" pipe full
1/2 Full 6" pipe – 2.0 times 3" pipe full

## MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

**Table 4** DISCHARGE IN CUBIC FEET PER SECOND (cfs) AND GALLONS PER MINUTE [illegible]

WEIRS, BY THE FRANCIS FORMULA: $Q = 3.33\ H^{3/2}$

(Adapted from King's "Handbook [illegible]

| Depth on Crest *(inches)* | Discharge per Foot of Weir Crest *cfs* | *gpm* |
|---|---|---|
| 1/2 | .03 | 13 |
| 3/4 | .05 | 24 |
| 1 | .08 | 36 |
| 1/4 | .11 | 50 |
| 1/2 | .15 | 66 |
| 3/4 | .19 | 83 |
| 2 | .23 | 102 |
| 1/4 | .27 | 122 |
| 1/2 | .32 | 142 |
| 3/4 | .36 | 164 |
| 3 | .42 | 187 |
| 1/4 | .47 | 211 |
| 1/2 | .53 | 236 |
| 3/4 | .58 | 262 |
| 4 | .64 | 287 |
| 1/4 | .70 | 315 |
| 1/2 | .77 | 343 |
| 3/4 | .83 | 372 |
| 5 | .90 | 402 |
| 1/4 | .97 | 433 |
| 1/2 | 1.03 | 463 |
| 3/4 | 1.10 | 495 |
| 6 | 1.18 | 528 |
| 1/4 | 1.25 | 562 |
| 1/2 | 1.33 | 596 |
| 3/4 | 1.41 | 631 |
| 7 | 1.48 | 665 |
| 1/4 | 1.56 | 702 |
| 1/2 | 1.65 | 738 |
| 3/4 | 1.73 | 776 |
| 8 | 1.81 | 814 |
| 1/4 | 1.90 | 853 |
| 1/2 | 1.98 | 890 |
| 3/4 | 2.07 | 930 |
| 9 | 2.16 | 971 |
| 1/4 | 2.25 | 1,012 |
| 1/2 | 2.35 | 1,053 |
| 3/4 | 2.44 | 1,096 |
| 10 | 2.53 | 1,136 |
| 1/4 | 2.63 | 1,179 |
| 1/2 | 2.73 | 1,223 |
| 3/4 | 2.82 | 1,268 |
| 11 | 2.92 | 1,312 |
| 1/4 | 3.03 | 1,358 |
| 1/2 | 3.12 | 1,401 |
| 3/4 | 3.23 | 1,448 |
| 12 | 3.33 | 1,495 |

The above table is to be used for measuring the discharge of water over damboards or other similar weirs. For practical purposes the width of a damboard fits the description of a sharp-crested weir. The discharge must be free falling to use this table. In practice the depth on the weir crest is measured to the top of the curl (see diagram) on the leading edge of a yardstick when the yardstick is placed on the leading edge of the top damboard. After measuring the depth on the crest, refer to the table and multiply the flow in gpm by the length of the weir in feet.

**Example:** A weir is 41 inches long and the depth on the crest is 1 3/4 in.
The flow is:
3.42 X 83 = 284 gpm

(41 inches = 3.42 feet)

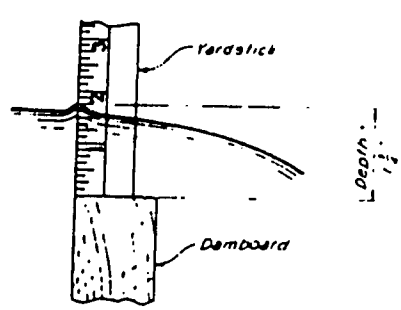



TABLE 4

## MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

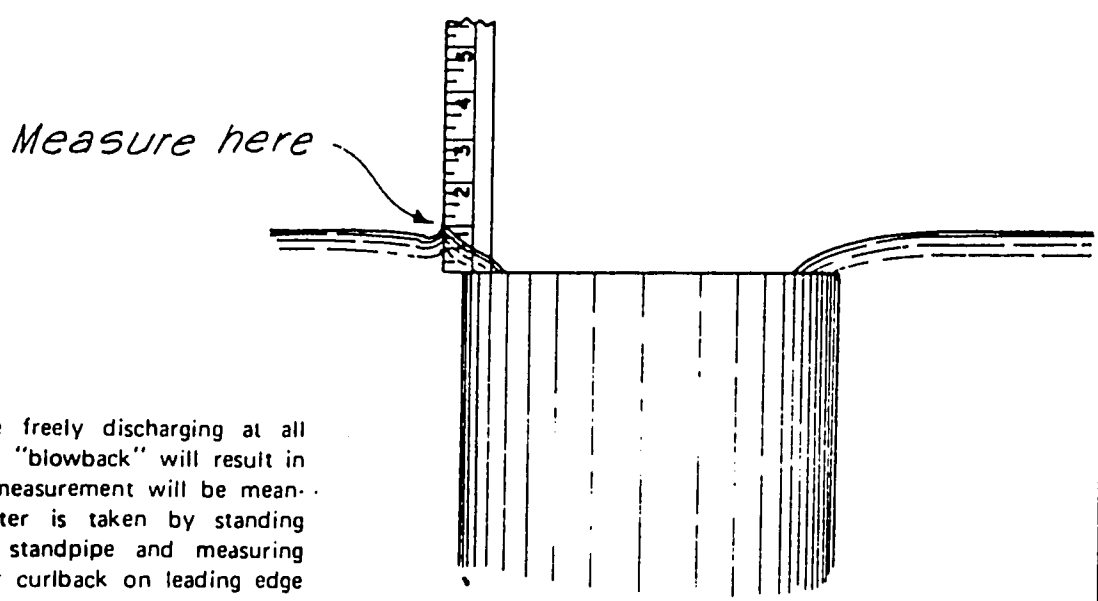
Table 5

### WATER DISCHARGE DOWN DRAIN SUMPS

12" diam = 3.14' crest
10" diam = 2.62' crest
8" diam = 2.09' crest
6" diam = 1.57' crest

Flow in gallons per minute

| Depth on Crest *(inches)* | *12" Sump* | *10" Sump* | *8" Sump* | *6" Sump* |
|---|---|---|---|---|
| 1/2 | 42 | 35 | 28 | 21 |
| 3/4 | 70 | 59 | 47 | 35 |
| 1 | 113 | 94 | 75 | 57 |
| 1/8 | 141 | 118 | 94 | 70 |
| 1/4 | 155 | 129 | 103 | 78 |
| 3/8 | 183 | 153 | 122 | 92 |
| 1/2 | 211 | 176 | 141 | 106 |
| 5/8 | 240 | 200 | 160 | 120 |
| 3/4 | 268 | 224 | 179 | 134 |
| 7/8 | 296 | 247 | 197 | 148 |
| 2 | 324 | 271 | 216 | 162 |
| 1/8 | 352 | 294 | 235 | 176 |
| 1/4 | 381 | 317 | 254 | 190 |
| 3/8 | 423 | 353 | 282 | 211 |
| 1/2 | 451 | 376 | 301 | 225 |
| 5/8 | 479 | 400 | 320 | 240 |
| 3/4 | 522 | 435 | 349 | 261 |
| 7/8 | 564 | 470 | 376 | 282 |
| 3 | 592 | 494 | 394 | 296 |




Note: Water must be freely discharging at all times down the sump; "blowback" will result in increased depth and measurement will be meaningless. Depth of water is taken by standing yardstick on lip of standpipe and measuring depth to top of water curlback on leading edge of yardstick.

TABLE 5

## MEASURING HYDROLOGIC CHARACTERISTICS (Continued)

**Measurement in Open Channels and Streams** – An approximation of fair accuracy of the flow in a channel or stream can be made with relatively little effort. You will need to measure off a length of the stream, preferable 100 ft; determine the average depth through this section (consider the entire length and width of the stream); and, determine the average width of this section of stream. You will then need to time the passage of a wood chip or float through the length of this section. The following formula is then used to calculate the volume of flow in cu ft/sec (cfs).

$$Q = \frac{WDal}{T}$$

Where: Q = volume of flow in cubic feet/sec (cfs)
W = average width of stream section in feet
D = average depth of stream section in feet
a = constant factor depending on type of stream bottom, use:
a · 0.9 for smooth sand, etc.
a = 0.8 for rough rocks, etc.
L = length of stream section measured
T = time in seconds for wood chip or float to travel the measured distance

Example: Suppose we find only a 50-foot length of stream that appears to have a uniform channel. By a series of measurements we determine the average depth to be 2 feet and the average width to be 15 feet. The bottom of the stream is mostly rocks and coarse gravel. We drop a wood chip at the upper end of the 50-foot section and find that it takes 65 seconds for the chip to reach the lower end of the section. Therefore:

W = 15 ft
D = 2 ft
a = 0.8
L = 50 ft
T = 65 secs

$$Q = \frac{15 \text{ ft} \times 2 \text{ ft} \times 0.8 \times 50 \text{ ft}}{65 \text{ secs}} = \frac{1200 \text{ cu ft}}{65 \text{ secs}}$$

= 18 cfs (approx)

**Measurement from Pond Refill Time** – The rate of water inflow to a pond may also be measured by determining the time necessary to refill a portion of the pond's volume. The method is valid providing no water leaves the pond during the refill period; if the pond leaks, you can't use this method. This method, however, may be the only one available to you if the water enters the pond through the upwelling chamber or through a flume or ditch at ground level.

It is necessary that the surface area of the pond be calculated. The pond is then drained down to a predetermined level below the top of the standpipe then allowed to refill. From the volume of water necessary to refill the discharged water, and the time necessary to do so, it is easy to calculate the rate of inflow.

Example: In a standard pond 80 ft long by 20 ft wide, the water is drained down 1 ft below the top of the standpipe. It takes 35 minutes to replace this top 1 ft of water.

Area of pond: 80 ft x 20 ft = 1,600 sq ft

Volume of top 1 ft: 1 ft x 1,600 sq ft = 1,600 cu ft; or, 7.48 gals/cu ft x 1,600 cu ft = 11,968 gals

Rate of inflow: 11,968 gals/35 mins = 342 gpm

ANALYTICAL METHOD

DISSOLVED OXYGEN

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 388-399.

# 421 OXYGEN (DISSOLVED)

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric method using membrane electrodes. The iodometric method[1] is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.[2] The choice of test procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

## 421 A. Iodometric Methods

### 1. Principle

Improved by variations in technic and equipment and aided by instrumentation, the iodometric test remains the most precise and reliable titrimetric procedure for DO analysis. The test is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions and acidification, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate.

The titration end point can be detected visually, with a starch indicator, or electrometrically, with potentiometric or dead-stop technics.[3] Experienced analysts can maintain a precision of ±50 μg/L with visual end-point detection and a precision of ±5 μg/L with electrometric end-point detection.[2,3]

The liberated iodine also can be determined directly by simple absorption spectrophotometers.[4] This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

### 2. Selection of Method

Before selecting a method consider the effect of interferences, oxidizing or reducing materials that may be present in the sample. Certain oxidizing agents liberate

DISSOLVED OXYGEN (continued)



iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Several modifications of the iodometric method are given to minimize the effect of interfering materials.[2] Among the more commonly used procedures are the azide modification,[5] the permanganate modification,[6] the alum flocculation modification,[7] and the copper sulfate-sulfamic acid flocculation modification.[8,9] The azide modification (B) effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (C) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85-87% phosphoric acid ($H_3PO_4$) instead of sulfuric acid ($H_2SO_4$) for acidification. This pro-

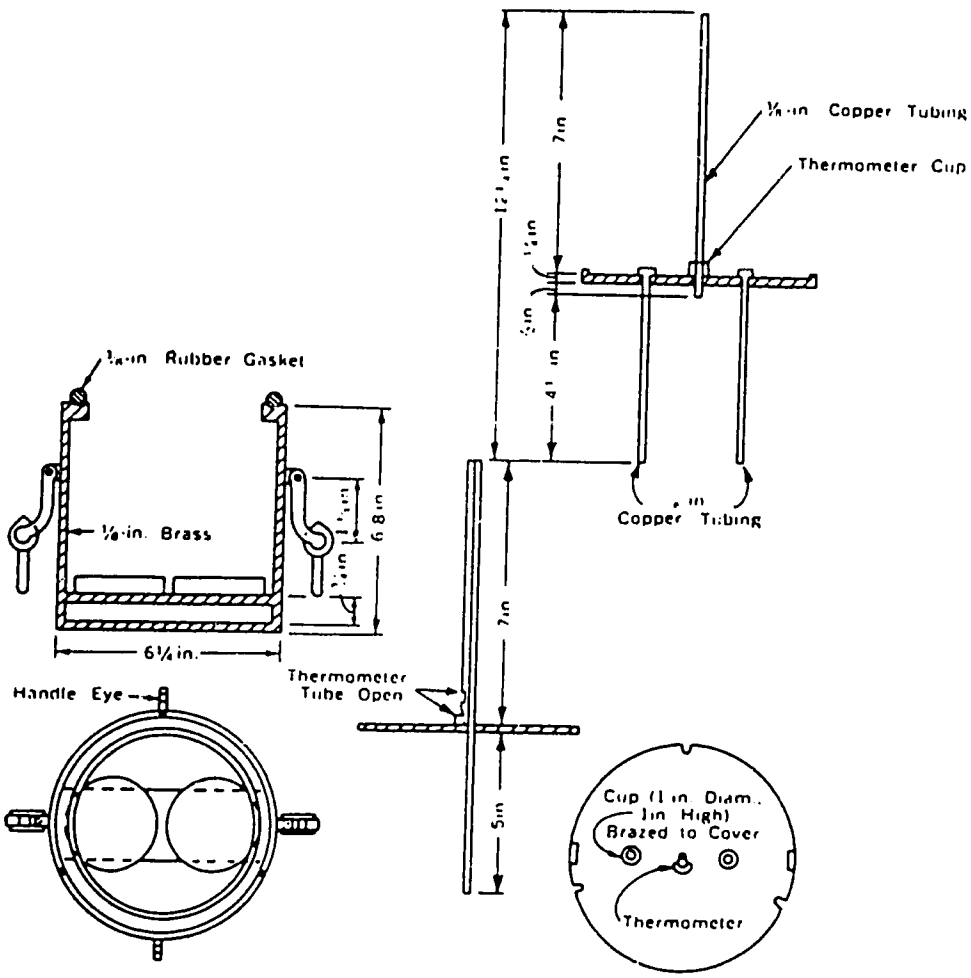



Figure 421:1. DO and BOD sampler assembly.

cedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (D) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (E) on activated-sludge mixed liquor.

### 3. Collection of Samples

Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams, rivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-I and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 421:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 sec), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

### 4. Preservation of Samples

Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after addition of manganous sulfate ($MnSO_4$) solution, alkali-iodide solution, and $H_2SO_4$, followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible.

For samples with an iodine demand, preserve for 4 to 8 hr by adding 0.7 mL conc $H_2SO_4$ and 1 mL sodium azide solution (2 g $NaN_3$/100 mL distilled water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20°C. As soon as possible, complete the procedure, using 2 mL $MnSO_4$ solution, 3 mL alkali-iodide solution, and 2 mL conc $H_2SO_4$.

## 421 B. Azide Modification

### 1. General Discussion

Use the azide modification for most sewage, effluent, and stream samples, especially if samples contain more than 50 µg $NO_2^-$-N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF so-

DISSOLVED OXYGEN (continued)



lution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

*a. Manganous sulfate solution:* Dissolve 480 g $MnSO_4 \cdot 4H_2O$, 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

*b. Alkali-iodide-azide reagent:* Dissolve 10 g $NaN_3$ in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate ($Na_2CO_3$), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

*c. Sulfuric acid,* $H_2SO_4$, conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

*d. Starch:* Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

*e. Standard sodium thiosulfate titrant:* Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water. Add 1.5 mL 6*N* NaOH or 0.4 g solid NaOH and dilute to 1,000 mL. Standardize with bi-iodate solution.

*f. Standard potassium bi-iodate solution, 0.0250N:* Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1,000 mL.

Standardization: Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6*N* $H_2SO_4$ or a few drops of conc $H_2SO_4$ and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.0250*N* $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.0250*N*.

*g. Potassium fluoride solution:* Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 mL.

3. Procedure

*a.* To the sample collected in a 250- to 300-mL bottle, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc $H_2SO_4$. Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of $MnSO_4$ and alkali-iodide-azide reagents in a 300-mL bottle, titrate 200 × 300/(300-2) = 201 mL.

*b.* Titrate with 0.0250*N* $Na_2S_2O_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0250*N* bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

*a.* For titration of 200 mL sample, 1 mL 0.0250*N* $Na_2S_2O_3$ = 1 mg DO/L.

DISSOLVED OXYGEN (continued)



TABLE 421:1. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR*

| Temperature C | Chloride Concentration in Water mg/L 0 | 5,000 | 10,000 | 15,000 | 20,000 |
|---|---|---|---|---|---|
| 0 | 14.60 | 13.72 | 12.90 | 12.13 | 11.41 |
| 1 | 14.19 | 13.35 | 12.56 | 11.81 | 11.11 |
| 2 | 13.81 | 12.99 | 12.23 | 11.51 | 10.83 |
| 3 | 13.44 | 12.65 | 11.91 | 11.22 | 10.56 |
| 4 | 13.09 | 12.33 | 11.61 | 10.94 | 10.30 |
| 5 | 12.75 | 12.02 | 11.32 | 10.67 | 10.05 |
| 6 | 12.43 | 11.72 | 11.05 | 10.41 | 9.82 |
| 7 | 12.12 | 11.43 | 10.78 | 10.17 | 9.59 |
| 8 | 11.83 | 11.16 | 10.53 | 9.93 | 9.37 |
| 9 | 11.55 | 10.90 | 10.29 | 9.71 | 9.16 |
| 10 | 11.27 | 10.65 | 10.05 | 9.49 | 8.96 |
| 11 | 11.01 | 10.40 | 9.83 | 9.28 | 8.77 |
| 12 | 10.76 | 10.17 | 9.61 | 9.08 | 8.58 |
| 13 | 10.52 | 9.95 | 9.41 | 8.89 | 8.41 |
| 14 | 10.29 | 9.73 | 9.21 | 8.71 | 8.24 |
| 15 | 10.07 | 9.53 | 9.01 | 8.53 | 8.07 |
| 16 | 9.85 | 9.33 | 8.83 | 8.36 | 7.91 |
| 17 | 9.65 | 9.14 | 8.65 | 8.19 | 7.78 |
| 18 | 9.45 | 8.95 | 8.48 | 8.03 | 7.61 |
| 19 | 9.26 | 8.77 | 8.32 | 7.88 | 7.47 |
| 20 | 9.07 | 8.60 | 8.16 | 7.73 | 7.33 |
| 21 | 8.90 | 8.44 | 8.00 | 7.59 | 7.20 |
| 22 | 8.72 | 8.28 | 7.85 | 7.45 | 7.07 |
| 23 | 8.56 | 8.12 | 7.71 | 7.32 | 6.95 |
| 24 | 8.40 | 7.97 | 7.57 | 7.19 | 6.83 |
| 25 | 8.24 | 7.83 | 7.44 | 7.06 | 6.71 |
| 26 | 8.09 | 7.69 | 7.31 | 6.94 | 6.60 |
| 27 | 7.95 | 7.55 | 7.18 | 6.83 | 6.49 |
| 28 | 7.81 | 7.42 | 7.06 | 6.71 | 6.38 |
| 29 | 7.67 | 7.30 | 6.94 | 6.60 | 6.28 |
| 30 | 7.54 | 7.17 | 6.83 | 6.49 | 6.18 |
| 31 | 7.41 | 7.05 | 6.71 | 6.39 | 6.08 |
| 32 | 7.28 | 6.94 | 6.61 | 6.29 | 5.99 |
| 33 | 7.16 | 6.82 | 6.50 | 6.19 | 5.90 |
| 34 | 7.05 | 6.71 | 6.40 | 6.10 | 5.81 |
| 35 | 6.93 | 6.61 | 6.30 | 6.01 | 5.72 |
| 36 | 6.82 | 6.51 | 6.20 | 5.92 | 5.64 |

* At a total pressure of 101.3 kPa. Under any other barometric pressure, $P$, obtain the solubility, $S$ (mg/L) from the corresponding value in the table by the equation:

$$S' = S \cdot \frac{P-p}{760-p}$$

in which $S$ is the solubility at 101.3 kPa and $p$ is the pressure (mm) of saturated water vapor at the water temperature. For elevations less than 1,000 m and temperatures below 25 C, ignore $p$. The equation then becomes:

$$S' = S \cdot \frac{P}{760} = S \cdot \frac{P}{29.92}$$

Dry air is assumed to contain 20.90% oxygen. (Calculations made by Whipple and Whipple, 1911. *J. Amer. Chem. Soc.* 33:362.)

DISSOLVED OXYGEN (continued)



TABLE 421:1. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR[a]

| Temperature C | Chloride Concentration in Water mg/L | | | | |
|---|---|---|---|---|---|
| | 0 | 5,000 | 10,000 | 15,000 | 20,000 |
| 37 | 6.71 | 6.40 | 6.11 | 5.83 | 5.56 |
| 38 | 6.61 | 6.31 | 6.02 | 5.74 | 5.48 |
| 39 | 6.51 | 6.21 | 5.93 | 5.66 | 5.40 |
| 40 | 6.41 | 6.12 | 5.84 | 5.58 | 5.33 |
| 41 | 6.31 | 6.03 | 5.76 | 5.50 | 5.25 |
| 42 | 6.22 | 5.94 | 5.68 | 5.42 | 5.18 |
| 43 | 6.13 | 5.85 | 5.60 | 5.35 | 5.11 |
| 44 | 6.04 | 5.77 | 5.52 | 5.27 | 5.04 |
| 45 | 5.95 | 5.69 | 5.44 | 5.20 | 4.98 |
| 46 | 5.86 | 5.61 | 5.37 | 5.13 | 4.91 |
| 47 | 5.78 | 5.53 | 5.29 | 5.06 | 4.85 |
| 48 | 5.70 | 5.45 | 5.22 | 5.00 | 4.78 |
| 49 | 5.62 | 5.38 | 5.15 | 4.93 | 4.72 |
| 50 | 5.54 | 5.31 | 5.08 | 4.87 | 4.66 |

*b*. To obtain results in milliliters oxygen gas per liter, corrected to 0 C and 101.3 kPa, multiply mg DO/L by 0.70.

*c*. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 421:1. Equations for correcting solubilities to barometric pressures other than mean sea level are given below the table.

5. Precision and Accuracy

DO can be determined with a precision, expressed as a standard deviation, of about 20 μg/L in distilled water and about 60 μg/L in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 μg/L. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

## 421 C. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the al-

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DISSOLVED OXYGEN (continued)



kali-hypochlorite modification.[10] At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

### 2 Reagents

All the reagents required for Method B, and in addition:

*a. Potassium permanganate solution:* Dissolve 6.3 g $KMnO_4$ in distilled water and dilute to 1 L.

*b. Potassium oxalate solution:* Dissolve 2 g $K_2C_2O_4 \cdot H_2O$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.

### 3. Procedure

*a.* To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc $H_2SO_4$, 1 mL $KMnO_4$ solution, and 1 mL KF solution. Stopper and mix by inversion. Never add more than 0.7 mL conc $H_2SO_4$ as the first step of pretreatment. Add acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient $KMnO_4$ solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional $KMnO_4$ solution, but avoid large excesses.

*b.* Remove permanganate color completely by adding 0.5 to 1.0 mL $K_2C_2O_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only an amount of $K_2C_2O_4$ that completely decolorizes the $KMnO_4$ without having an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

*c.* From this point the procedure closely parallels that in Section 421B.3. Add 1 mL $MnSO_4$ solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a short time; acidify with 2 mL conc $H_2SO_4$. When 0.7 mL acid, 1 mL $KMnO_4$ solution, 1 mL $K_2C_2O_4$ solution, 1 mL $MnSO_4$ solution, and 3 mL alkali-iodide-azide (or a total of 6.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300/(300 - 6.7) = 205$ mL for titration.

This correction is slightly in error because the $KMnO_4$ solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more $KMnO_4$ solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

## 421 D. Alum Flocculation Modification

### 1. General Discussion

Samples high in suspended solids may consume appreciable quantities of iodine in acid solution. The interference due to solids may be removed by alum flocculation.

### 2. Reagents

All the reagents required for the azide modification (Section 421B.2) and in addition:

*a. Alum solution:* Dissolve 10 g aluminum potassium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$,

in distilled water and dilute to 100 mL.

*b. Ammonium hydroxide*, $NH_4OH$, conc.

### 3. Procedure

Collect sample in a glass-stoppered bottle of 500 to 1,000 mL capacity, using the same precautions as for regular DO samples. Add 10 mL alum solution and 1 to 2 mL conc $NH_4OH$. Stopper and invert gently for about 1 min. Let sample settle for about 10 min and siphon clear supernate into a 250- to 300-mL DO bottle until it overflows. Avoid sample aeration and keep siphon submerged at all times. Continue sample treatment as in Section 421B.3 or an appropriate modification.

## 421 E. Copper Sulfate-Sulfamic Acid Flocculation Modification

### 1. General Discussion

This modification is used for biological flocs such as activated sludge mixtures, which have high oxygen utilization rates.

### 2. Reagents

All the reagents required for the azide modification (Section 421B.2) and, in addition:

*Copper sulfate-sulfamic acid inhibitor solution:* Dissolve 32 g technical-grade $NH_2SO_3OH$ without heat in 475 mL distilled water. Dissolve 50 g $CuSO_4 \cdot 5H_2O$ in 500 mL distilled water. Mix the two solutions and add 25 mL conc acetic acid.

### 3. Procedure

Add 10 mL $CuSO_4$-$NH_2SO_3OH$ inhibitor to a 1-L glass-stoppered bottle. Insert bottle in a special sampler designed so that bottle fills from a tube near bottom and overflows only 25 to 50% of bottle capacity. Collect sample, stopper, and mix by inverting. Let suspended solids settle and siphon relatively clear supernatant liquor into a 250- to 300-mL DO bottle. Continue sample treatment as rapidly as possible by the azide (Section 421B.3) or other appropriate modification.

## 421 F. Membrane Electrode Method

### 1. General Discussion

Various modifications of the iodometric method have been developed to eliminate or minimize effects of interferences; nevertheless, the method still is inapplicable to a variety of industrial and domestic wastewaters.[11] Moreover, the iodometric method is not suited for field testing and cannot be adapted easily for continuous monitoring or for DO determinations in situ.

Polarographic methods using the dropping mercury electrode or the rotating platinum electrode have not been reliable always for the DO analysis in domestic and industrial wastewaters because impurities in the test solution can cause electrode poisoning or other interferences.[12,13] With membrane-covered electrode systems these problems are minimized, because the sensing element is protected by an oxygen-permeable plastic membrane that serves as a diffusion barrier against

impurities.[14-16] Under steady-state conditions the current is directly proportional to the DO concentration.*

Membrane electrodes of the polarographic[14] as well as the galvanic[15] type have been used for DO measurements in lakes and reservoirs,[17] for stream survey and control of industrial effluents,[18,19] for continuous monitoring of DO in activated sludge units,[20] and for estuarine and oceanographic studies.[21] Being completely submersible, membrane electrodes are suited for analysis in situ. Their portability and ease of operation and maintenance make them particularly convenient for field applications. In laboratory investigations, membrane electrodes have been used for continuous DO analysis in bacterial cultures, including the BOD test.[15,22]

Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. They are recommended for use especially under conditions that are unfavorable for use of the iodometric method, or when that test and its modifications are subject to serious errors caused by interferences.

*a. Principle:* Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are used commonly because they are permeable to molecular oxygen and are relatively rugged.

Membrane electrodes are commercially available in some variety. In all these instruments the "diffusion current" is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by a number of calibration procedures.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability.[16] The effect of temperature on the electrode sensitivity, $\phi$ (microamperes per milligram per liter), can be expressed by the following simplified relationship:[16]

$$\log \phi = 0.43\, mt + b$$

where:

$t$ = temperature, degrees C,
$m$ = constant that depends on the membrane material, and
$b$ = constant that largely depends on membrane thickness.

If values of $\phi$ and $m$ are determined for one temperature ($\phi_0$ and $t_0$), it is possible to calculate the sensitivity at any desired temperature ($\phi$ and $t$) as follows:

$$\log \phi = \log \phi_0 + 0.43\, m\, (t - t_0)$$

Nomographic charts for temperature correction can be constructed easily[2] and are available from some manufacturers. An example is shown in Figure 421:2, in which, for simplicity, sensitivity is plotted versus temperature in degrees Celsius on semilogarithmic coordinates. Check one or two points frequently to confirm original calibration. If calibration changes, the new calibration should be parallel to the original, provided that the same membrane material is used.

Temperature compensation also can be made automatically by using thermistors in the electrode circuit.[14] However, thermistors may not compensate fully over a wide temperature range. For certain applications where high accuracy is required,

*Fundamentally the current is directly proportional to the activity of molecular oxygen.[2]

DISSOLVED OXYGEN (continued)



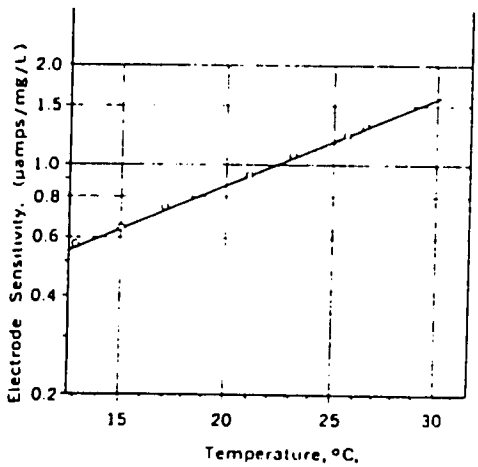


**Figure 421:2. Effect of temperature on electrode sensitivity.**

use calibrated nomographic charts to correct for temperature effect.

To use the DO membrane electrode in estuarine waters or in wastewaters with varying ionic strength, correct for effect of salting-out on electrode sensitivity.[2,16] This effect is particularly significant for large changes in salt content. Electrode sensitivity varies with salt concentration according to the following relationship:

$$\log \phi_s = 0.43\, m_s C_s + \log \phi_0$$

where:

$\phi_s, \phi_0$ = sensitivities in salt solution and distilled water, respectively,
$C_s$ = salt concentration (preferably ionic strength), and
$m_s$ = constant (salting-out coefficient).

If $\phi_0$ and $m_s$ are determined, it is possible to calculate sensitivity for any value of $C_s$. Conductivity measurements can be used to approximate salt concentration ($C_s$). This is particularly applicable to estuarine waters. Figure 421:3 shows calibration curves for sensitivity of varying salt solutions at different temperatures.

*b. Interference:* Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide ($H_2S$) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

*c. Sampling:* Because membrane electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, use the same precautions suggested for the iodometric method.

## 2. Apparatus

*Oxygen-sensitive membrane electrode,* polarographic or galvanic, with appropriate meter.

## 3. Procedure

*a. Calibration:* Follow manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy. Generally, calibrate membrane electrodes by reading against air or a sample of known DO concentration (determined by iodometric method) as well as in a sample with

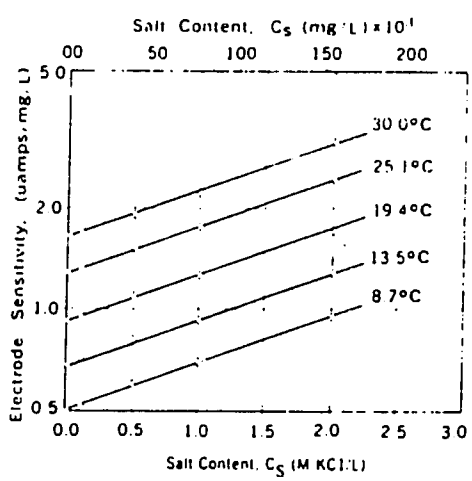


**Figure 421:3. The salting-out effect at different temperatures.**

DISSOLVED OXYGEN (continued)



zero DO. (Add excess sodium sulfite, $Na_2SO_3$, and a trace of cobalt chloride, $CoCl_2$, to bring DO to zero.) Preferably calibrate with samples of water under test. Avoid an iodometric calibration where interfering substances are suspected. The following illustrate the recommended procedures:

1) Fresh water—For unpolluted samples where interfering substances are absent, calibrate in the test solution or distilled water, whichever is more convenient.

2) Salt water—Calibrate directly with samples of seawater or waters having a constant salt concentration in excess of 1,000 mg/L.

3) Fresh water containing pollutants or interfering substances—Calibrate with distilled water because erroneous results occur with the sample.

4) Salt water containing pollutants or interfering substances—Calibrate with a sample of clean water containing the same salt content as the sample. Add a concentrated potassium chloride (KCl) solution (see Conductivity, Section 205 and Table 205:1) to distilled water to produce the same specific conductance as that in the sample. For polluted ocean waters, calibrate with a sample of unpolluted seawater.

5) Estuary water containing varying quantities of salt—Calibrate with a sample of uncontaminated seawater or distilled or tap water. Determine sample chloride or salt concentration and revise calibration to account for change of oxygen solubility in the estuary water.[2]

*b. Sample measurement:* Follow all precautions recommended by manufacturer to insure acceptable results. Take care in changing membrane to avoid contamination of sensing element and also trapping of minute air bubbles under the membrane, which can lead to lowered response and high residual current. Provide sufficient sample flow across membrane surface to overcome erratic response (see Figure 421:4 for a typical example of the effect of stirring).

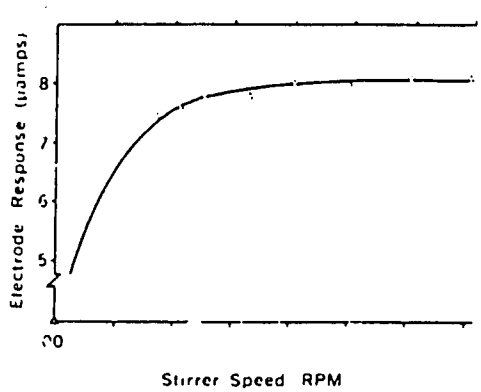


**Figure 421:4. Typical trend of effect of stirring on electrode response.**

*c. Validation of temperature effect:* Check frequently one or two points to verify temperature correction data.

4. Precision and Accuracy

With most commercially available membrane electrode systems an accuracy of ±0.1 mg DO/L and a precision of ±0.05 mg DO/L can be obtained.

## 421 G. References

1. WINKLER, L.W. 1888. The determination of dissolved oxygen in water. *Berlin. Deut. Chem. Ges.* 21:2843.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of dissolved oxygen in natural and waste waters. USPHS Publ. No. 999-WP-37. Washington, D.C.
3. POTTER, E.C. & G.E. EVERITT. 1957. Ad-

DISSOLVED OXYGEN (continued)



vances in dissolved oxygen microanalysis. *J. Appl. Chem.* 9:642.

4. OULMAN, C.S. & E.R. BAUMANN. 1956. A colorimetric method for determining dissolved oxygen. *Sewage Ind. Wastes* 28:1461.
5. ALSTERBERG, G. 1925. Methods for the determination of elementary oxygen dissolved in water in the presence of nitrite. *Biochem. Z.* 159:36.
6. RIDEAL, S. & G.G. STEWART. 1901. The determination of dissolved oxygen in waters in the presence of nitrites and of organic matter. *Analyst* 26:141.
7. RUCHHOFT, C.C. & W.A. MOORE. 1940. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem.* Anal. Ed. 12:711.
8. PLACAK, O.R. & C.C. RUCHHOFT. 1941. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem.*, Anal. Ed. 13:12.
9. RUCHHOFT, C.C. & O.R. PLACAK. 1942. Determination of dissolved oxygen in activated-sludge sewage mixtures. *Sewage Works J.* 14:638.
10. THERIAULT, E.J. & P.D. MCNAMEE. 1932. Dissolved oxygen in the presence of organic matter, hypochlorites, and sulfite wastes. *Ind. Eng. Chem.*, Anal. Ed. 4:59.
11. MCKEOWN, J.J., L.C. BROWN & G.W. GOVE. 1967. Comparative studies of dissolved oxygen analysis methods. *J. Water Pollut. Control Fed.* 39:1323.
12. LYNN, W.R. & D.A. OKUN. 1955. Experience with solid platinum electrodes in the determination of dissolved oxygen. *Sewage Ind. Wastes* 27:4.
13. MANCY, K.H. & D.A. OKUN. 1960. Automatic recording of dissolved oxygen in aqueous systems containing surface active agents. *Anal. Chem.* 32:108.
14. CARRITT, D.E. & J.W. KANWISHER. 1959. An electrode system for measuring dissolved oxygen. *Anal. Chem.* 31:5.
15. MANCY, K.H. & W.C. WESTGARTH. 1962. A galvanic cell oxygen analyzer. *J. Water Pollut. Control Fed.* 34:1037.
16. MANCY, K.H., D.A. OKUN & C.N. REILLEY. 1962. A galvanic cell oxygen analyzer. *J. Electroanal. Chem.* 4:65.
17. WEISS, C.M. & R.T. OGLESBY. 1963. Instrumentation for monitoring water quality in reservoirs. Amer. Water Works Ass. 83rd Annual Conf., New York, N.Y.
18. CLEARY, E.J. 1962. Introducing the OR-SANCO robot monitor. *Proc. Water Quality Meas. Instrum.* Publ. No. 108, USPHS, Washington, D.C.
19. MACKERETH, F.J.H. 1964. An improved galvanic cell for determination of oxygen concentrations in fluids. *J. Sci. Instrum.* 41:38.
20. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D.O. recording in activated sludge. *Water Sewage Works* 109:376.
21. DUXBURY, A.C. 1963. Calibration and use of a galvanic type oxygen electrode in field work. *Limnol. Oceanogr.* 8:483.
22. LIPNER, H.J., L.R. WITHERSPOON & V.C. CHAMPEAUS. 1964. Adaptation of a galvanic cell for microanalysis of oxygen. *Anal. Chem.* 36:204.
23. POSTMA, H. & A. SYANSSON, H. LABOMBE & K. GRASSHOFF. 1976. Letter to the Editors—The International Oceanographic Tables for the Solubility of Oxygen in Sea Water. *J. Cons. Int. Explor. Mer.* 36:295.

ANALYTICAL METHOD

ALKALINITY

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 253-257.

# 403 ALKALINITY

Alkalinity of a water is its quantitative capacity to react with a strong acid to a designated pH. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constitutents. The measured values may include contributions from borates, phosphates, or silicates if these are present. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of a water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than or only slightly greater than that of the water supply. Properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2,000 to 4,000 mg calcium carbonate ($CaCO_3$)/L.[1]

## 1. General Discussion

*a. Principle:* Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Section 402.1*a*.

For samples of low alkalinity (less than 20 mg $CaCO_3$/L) use an extrapolation technic based on the near proportionality of concentration of hydrogen ions to excess of titrant beyond the equivalence point. The amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a

ALKALINITY (continued)



simple extrapolation can be made to the equivalence point.[2,4]

*b. End points:* When alkalinity is due entirely to hydroxide, carbonate, or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide ($CO_2$) at that stage. $CO_2$ concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The following pH values are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams $CaCO_3$ per liter:

| | End point pH | |
|---|---|---|
| | Total | Phenolphthalein |
| Alkalinity, mg $CaCO_3$/L: | | |
| 30 | 5.1 | 8.3 |
| 150 | 4.8 | 8.3 |
| 500 | 4.5 | 8.3 |
| Silicates, phosphates known or suspected | 4.5 | 8.3 |
| Routine or automated analyses | 4.5 | 8.3 |
| Industrial waste or complex system | 3.7 | 8.3 |

*c. Interferences:* Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium. Do not filter, dilute, concentrate, or alter sample.

*d. Selection of method:* Determine sample alkalinity from volume of standard acid required to titrate a portion to a designated pH taken from ¶ 1*b*. Titrate at room temperature with a properly calibrated pH meter or electrically operated titrator, or use color indicators.

Report alkalinity less than 20 mg $CaCO_3$/L only if it has been determined by the low-alkalinity method of ¶ 4*d*.

Construct a titration curve for standardization of reagents.

Color indicators may be used for routine and control titrations in the absence of interfering color and turbidity and for preliminary titrations to select sample size and strength of titrant (see below).

*e. Sample size:* See Section 402.1*e* for selection of size sample to be titrated and normality of titrant, substituting 0.02 *N* or 0.1*N* sulfuric ($H_2SO_4$) or hydrochloric (HCl) acid for the standard alkali of that method. For the low-alkalinity method, titrate a 200-mL sample with 0.02*N* $H_2SO_4$ from a 10-mL buret.

*f. Sampling and storage:* See Section 402.1*f*.

2. Apparatus

See Section 402.2.

3. Reagents

*a. Sodium carbonate solution,* approximately 0.05*N*: Dry 3 to 5 g primary standard $Na_2CO_3$ at 250 C for 4 hr and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, and dissolve and mix reagent. Do not keep longer than 1 wk.

*b. Standard sulfuric acid or hydrochloric acid,* 0.1 *N*: Dilute 3.0 mL conc $H_2SO_4$ or 8.3 mL conc HCl to 1 L with distilled or deionized water. Standardize against 40.00 mL 0.05 *N* $Na_2CO_3$ solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to the pH inflection point. Calculate normality:

$$\text{Normality, } N = \frac{A \times B}{53.00 \times C}$$

where:

$A$ = g $Na_2CO_3$ weighed into 1 L flask,

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ALKALINITY (continued)



$B$ = mL $Na_2CO_3$ solution taken for titration, and
$C$ = mL acid used.

Use measured normality in calculations or adjust to 0.1000$N$; 1 mL 0.1000 $N$ solution = 5.00 mg $CaCO_3$.

*c. Standard sulfuric acid or hydrochloric acid, 0.02N:* Dilute 200.00 mL 0.1000$N$ standard acid to 1,000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05$N$ $Na_2CO_3$ according to the procedure of ¶ 3*b*; 1 mL = 1.00 mg $CaCO_3$.

*d. Mixed bromcresol green-methyl red indicator solution:* Use either the aqueous or the alcoholic solution:

1) Dissolve 100 mg bromcresol green sodium salt and 20 mg methyl red sodium salt in 100 mL distilled water.

2) Dissolve 100 mg bromcresol green and 20 mg methyl red in 100 mL 95% ethyl alcohol or isopropyl alcohol.

*e. Methyl orange solution.*

*f. Phenolphthalein solution, alcoholic.*

*g. Sodium thiosulfate, 0.1N.* See Section 402.3*b*.

4. Procedure

*a. Color change:* See Section 402.4*a*. The color response of the mixed bromcresol green-methyl red indicator is approximately as follows: above pH 5.2, greenish blue; pH 5.0, light blue with lavender gray; pH 4.8, light pink-gray with bluish cast; and pH 4.6, light pink. Check color changes against reading of a pH meter under the conditions of the titration. Because colors are difficult to distinguish, the method is subject to relatively large operator error.

*b. Potentiometric titration curve:* Follow the procedure for determining acidity (Section 402.4*b*), substituting the appropriate normality of standard acid solution for standard NaOH, and continue titration to pH 3.7 or lower. Do not filter, dilute, concentrate, or alter the sample.

*c. Potentiometric titration to preselected pH:* Determine the appropriate end-point pH according to ¶ 1*b*. Prepare sample and titration assembly (Section 402.4*b*). Titrate to the end-point pH without recording intermediate pH values and without undue delay. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

*d. Potentiometric titration of low alkalinity:* For alkalinities less than 20 mg/L titrate 100 to 200 mL according to the procedure of ¶ 4*c*, above, using a 10-mL microburet and 0.02$N$ standard acid solution. Stop the titration at a pH in the range 4.3 to 4.7 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume.

5. Calculations

*a. Potentiometric titration to end-point pH:*

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{\text{mL sample}}$$

where:
$A$ = mL standard acid used and
$N$ = normality of standard acid

or

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times t \times 1{,}000}{\text{mL sample}}$$

where:
$t$ = titer of standard acid, mg $CaCO_3$/mL.

Report pH of end point used as follows: "The alkalinity to pH _____ = _____ mg $CaCO_3$/L" and indicate clearly if this pH corresponds to an inflection point of the titration curve.

*b. Potentiometric titration of low alkalinity:*

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ALKALINITY (continued)



Total alkalinity, mg $CaCO_3$/L

$$= \frac{(2B - C) \times N \times 50{,}000}{\text{mL sample}}$$

where:

$B$ = mL titrant to first recorded pH,
$C$ = total mL titrant to reach pH 0.3 unit lower, and
$N$ = normality of acid.

*c. Calculation of alkalinity relationships:* The results obtained from the phenolphthalein and total alkalinity determinations offer a means for stoichiometric classification of the three principal forms of alkalinity present in many waters. The classification ascribes the entire alkalinity to bicarbonate, carbonate, and hydroxide, and assumes the absence of other (weak) inorganic or organic acids, such as silicic, phosphoric, and boric acids. It further presupposes the incompatibility of hydroxide and bicarbonate alkalinities. Because the calculations are made on a stoichiometric basis, ion concentrations in the strictest sense are not represented in the results, which may differ significantly from actual concentrations especially at pH >10. According to this scheme:

1) Carbonate ($CO_3^{2-}$) alkalinity is present when phenolphthalein alkalinity is not zero but is less than total alkalinity.

2) Hydroxide ($OH^-$) alkalinity is present if phenolphthalein alkalinity is more than half the total alkalinity.

3) Bicarbonate ($HCO_3^-$) ions are present if phenolphthalein alkalinity is less than half the total alkalinity. These relationships may be calculated by the following scheme, where $P$ is phenolphthalein alkalinity and $T$ is total alkalinity (¶ 1*b*):

Select the smaller value of $P$ or $(T\text{-}P)$. Then, carbonate alkalinity equals twice the smaller value. When the smaller value is $P$, the balance $(T\text{-}2P)$ is bicarbonate. When the smaller value is $(T\text{-}P)$, the balance $(2P\text{-}T)$ is hydroxide. All results are expressed as $CaCO_3$. The mathematical conversion of the results is shown in Table 403:I.

TABLE 403:I. ALKALINITY RELATIONSHIPS*

| Result of Titration | Hydroxide Alkalinity as $CaCO_3$ | Carbonate Alkalinity as $CaCO_3$ | Bicarbonate Concentration as $CaCO_3$ |
|---|---|---|---|
| P = 0 | 0 | 0 | T |
| P < ½T | 0 | 2P | T - 2P |
| P = ½T | 0 | 2P | 0 |
| P > ½T | 2P - T | 2(T - P) | 0 |
| P = T | T | 0 | 0 |

*Key: P—phenolphthalein alkalinity; T—total alkalinity

Alkalinity relationships also may be computed nomographically (see Carbon Dioxide, Section 406). Accurately measure pH, calculate $OH^-$ concentration as milligrams $CaCO_3$ per liter, and calculate concentrations of $CO_3^{2-}$ and $HCO_3^-$ as milligrams $CaCO_3$ per liter from the $OH^-$ concentration, and the phenolphthalein and total alkalinities by the following equations:

$$CO_3^{2-} = 2P - 2[OH^-]$$

$$HCO_3^- = T - 2P + [OH^-]$$

Similarly, if difficulty is experienced with the phenolphthalein end point, or if a check on the phenolphthalein titration is desired, calculate phenolphthalein alkalinity as $CaCO_3$ from the results of the nomographic determinations of carbonate and hydroxide ion concentrations:

$$P = 1/2\,[CO_3^{2-}] + [OH^-]$$

## 6. Precision and Accuracy

No general statement can be made about precision because of the great variation in sample characteristics. The precision of the titration is likely to be much greater than the uncertainties involved in

ALKALINITY (continued)



sampling and sample handling before the analysis.

In the range of 10 to 500 mg/L, when the alkalinity is due entirely to carbonates or bicarbonates, a standard deviation of 1 mg $CaCO_3$/L can be achieved. Forty analysts in 17 laboratories analyzed synthetic samples containing increments of bicarbonate equivalent to 120 mg $CaCO_3$/L. The titration procedure of ¶ 4*b* was used, with an end point pH of 4.5. The standard deviation was 5 mg/L and the average bias (lower than the true value) was 9 mg/L.[4]

## 7. References


1. Pohland, F.G. & D.E. Bloodgood. 1963. Laboratory studies on mesophilic and thermophilic anaerobic sludge digestion. *J. Water Pollut. Control Fed.* 35:11.
2. Larson, T.E. & L.M. Henley. 1955. Determination of low alkalinity or acidity in water. *Anal. Chem.* 27:851.
3. Thomas, J.F.J. & J.J. Lynch. 1960. Determination of carbonate alkalinity in natural waters. *J. Amer. Water Works Ass.* 52:259
4. Winter, J.A. & M.R. Midgett. 1969. FWPCA Method Study 1. Mineral and Physical Analyses. FWPCA, Washington, D.C.


## 8. Bibliography


American Society for Testing & Materials. 1970. Standard Methods for Acidity or Alkalinity of Water. ASTM Publ. D1067-70, Philadelphia, Pa.

Brown, E., M.W. Skougstad & M.J. Fishman. 1970. Methods of collection and analysis of water sample for dissolved minerals and gases. Chapter A1 *in* Book 5, Techniques of Water-Resources Investigation of the United States Geological Survey. U.S. Geol. Surv., Washington, D.C.

ANALYTICAL METHOD

HARDNESS

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 194-199.

## 314 HARDNESS

Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present. Other polyvalent cations also may precipitate soap, but they often are in complex forms, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. In conformity with current practice, total hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter.

When hardness numerically is greater than the sum of carbonate and bicarbonate alkalinity, that amount of hardness equivalent to the total alkalinity is called "car-

HARDNESS (continued)



bonate hardness"; the amount of hardness in excess of this is called "noncarbonate hardness." When the hardness numerically is equal to or less than the sum of carbonate and bicarbonate alkalinity, all hardness is carbonate hardness and noncarbonate hardness is absent. The hardness may range from zero to hundreds of milligrams per liter in terms of calcium carbonate, depending on the source and treatment to which the water has been subjected.

1. Selection of Method

Two methods are presented for the determination of hardness. Method A, hardness by calculation, is applicable to all waters and yields the higher accuracy. If a mineral analysis is performed, hardness by calculation can be reported. Method B, the EDTA titration method, measures the calcium and magnesium ions and may be applied with appropriate modification to any kind of water. The procedure described affords a means of rapid analysis.

2. Reporting Results

When reporting hardness, state the method used, for example, "hardness (calc.)" or "hardness (EDTA)".

## 314 A. Hardness by Calculation

1. Discussion

The preferred method for determining hardness is to compute it from the results of separate determinations of calcium and magnesium.

2. Calculation

Hardness, mg equivalent $CaCO_3$/L
$= 2.497\,[Ca, mg/L] + 4.118\,[Mg, mg/L]$

## 314 B. EDTA Titrimetric Method

1. General Discussion

*a. Principle:* Ethylenediaminetetraacetic acid and its sodium salts (abbreviated EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0 ± 0.1, the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of the magnesium and calcium has been complexed the solution turns from wine red to blue, marking the end point of the titration. Magnesium ion must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer, this automatically introduces sufficient magnesium and obviates the need for a blank correction.

The sharpness of the end point increases with increasing pH. However, the pH cannot be increased indefinitely because of the danger of precipitating calcium carbonate, $CaCO_3$, or magnesium hydroxide, $Mg(OH)_2$, and because the dye changes color at high pH values. The specified pH of 10.0 ± 0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of

the titration to minimize the tendency toward $CaCO_3$ precipitation.

*b. Interference:* Some metal ions interfere by causing fading or indistinct end points or by stoichiometric consumption of EDTA. Reduce this interference by adding certain inhibitors before titration. Adding MgCDTA [see 2*b*3)], which is *not* an inhibitor, permits titrating all polyvalent cations listed in Table 314:1 but yields erroneously high hardness values in proportion to the concentration of such cations. Because it is nontoxic it is the complexing agent of choice if the interferences are known to be so low that no significant addition to the hardness will result from their titration. When the indicated cations are present in significant concentrations, use the inhibitors listed in Table 314:1. The figures in Table 314:1 are intended as a rough guide only and are based on using a 25-mL sample diluted to 50 mL.

TABLE 314:1. MAXIMUM CONCENTRATIONS OF INTERFERENCES PERMISSIBLE WITH VARIOUS INHIBITORS*

| Interfering Substance | Max. Interference Concentration *mg/L* | |
|---|---|---|
| | Inhibitor I | Inhibitor II |
| Aluminum | 20 | 20 |
| Barium | † | † |
| Cadmium | † | 20 |
| Cobalt | over 20 | 0.3 |
| Copper | over 30 | 20 |
| Iron | over 30 | 5 |
| Lead | † | 20 |
| Manganese ($Mn^{2+}$) | † | 1 |
| Nickel | over 20 | 0.3 |
| Strontium | † | † |
| Zinc | † | 200 |
| Polyphosphate | | 10 |

*Based on 25-mL sample diluted to 50 mL.
†Titrates as hardness.

Suspended or colloidal organic matter also may interfere with the end point. Eliminate this interference by evaporating the sample to dryness on a steam bath and heating in a muffle furnace at 550 C until the organic matter is completely oxidized. Dissolve the residue in 20 mL *1N* hydrochloric acid (HCl), neutralize to pH 7 with *1N* sodium hydroxide (NaOH), and make up to 50 mL with distilled water; cool to room temperature and continue according to the general procedure.

*c. Titration precautions:* Conduct titrations at or near normal room temperature. The color change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition becomes a problem in hot water.

The specified pH may produce an environment conducive to $CaCO_3$ precipitation. Although the titrant slowly redissolves such precipitates, a drifting end point often yields low results. Completion of the titration within 5 min minimizes the tendency for $CaCO_3$ to precipitate. The following three methods also reduce precipitation loss:

1) Dilute the sample with distilled water to reduce the $CaCO_3$ concentration. This simple expedient has been incorporated in the procedure. If precipitation occurs at this dilution of 1 + 1 use modification 2) or 3). Using too small a sample contributes a systematic error due to the buret-reading error.

2) If the approximate hardness is known or is determined by a preliminary titration, add 90% or more of titrant to sample *before* adjusting the pH with buffer.

3) Acidify the sample and stir for 2 min to expel $CO_2$ *before* pH adjustment. Determine alkalinity to indicate the amount of acid to be added.

HARDNESS (continued)



2. Reagents

*a. Buffer solution:*

1) Dissolve 16.9 g ammonium chloride ($NH_4Cl$) in 143 mL conc ammonium hydroxide ($NH_4OH$). Add 1.25 g magnesium salt of EDTA (available commercially) and dilute to 250 mL with distilled water.

2) If the magnesium salt of EDTA is unavailable, dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (analytical reagent grade) and 780 mg magnesium sulfate ($MgSO_4 \cdot 7H_2O$) or 644 mg magnesium chloride ($MgCl_2 \cdot 6H_2O$) in 50 mL distilled water. Add this solution to 16.9 g $NH_4Cl$ and 143 mL conc $NH_4OH$ with mixing and dilute to 250 mL with distilled water. To attain the highest accuracy, adjust to exact equivalence through appropriate addition of a small amount of EDTA or $MgSO_4$ or $MgCl_2$.

Store Solution 1) or 2) in a plastic or resistant-glass container for no longer than 1 month. Stopper tightly to prevent loss of ammonia ($NH_3$) or pickup of carbon dioxide ($CO_2$). Dispense buffer solution by means of a bulb-operated pipet. Discard buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ± 0.1 at the titration end point.

3) Satisfactory alternate "odorless buffers" also are available commercially. They contain the magnesium salt of EDTA and have the advantage of being relatively odorless and more stable than the $NH_4Cl$-$NH_4OH$ buffer. They usually do not provide as good an endpoint as $NH_4Cl$-$NH_4OH$ because of slower reactions and they may be unsuitable when this method is automated. Prepare one of these buffers by mixing 55 mL conc HCl with 400 mL distilled water and then, slowly and with stirring, adding 300 mL 2-aminoethanol (free of aluminum and heavier metals). Add 5.0 g magnesium salt of EDTA and dilute to 1 L with distilled water.

*b. Complexing agents:* For most waters no complexing agent is needed. Occasionally water containing interfering ions requires the addition of an appropriate complexing agent to give a clear, sharp change in color at the end point. The following are satisfactory:

1) *Inhibitor I:* Adjust acid samples to pH 6 or higher with buffer or 0.1*N* NaOH. Add 250 mg sodium cyanide (NaCN) in powder form to the sample. Add sufficient buffer to adjust to pH 10.0 ± 0.1 (CAUTION: *NaCN is extremely poisonous. Take extra precautions in its use.* Flush solutions containing this inhibitor down the drain with large quantities of water after insuring that no acid is present to liberate volatile poisonous hydrogen cyanide.)

2) *Inhibitor II:* Dissolve 5.0 g sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) or 3.7 g $Na_2S \cdot 5H_2O$ in 100 mL distilled water. Exclude air with a tightly fitting rubber stopper. This inhibitor deteriorates through air oxidation. It produces a sulfide precipitate that obscures the end point when appreciable concentrations of heavy metals are present. Use 1 mL in ¶ 3*b* below.

3) *MgCDTA:* Magnesium salt of 1, 2-cyclohexanediaminetetraacetic acid. Add 250 mg per 100 mL sample and dissolve completely before adding buffer solution. Use this complexing agent to avoid using toxic or odorous inhibitors when interfering substances are present in concentrations that affect the end point but will not contribute significantly to the hardness value.

Commercial preparations incorporating a buffer and a complexing agent are available. Such mixtures must maintain pH 10.0 ± 0.1 during the titration and give a clear, sharp end point when the sample is titrated.

*c. Indicators:* Many types of indicator solutions have been advocated and may be used if the analyst demonstrates that they yield accurate values. The prime difficulty with indicator solutions is deterioration with aging, giving indistinct end points. For example, alkaline solutions of Erio-

chrome Black T are sensitive to oxidants and aqueous or alcoholic solutions are unstable. In general, use the least amount of indicator providing a sharp end point. It is the analyst's responsibility to determine individually the optimal indicator concentration.

1) *Eriochrome Black T:* Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid; No. 203 in the Color Index. Dissolve 0.5 g dye in 100 g 2,2',2"-nitrilotriethanol (also called triethanolamine) or 2-methoxymethanol (also called ethylene glycol monomethyl ether). Add 2 drops per 50 mL solution to be titrated. Adjust volume if necessary.

2) *Calmagite:* 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid. This is stable in aqueous solution and produces the same color change as Eriochrome Black T, with a sharper end point. Dissolve 0.10 g Calmagite in 100 mL distilled water. Use 1 mL per 50 mL solution to be titrated. Adjust volume if necessary.

3) Indicators 1 and 2 can be used in dry powder form if care is taken to avoid excess indicator. Prepared dry mixtures of these indicators and an inert salt are available commercially.

If the end point color change of these indicators is not clear and sharp, it usually means that an appropriate complexing agent is required. If NaCN inhibitor does not sharpen the end point, the indicator probably is at fault.

*d. Standard EDTA titrant, 0.01M:* Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate, also called (ethylenedinitrilo)-tetraacetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1,000 mL. Standardize against standard-calcium solution (¶ 2*e*) as described in ¶ 3*b* below.

Because the titrant extracts hardness-producing cations from soft-glass containers, store in polyethylene (preferable) or borosilicate glass bottles. Compensate for gradual deterioration by periodic restandardization and by using a suitable correction factor.

*e. Standard calcium solution:* Weigh 1.000 g anhydrous $CaCO_3$ powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL erlenmeyer flask. Place a funnel in the flask neck and add, a little at a time, 1 + 1 HCl until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel $CO_2$. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3*N* $NH_4OH$ or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1,000 mL with distilled water; 1 mL = 1.00 mg $CaCO_3$.

*f. Sodium hydroxide,* NaOH, 0.1 *N*.

## 3 Procedure

*a. Pretreatment of polluted water and wastewater samples:* Follow the procedure described in Section 302E or F.

*b. Titration of sample:* Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from the time of buffer addition.

Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point color change in the titration usually means that an inhibitor must be added at this point in the procedure (¶ 2*b* et seq.) or that the indicator has deteriorated.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation [¶ 2*c*-3)]. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears from the solution. Add the last few drops at 3- to 5-sec intervals. At the end point the solution normally is blue. Daylight or a daylight fluorescent lamp is highly recom-

mended because ordinary incandescent lights tend to produce a reddish tinge in the blue at the end point.

If sufficient sample is available and interference is absent, improve accuracy by increasing sample size, as described in ¶ 3*c* below.

*c. Low-hardness sample:* For ion-exchanger effluent or other softened water and for natural waters of low hardness (less than 5 mg/L), take a larger sample, 100 to 1,000 mL, for titration and add proportionately larger amounts of buffer, inhibitor, and indicator. Add standard EDTA titrant slowly from a microburet and run a blank, using redistilled, distilled, or deionized water of the same volume as the sample, to which identical amounts of buffer, inhibitor, and indicator have been added. Subtract volume of EDTA used for blank from volume of EDTA used for sample.

4. Calculation

Hardness (EDTA) as mg $CaCO_3$/L

$$= \frac{A \times B \times 1{,}000}{\text{mL sample}}$$

where:

$A$ = mL titration for sample and
$B$ = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant.

5. Precision and Accuracy

A synthetic sample containing 610 mg/L total hardness as $CaCO_3$ contributed by 108 mg Ca/L and 82 mg Mg/L, and the following supplementary substances: 3.1 mg K/L, 19.9 mg Na/L, 241 mg Cl/L, 0.25 mg $NO_2^-$-N/L, 1.1 mg $NO_3^-$-N/L, 259 mg sulfate/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 56 laboratories by the EDTA titrimetric method with a relative standard deviation of 2.9% and a relative error of 0.8%.

## 314 C. Bibliography


CONNORS, J.J. 1950. Advances in chemical and colorimetric methods. *J. Amer. Water Works Ass.* 42:33.

DIEHL, H., C.A. GOETZ & C.C. HACH. 1950. The versenate titration for total hardness. *J. Amer. Water Works Ass.* 42:40.

BETZ, J.D. & C.A. NOLL. 1950. Total hardness determination by direct colorimetric titration. *J. Amer. Water Works Ass.* 42:49

GOETZ, C.A., T.C. LOOMIS & H. DIEHL. 1950. Total hardness in water: The stability of standard disodium dihydrogen ethylenediaminetetraacetate solutions. *Anal. Chem.* 22:798.

DISKANT, E.M. 1952. Stable indicator solutions for complexometric determination of total hardness in water. *Anal. Chem.* 24:1856.

BARNARD, A.J., JR., W.C. BROAD & H. FLASCHKA. 1956 & 1957. The EDTA titration. *Chemist Analyst* 45:86 & 46:46.

GOETZ, C.A. & R.C. SMITH. 1959. Evaluation of various methods and reagents for total hardness and calcium hardness in water. *Iowa State J. Sci.* 34:81 (Aug. 15).

SCHWARZENBACH, G. & H. FLASCHKA. 1969. Complexometric Titrations. 2nd ed. Barnes & Noble, Inc., New York, N.Y.

ANALYTICAL METHOD

Sample Preservation, Nitrogen Forms

REFERENCE

Limnological Research Laboratory, Department of Fisheries and Wildlife, Michigan State University.

Nitrate-Nitrite-N:

Samples should be analyzed for Nitrate-N or Nitrite-N within 24 hrs of collection. During this time samples should be stored at 4°C. If a combined Nitrate-Nitrite-N determination is desired and analysis within 24 hrs is not possible, add sufficient sulfuric acid, $H_2SO_4$, to bring the pH to less than 2 and store at 4°C. Note that the addition of acid requires neutralization before the cadmium reduction procedure and that separate determinations of nitrate and nitrite are no longer meaningful. Appropriately cleaned glass or plastic storage containers are permitted (U.S.E.P.A. 1979). Do not use mercuric chloride as a preservative for samples to be passed through the reduction column.

Ammonia-N:

Samples for ammonia determination should be analyzed as soon as possible after collection. Samples to be analyzed within 24 hrs can be preserved by storage at 4°C. Although the addition of conc. sulfuric acid (to pH less than 2) and storage at 4°C is suggested as a preservation technique, some conversion of organic nitrogen to ammonia may occur (U.S.E.P.A. 1979). Short term storage is permitted in appropriately cleaned glass or plastic storage containers. The container should be full and tightly capped. Protect samples for ammonia-N from the laboratory atmosphere and fumes of $NH_4OH$ (i.e., nitrate-nitrite-N reagents).

Total Kjeldahl-N:

Samples may be preserved by the addition of conc. sulfuric acid, $H_2SO_4$, to bring the pH to less than 2 and stored at 4°C. Avoid prolonged storage. Protect samples for TKN from the laboratory atmosphere and fumes of $NH_4OH$ (i.e., nitrate-nitrite-N reagents). Appropriately cleaned glass or plastic storage containers are permitted.

Total Nitrogen:

Total Nitrogen is calculated as the sum of Total Kjeldahl-N and Nitrate-Nitrite-N determinations. It is recommended that samples be acidified with $H_2SO_4$ to a pH less than 2 and stored at 4°C. The maximum holding time before significant loss or gain of nitrogen is unknown. However, the holding time should not exceed 7 days. The same cautions apply here as for the Nitrate-Nitrite-N and Total Kjeldahl-N procedures given above.

It is important to remember that any preservation method is less than perfect and that it is best to analyze samples immediately after collection. This is, of course, not always practical, so compromises must be made.

REFERENCE

U.S.E.P.A. 1979. Methods for chemical analysis of water and wastes. U.S. Environmental Protection Agency, Cincinatti, OH 45268. EPA 600/4-79-020.

JRC
30 January 1985

ANALYTICAL METHOD

AMMONIA NITROGEN DETERMINATION

REFERENCE

Limnological Research Laboratory, Department of Fisheries and Wildlife, Michigan State University.

METHOD

This method estimates the ammonia nitrogen in a natural water sample. Ammonia is estimated colorimetrically after steam distillation by the Nessler reaction. Distillation is necessary for nearly all fresh waters to avoid interfering substances. The distillation may also be used to concentrate the ammonia. The analytical procedure described below is an application of methods for natural waters described by Golterman et al. (1978) and U.S.E.P.A. (1979) to the semi-micro Kjeldahl digestion and distillation apparatus of Kontes Scientific Glassware (Vineland, NJ). Note that this same distillation glassware is used for the determination of total Kjeldahl nitrogen as ammonia following an acid digestion.

EQUIPMENT

Transformer, variable voltage, input: to match voltage at project site; output: 115 V, 50/60 Hz.

GLASSWARE

Distillation apparatus, Kjeldahl, Kontes (cat. No. K-551100-0000)
Flasks, erlenmeyer, 50 ml, with ground glass stoppers (accurately determine the level of 50.0 ml for each flask and permanently mark the side of the flasks with a file)
Flask, Kjeldahl digestion/distillation, 100 ml, Kontes (cat. no. K-551600-0100)

SUPPLIES

Glasses, safety
Granules, micro, for smooth boiling, 10 mesh, plain, Hengar (cat. no. 136-CC) (Amer. Sci. Products cat. no. N3916-2) (Preparation for use: soak overnight in 0.1 N HCl, rinse thoroughly with ammonia-free deionized distilled water, dry and store closed to the atmosphere)
Grease, silicone, high vacuum, Dow Corning
Protector, Hot-Hand, silicone rubber (Fisher Scientific cat. no. 11-394-300)
Ring stands, 3-prong clamps, clamp holders (for distillation glassware)
Tubing, laboratory, Tygon, 5/8 in ID, 1/16 in wall and 1/4 in ID, 1/16 in wall

AMMONIA NITROGEN DETERMINATION (Continued)

GLASSWARE PREPARATION

and is rinsed with tap water.

2. The glassware is soaked in 0.1 N HCl until ready for use. Glassware to be used is rinsed with distilled water with a final rinse with ammonia-free deionized distilled water for low-level Kjeldahl-N or ammonia-N determinations. The glassware is best protected from pick-up of ammonia from the laboratory atmosphere by storage in the 0.1 N HCl acid bath.

REAGENTS

Borate buffer solution:
Add 88 ml of 0.1 N sodium hydroxide solution to 500 ml 0.025 M sodium tetraborate solution and dilute to 1 liter with ammonia-free deionized distilled water.

Boric acid solution:
Dissolve 20 g of boric acid, $H_3BO_3$ in ammonia-free deionized distilled water and diluted to 1 liter.

Nessler reagent:
Dissolve 100 g of mercuric iodide, $HgI_2$, (Fisher M-166 or equivalent) and 70 g potassium iodide, KI, in a small volume of ammonia-free deionized distilled water. Add this mixture slowly with stirring, to a cooled solution of 160 g of sodium hydroxide in 500 ml of ammonia-free deionized distilled water. Dilute this mixture to 1 liter. Allow to stand for 24-48 hours and decant off the clear solution for use. This solution is stable for at least 1 year if stored in a pyrex bottle out of direct sunlight.

Nitrogen stock solution, 1000 microgram $ml^{-1}$ $NH_3$-N:
Dissolve 3.819 g of ammonium chloride, $NH_4Cl$, in ammonia-free deionized distilled water and dilute to 1000 ml. Preserve with 1 ml of chloroform. Store at 5°C.

Nitrogen standard solution, 20 [illegible] $ml^{-1}$ [illegible]
Dilute 20.0 ml of nitrogen stock solution with ammonia-free deionized distilled water to 1000 ml. Prepare fresh for each set of samples.

Nitrogen working standard solutions:
Using 200 ml volumetric flasks, dilute the following volumes of the 20 microgram $ml^{-1}$ $NH_3$-N standard solution to volume with ammonia-free deionized distilled water. Prepare fresh for each set of samples.

AMMONIA NITROGEN DETERMINATION (Continued)

| ml of 20 microgram $ml^{-1}$ $NH_3$-N standard solution diluted to 200 ml | mg $NH_3$-N / 50 ml |
|---|---|
| 0.0 | 0.000 (Blank) |
| 2.0 | 0.010 |
| 6.0 | 0.030 |
| 10.0 | 0.050 |
| 15.0 | 0.075 |
| 20.0 | 0.100 |

Sodium hydroxide solution, 1 N:
Dissolve 40 g of sodium hydroxide, NaOH, in ammonia-free deionized distilled water and dilute to 1 liter.

Sodium hydroxide solution, 0.1 N:
Dilute 100 ml of 1 N sodium hydroxide solution to 1 liter with ammonia-free deionized distilled water.

Sodium tetraborate solution, 0.025 M:
Dissolve 5.0 g of sodium tetraborate, $Na_2B_4O_7$, (or 9.5 g of $Na_2B_4O_7 \cdot 10H_2O$) in ammonia-free deionized distilled water and dilute to 1 liter.

DISTILLATION PROCEDURE

1. Fill the steam generator flask with ammonia-free deionized distilled water. Turn on the heating element and the condenser cooling water. Adjust the velocity at which the steam is generated by changing the output voltage of the variable voltage transformer.
2. The distillation apparatus should be pre-steamed before use. Attach a clean Kjeldahl flask with about 30 ml of ammonia-free deionized distilled water and 10 ml of 1 N sodium hydroxide solution. Adjust the vent plug on the steam generator flask and the three-way steam control stopcock (stopcock B) on the distillation head to allow steam to enter the flask. The entrance tube stopcock (stopcock A) should be closed. After 10 minutes, begin collecting the effluent and testing for ammonia by the addition of the Nessler reagent. Continue steaming the apparatus until the effluent is ammonia-free. Repeat this procedure if the apparatus is idle for more than 4 hours.
3. Adjust the vent plug on the steam generator flask to the open position to allow the generated steam to exit through the vent tube. Close both stopcocks (A and B) on the distillation head.
4. Transfer 50.0 ml of sample or an aliquot diluted to 50.0 ml into a 100 ml Kjeldahl flask and add 3-5 Hengar micro granules (boiling chips). Prepare the ammonia standard solutions and at least one blank of ammonia-free deionized distilled water in the same manner.

AMMONIA NITROGEN DETERMINATION (Continued)

5. Add 5 ml of boric acid solution into a 50 ml erlenmeyer flask and set the ground glass stopper aside. Place the flask with the tip of the extension tube of the condenser below the level of the boric acid solution. The erlenmeyer flask is considered in the "up" position when the condenser tip is submerged in the boric acid solution.
6. Attach a Kjeldahl flask containing a sample, standard or blank(s) to the distillation head. Dispense 3 ml of borate buffer solution into the reservoir above the entrance tube stopcock (stopcock A). If necessary, add (dropwise) sufficient 1 N sodium hydroxide (or 5 N sulfuric acid) to the borate buffer to insure that the pH of the samples is in the range 9.5-9.8 after the addition of the buffer. This should be checked using a pH meter or narrow range pH indicator paper on a second aliquot of a representative sample. Record the number of drops of sodium hydroxide (or sulfuric acid) necessary to bring the pH into the proper range. This volume of sodium hydroxide (or sulfuric acid) can be added to the buffer for subsequent samples. Open the entrance tube stopcock and allow the borate buffer solution to slowly enter the digestion flask without mixing. Close the stopcock immediately. Do **NOT** allow steam to enter the Kjeldahl flask before or during the addition of the borate buffer solution, otherwise a bumping may occur when that alkaline solution is added.
7. Adjust the three-way steam control stopcock (stopcock B) to permit steam to enter the Kjeldahl flask through the steam entrance tube. Adjust the vent control plug on the steam generator to the closed position to allow the generated steam to go to the distillation head.
8. Distill about 35 ml of distillate at a rate of 6-10 ml $min^{-1}$ with the erlenmeyer flask to the "down" position so that the tip of the extension tube of the condenser is above the level of the boric acid. This will permit the distillate to wash the tip of the tube. Collect about 10 ml of additional distillate. It may be necessary to adjust the rate of distillation or the volumes collected in the "up" and "down" position to insure maximum recovery of ammonia with a minimum distillation time.
9. Dilute the collected distillate to 50.0 ml and replace the ground-glass stopper on the flask. Set the flasks aside until all samples, standards and blanks have been distilled.

NESSLERIZATION

1. To a 50.0 ml sample or an aliquot diluted to 50.0 ml, add 2 ml of Nessler reagent and mix.
2. After 20 minutes read the absorbance at 425 nm against an ammonia-free deionized distilled water reference. Subtract the mean absorbance of the blanks from the absorbances of the samples and standards. From the values obtained prepare a standard curve of absorbance vs. mg $NH_3$-N.
3. Calculate the Ammonia-N in the original sample as follows:

$$NH_3\text{-}N\ (mg\ l^{-1}) = \frac{A \times 1000 \times B}{C \times D}$$

AMMONIA NITROGEN DETERMINATION (Continued)

where

A = mg $NH_3$-N read from standard curve.
B = ml of total distillate collected including the boric acid and dilution.
C = ml of distillate taken for nesslerization.
D = ml of original sample taken for distillation

REFERENCES

Golterman, H. L., R. S. Clymo and M.A.M. Ohnstad. 1978. Methods for physical and chemical analysis of fresh waters. IBP Handbook No. 8, 2nd. ed. Blackwell Scientific Pub., Oxford. 213 pp.

U.S.E.P.A. 1979. Methods for chemical analysis of water and wastes. U.S. Environmental Protection Agency, Cincinnati, OH 45268. EPA 600/4-79-020.

JRC
29 January 1985

ANALYTICAL METHOD

TOTAL KJELDAHL NITROGEN DETERMINATION

REFERENCE

Limnological Research Laboratory, Department of Fisheries and Wildlife, Michigan State University.

METHOD

This method estimates the total organic nitrogen plus the ammonia nitrogen in a natural water sample. The classical Kjeldahl digestion converts the organic nitrogen to ammonium nitrogen. The sample is digested with sulfuric acid containing potassium sulfate to raise the digestion temperature and mercuric oxide, a required catalyst. The ammonia is estimated colorimetrically after steam distillation by Nesslerization. The analytical procedure described below is an application of methods for natural waters described by Golterman et al. (1978) and U.S.E.P.A. (1979) to the semi-micro Kjeldahl digestion and distillation apparatus of Kontes Scientific Glassware (Vineland, NJ). Note that this same glassware can be used to determine ammonia nitrogen separately if the digestion step is omitted. In addition, the Kjeldahl method for nitrogen and its compounds in biological materials and the apparatus provided by Kontes permits nitrogen determinations to be made on a wide range of sample matrices, i.e., soils, soil extracts, feeds, fertilizers, plant and animal tissues, rainwater, fresh and sea waters.

EQUIPMENT

Digestion apparatus, Kjeldahl, rotary, Kontes (cat. no. K-551000-0000) (Kontes Scientific Glassware, Spruce Street, P.O. Box 729, Vineland, NJ 08360)
Transformer, variable voltage, input: to match voltage at project site; output: 115 V, 50/60 Hz.

GLASSWARE

Distillation apparatus, Kjeldahl, Kontes (cat. no. K-551100-0000)
Flasks, erlenmeyer, 50 ml, with ground glass stoppers
Flask, Kjeldahl digestion/distillation, 100 ml, Kontes (cat. no. K-551600-0100)
Fume hood, Kjeldahl digestion, Kontes (cat. no. K-551001-0030), with 30 mm dia. holes to accept K-551600 30 and 100 ml flasks. (Accessory permits Kjeldahl digestion outside of a laboratory fume hood, requires proximity to a faucet for water aspirator).

SUPPLIES

Aspirator, water (required for use of K-551001-0030 fume hood)
Glasses, safety
Granules, micro, for smooth boiling, 10 mesh, plain, Hengar (cat. no. 136-CC) (Amer. Sci. Products cat. no. N3916-2)

TOTAL KJELDAHL NITROGEN DETERMINATION (Continued)

Grease, silicone, high vacuum, Dow Corning
Protector, Hot-Hand, silicone rubber (Fisher Scientific cat. no. 11-394-300)
Ring stands, 3-prong clamps, clamp holders (for distillation glassware)
Tubing, laboratory, Tygon, 5/8 in ID, 1/16 in wall and 1/4 in ID, 1/16 in wall

GLASSWARE PREPARATION

1. The glassware is soaked overnight in a solution of Liqui-Nox detergent and is rinsed with tap water.
2. The glassware is soaked in 0.1 N HCl until ready for use. Glassware to be used is rinsed with distilled water with a final rinse with deionized distilled water for low-level Kjeldahl-N or ammonia-N determinations. The glassware is best protected from pick-up of ammonia from the laboratory atmosphere by storage in the 0.1 N HCl acid bath.

REAGENTS

Boric acid solution:
Dissolve 20 g of boric acid, $H_3BO_3$ in deionized distilled water and dilute to 1 liter.

Digestion (sulfuric acid-mercuric sulfate-potassium sulfate) solution:
Dissolve 133.5 g of potassium sulfate, $K_2SO_4$, in 650 ml of deionized distilled water and 200 ml of concentrated sulfuric acid. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Mercuric sulfate solution:
Dissolve 8 g of mercuric oxide, red, HgO, in 50 ml of 1:4 sulfuric acid (10 ml concentrated sulfuric acid: 40 ml deionized distilled water) and dilute to 100 ml with deionized distilled water.

Nessler reagent:
Dissolve 100 g of mercuric iodide, $HgI_2$, (Fisher M-166 or equivalent) and 70 g potassium iodide, KI, in a small volume of deionized distilled water. Add this mixture slowly with stirring, to a cooled solution of 160 g of sodium hydroxide in 500 ml of deionized distilled water. Dilute this mixture to 1 liter. Allow to stand for 24-48 hours and decant off the clear solution for use. This solution is stable for at least 1 year if stored in a pyrex bottle out of direct sunlight.

Nitrogen stock solution, 1000 microgram $ml^{-1}$ $NH_3$-N:
Dissolve 3.819 g of ammonium chloride, $NH_4Cl$, in deionized distilled water and dilute to 1000 ml. Preserve with 1 ml of chloroform.

Nitrogen standard solution, 20 microgram $ml^{-1}$ $NH_3$-N:
Dilute 20.0 ml of nitrogen stock solution with deionized distilled water to 1000 ml. Prepare fresh for each set of samples.

Nitrogen working standard solutions:
Using 200 ml volumetric flasks, dilute the following volumes of the 20 microgram $ml^{-1}$ $NH_3$-N standard solution to volume with deionized distilled water. Prepare fresh for each set of samples.

TOTAL KJELDAHL NITROGEN DETERMINATION (Continued)

| ml of 20 microgram $ml^{-1}$ $NH_3$-N standard solution diluted to 200 ml | mg $NH_3$-N / 50 ml |
|---|---|
| 0.0 | 0.00 (Blank) |
| 2.0 | 0.01 |
| 10.0 | 0.05 |
| 20.0 | 0.10 |
| 50.0 | 0.25 |
| 100.0 | 0.50 |

Sodium hydroxide-sodium thiosulfate solution:
Dissolve 500 g of sodium hydroxide, NaOH, and 16 g of anhydrous sodium thiosulfate, $Na_2S_2O_3$, in deionized distilled water and dilute to 1 liter.

DIGESTION PROCEDURE

1. Transfer 50.0 ml of a well homogenized sample or an aliquot diluted to 50.0 ml into a 100 ml Kjeldahl flask.
2. Add 10 ml of digestion (sulfuric acid-mercuric sulfate-potassium sulfate) solution and 3-5 Hengar granules. The Hengar granules are soaked in 0.1 N HCl overnight, rinsed with deionized distilled water, dried and stored closed to the atmosphere.
3. Place the prepared samples on the digestion apparatus in a laboratory fume hood or outfit the digestion apparatus with the accessory glass fume hood (Kontes). This glass fume hood is attached to a water aspirator.
4. The heat controls on the digestion apparatus are set near the "high" to boil off the water (about 1.5-2.0 hours).
5. When white, cloudy fumes of $SO_3$ are given off, increase the heat control setting to "high" and begin timing the digestion.
6. Allow the digestion to proceed for 40 minutes or until the digestion is complete. During this time, rotate and gently swirl the flasks at intervals to dislodge any material adhering to the walls of the flask and to bring it into contact with the acid. The digestion is complete when the digest becomes clear, colorless or pale yellow and there are no particles of charred material remaining.
7. After the flasks have cooled, add about 30 ml of deionized distilled water to each flask to dissolve the cake of material. It may be necessary to warm the flask gently to complete the dissolution.
8. Digest the standards, and at least, 2 blanks by the same procedure.

TOTAL KJELDAHL NITROGEN DETERMINATION (Continued)

DISTILLATION PROCEDURE

1. Fill the steam generator flask with deionized distilled water. Turn on the heating element and the condenser cooling water. Adjust the velocity at which the steam is generated by changing the output voltage of the variable voltage transformer.
2. The distillation apparatus should be pre-steamed before use. Attach a clean Kjeldahl flask with about 30 ml of deionized distilled water and 10 ml of sodium hydroxide-sodium thiosulfate solution. Adjust the vent plug on the steam generator flask and the three-way steam control stopcock (stopcock B) on the distillation head to allow steam to enter the flask. The entrance tube stopcock (stopcock A) should be closed. After 10 minutes, begin collecting the effluent and testing for ammonia by the addition of the Nessler reagent. Continue steaming the apparatus until the effluent is ammonia-free. Repeat this procedure if the apparatus is idle for more than 4 hours.
3. Adjust the vent plug on the steam generator flask to the open position to allow the generated steam to exit through the vent tube. Close both stopcocks (A and B) on the distillation head.
4. Add 5 ml of boric acid solution into a 50 ml erlenmeyer flask and set the ground glass stopper aside. Place the flask with the tip of the extension tube of the condenser below the level of the boric acid solution. The erlenmeyer flask is considered in the "up" position when the condenser tip is submerged in the boric acid solution.
5. The distillation can be carried out directly from the Kjeldahl digestion flask. Dispense 10 ml of sodium hydroxide-sodium thiosulfate solution into the reservoir above the entrance tube stopcock (stopcock A). Open the entrance tube stopcock and allow the sodium hydroxide-sodium thiosulfate solution to slowly enter the digestion flask without mixing. Close the stopcock immediately. No NOT allow steam to enter the Kjeldahl flask before or during the addition of the sodium hydroxide-sodium thiosulfate solution, otherwise a violent bumping may occur when that alkaline solution is added.
6. Adjust the three-way steam control stopcock (stopcock B) to permit steam to enter the Kjeldahl flask through the steam entrance tube. Adjust the vent control plug on the steam generator to the closed position to allow the generated steam to go to the distillation head.
7. Distill about 35 ml of distillate at a rate of 6-10 ml $min^{-1}$ with the erlenmeyer flask in the up position. Lower the erlenmeyer flask to the "down" position so that the tip of the extension tube of the condenser is above the level of the boric acid. This will permit the distillate to wash the tip of the tube. Collect about 20 ml of additional distillate. It may be necessary to adjust the rate of distillation or the volumes collected in the "up" and "down" position to insure maximum recovery of ammonia with a minimum distillation time.
8. Dilute the collected distillate to 50.0 ml and replace the ground-glass stopper on the flask. Set the flasks aside until all samples, standards and blanks have been distilled.

TOTAL KJELDAHL NITROGEN DETERMINATION (Continued)

NESSLERIZATION

1. To a 50.0 ml sample or an aliquot diluted to 50.0 ml, add 2 ml of Nessler reagent and mix.
2. After 20 minutes read the absorbance at 425 nm against a deionized distilled water reference. Subtract the mean absorbance of the blanks from the absorbances of the samples and standards. From the values obtained prepare a standard curve of absorbance vs. mg $NH_3$-N.
3. Calculate the Total Kjeldahl-N in the original sample as follows:

$$\text{TKN (mg l}^{-1}) = \frac{A \times 1000 \times B}{C \times D}$$

where:

A = mg $NH_3$-N read from standard curve.
B = ml of total distillate collected including the boric acid and dilution.
C = ml of distillate taken for Nesslerization.
D = ml of original sample taken for digestion.

REFERENCES

Golterman, H. L., R. S. Clymo and M.A.M. Ohnstad. 1978. Methods for physical and chemical analysis of fresh waters. IBP Handbook No. 8, 2nd. ed. Blackwell Scientific Pub., Oxford. 213 pp.

U.S.E.P.A. 1979. Methods for chemical analysis of water and wastes. U.S. Environmental Protection Agency, Cincinnati, OH 45268. EPA 600/4-79/020.

JRC
30 January 1985

ANALYTICAL METHOD

NITRATE-NITRITE NITROGEN

REFERENCE

Michigan State University Limnological Research Laboratory, 1984. Unpublished memo on nitrate-nitrite nitrogen determination. East Lansing, MI. 5 pp.

Nitrate is quantitatively reduced to nitrite by a cadmium-copper couple in an alkaline buffered solution (pH 8.5). This nitrite and any nitrite originally present in the sample is reacted with sulphanilamide in a strongly acidic medium to form a diazonium compound which reacts quantitatively with N-(1-napthyl)ethyl-enediamine dihydrochloride to form a strongly colored azo compound. The procedure described below is an application of the methods of the American Public Health Association (1980), Strickland and Parsons (1972) and Wood et al. (1967). If separate, rather than combined nitrate-nitrite values are required, nitrite standards can be prepared and nitrite can be determined without the reduction step. Nitrate is obtained by subtraction.

Sensitivity: 0.01 mg $NO_3$-N $l^{-1}$
Working range: 0.01 to 1.0 mg $NO_3$-N $l^{-1}$

GLASSWARE:

Flasks, erlenmeyer, 125 ml, pyrex (sufficient number for blanks, standards and samples)
Cylinders, graduated, 50 ml
Pipets, transfer, 2, 5, 10, 20, 25 and 75 ml
Reduction columns (custom construction by glassblowing shop or from Fisher Scientific, Cat. No. 13-942) (see: American Public Health Association (1980) Standard Methods, 15th ed., p. 371, for construction details)
Ring stand and buret holders

GLASSWARE PREPARATION:

1. Glassware is soaked overnight in a solution of Liqui-Nox detergent and is rinsed with tap water.
2. The glassware is soaked in 1 N hydrochloric acid for a minimum of 15 minutes and is rinsed with deionized distilled water.
3. After the glassware has dried in an inverted position, it is capped with aluminum foil to protect the interior from dust.
4. Chromic acid cleaning solution may be used to clean this glassware initially or as necessary.

NOTE: Do not use nitric acid to clean this glassware.

NITRATE (Continued)

REAGENTS:

Cadmium metal:

Cadmium course powder, for filling reductors, EM Reagents (Cat. No. 2001) (particle size: 0.3-1.5 mm) (available from: VWR Scientific, Cat. No. EM-2001-2, 250 g/btl.)

Color Reagent:

Dissolve 10 g of sulfanilamide (Baker V153, Fisher 0.4525 or equivalent) and 1 g of N-(1-naphthyl)ethylenediamine dihydrochloride, reagent ACS grade (Kodak 4835 or equivalent) in a mixture of 100 ml concentrated phosphoric acid and 800 ml deionized distilled water and dilute to 1 liter. Store in a low actinic glass bottle (dark brown or deep red bottle). Renew once a month or immediately if reagent develops a dark brown coloration.

Ammonium Chloride-EDTA Solution:

Dissolve 13 g of ammonium chloride and 1.7 g of disodium ethylenediamine tetracetate (EDTA) in 900 ml of deionized distilled water. Adjust the pH to 8.5 with concentrated ammonium hydroxide and dilute to 1 liter.

Dilute Ammonium Chloride-EDTA Solution:

Dilute 300 ml of ammonium chloride-EDTA solution to 500 ml with deionized distilled water.

Copper Sulfate Solution, 2%:

Dissolve 20 g of cupric sulfate penta-hydrate ($CuSO_4 \cdot 5H_2O$) in 500 ml of deionized distilled water and dilute to 1 liter.

Hydrochloric Acid, 6 N:

Carefully dilute 50 ml of concentrated hydrochloric acid to 100 ml with deionized distilled water.

Nitrate Stock Standard Solution, 1000 microgram $ml^{-1}$ $NO_3$-N:

Dissolve 7.218 g of potassium nitrate, $KNO_3$, (dried for 1 hour at 110°C) in deionized distilled water and dilute to 1000 ml. Preserve with 2 ml of chloroform per liter. This solution is stable for at least 6 months.

Nitrate Intermediate Standard Solution, 10 microgram $ml^{-1}$ $NO_3$-N:

Dilute 10.0 ml of nitrate stock standard solution (1000 microgram $ml^{-1}$) to 1000 ml with deionized distilled water. Prepare fresh for each new set of samples.

NITRATE (Continued)

Nitrite Stock Standard Solution, 1000 microgram $ml^{-1}$ $NO_2$-N:

Dissolve 4.926 g of sodium nitrite, $NaNO_2$, in deionized distilled water and dilute to 1000 ml. Preserve with 2 ml chloroform per liter. This solution is stable for 2 months.

Nitrite Intermediate Standard Solution, 10 microgram $ml^{-1}$ $NO_2$-N:

Dilute 10.0 ml of nitrite stock solution (1000 microgram $ml^{-1}$) to 1000 ml with deionized distilled water. Prepare fresh for each new set of samples.

REDUCTION COLUMN PREPARATION:

1. Prepare cadmium for all columns in one batch. Prepare enough cadmium to be used to a depth of 20 cm. Four reduction columns are a convenient number for use.
2. Wash the cadmium with 100-150 ml of 6 N hydrochloric acid and rinse well with distilled water until the wash is no longer acid (pH greater than 5). Decant as much of the final liquid as possible.
3. Stir the cadmium in 100-150 ml of the 2% cupric sulfate solution until all the blue color has disappeared from the solution and brown, semi-colloidal copper particles begin to appear in the supernatant liquid. After the addition of the cupric sulfate solution, the cadmium particles should never be exposed to the air. Wash the cadmium with distilled water until the brown copper particles are removed.
4. Insert a small plug made of fine copper turnings into the bottom of each reduction column.
5. Fill the column with dilute ammonium chloride solution and pour in sufficient cadmium to produce a column 20 cm in length. Add the cadmium a little at a time, pausing to tap the column after each addition to make sure that the cadmium is well settled. Wash the column thoroughly with the dilute ammonium chloride solution. During this washing procedure, check the flow rate of the packed column. The flow rate should be such that 100 ml of solution takes between 8 and 12 minutes to pass through the column. If the 100 ml of solution takes less than 8 minutes, slow the column by restricting the outlet by adding a short piece of tygon tubing to the outlet and using a clamp-screw compressor (nylon, Bel-Art Products, Cat. No. F18225, doz.). If the flow takes more than 12 minutes, loosen the plug at the bottom of the column. Add a plug of copper turnings to the top of the column to prevent cadmium particles from being washed into the reservoir when solutions are added to the column. When the columns are not in use, the cadmium must be completely covered with dilute ammonium chloride solution.

PREPARATION OF WORKING STANDARDS

Nitrate and/or Nitrite Standard Solutions:

Using 200 ml volumetric flasks, dilute the following volumes of the 10 microgram $ml^{-1}$ intermediate standard solution. Prepare fresh for each new set of samples.

| ml of 10 microgram $ml^{-1}$ intermediate standard solution diluted to 200 ml | mg $NO_3$-$NO_2$-N $l^{-1}$ or mg $NO_2$-N $l^{-1}$ |
|---|---|
| 0.0 | 0.00 (Blank) |
| 2.0 | 0.10 |
| 5.0 | 0.25 |
| 10.0 | 0.50 |
| 15.0 | 0.75 |
| 20.0 | 1.00 |

PROCEDURE

1. Activate the columns by passing through each column 100 ml of solution composed of 25 ml of 1.0 mg $NO_3$-$NO_2$-N standard and 75 ml of ammonium chloride-EDTA solution.
2. Filter the samples through a glass fiber filter or 0.45 micrometer membrane filter to remove suspended matter. This avoids potential clogging of the top of the columns.
3. To a 25.0 ml sample, add 75 ml of the ammonium chloride-EDTA solution and mix. The pH of this prepared sample should be 8.5 after the addition of the ammonium chloride-EDTA solution (Note 1).
4. Pour about 5 ml of the sample into the reduction column and allow it to pass through the column. This insures that the liquid at the top of the column has the same composition as the sample avoiding possible dilution. Add the balance of the sample to the column.
5. Discard the first 35 ml of column effluent.
6. Collect the next 50 ml in a graduated cylinder and return that volume to the original sample flask.
7. Collect the remaining 10-15 ml of sample (until flow from the column ceases) in the graduated cyclinder and save temporarily. If the concentration of the sample exceeds 1.0 mg $NO_3$-$NO_2$-N $l^{-1}$, this remaining volume of reduced sample can be used to make an appropriate dilution. Using a second graduated cylinder for this column, begin passing a new sample through the column (step 4). This will save time while waiting for the color development of the previous sample.

NITRATE (Continued)

8. Within 15 minutes after passage of the sample through the column, add 2.0 ml of color reagent to 50.0 ml of sample. Allow 10 minutes for complete color development. Measure the absorbance at 540 nm against deionized distilled water. The color is stable for at least 2 hrs.
9. If the absorbance of a sample appears to exceed that of the highest standard (1.00 mg $NO_3$-$NO_2$-N $l^{-1}$), make a dilution with deionized distilled water of a portion of the reduced sample saved. Add color reagent and read the absorbance after the color development period. Record the dilution used.
10. Carry out the reduction of the nitrate standards and a blank exactly as described for the samples. A complete set of standards and a blank should be run through each column in use.
11. At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration (preferably the 1.0 mg $NO_3$-N $l^{-1}$ standard) to verify the efficiency of the reduction columns. The efficiency should be greater than 95%. If not, repack the column according to the reduction column preparation procedure given above. That same nitrite standard may also be passed through the column to check on possible reduction past the $NO_2$ oxidation state. This is especially important if the nitrite concentration exceeds 30% of the nitrate-nitrite concentration.
12. Subtract the mean absorbance of all blanks run from the absorbances of the samples and standards. In addition, subtract any absorbance at 540 nm due to humic and fulvic substances from the samples absorbances (Note 2). From the values obtained, prepare a standard curve of absorbance vs. mg $NO_3$-$NO_2$-N $l^{-1}$. If nitrite is not determined separately, report the results as mg $NO_3$-$NO_2$-N $l^{-1}$.

NOTES:

1. If samples are acidified for transport or storage, they should be brought to a pH of 7-8 with concentrated ammonium hydroxide (keep this reagent away from ammonia or kjeldahl nitrogen glassware and apparatus). The strong acid added for preservation will exceed the base buffering capacity of the ammonium chloride-EDTA solution. The sample solution pH must be 8.5 or greater for reduction to take place in the column. Samples preserved with acid cannot be later analyzed for nitrite since the acid treatment alters the proportions of nitrate and nitrite in the sample. However, a nitrate-nitrite-nitrogen determination can be made.
2. If the samples are visibly brownish in their natural color due to humic or fulvic acids, the absorbance of each sample must be measured separately and subtracted from the absorbance of that sample after color development.

NITRATE (Continued)

REFERENCES:

American Public Health Association, 1980. Standard methods for the examination of water and wastewater, 15th ed. APHA, Washington, D.C. pp. 350-388.

Strickland, J.D.H. and T. R. Parsons, 1972. A practical handbook of seawater analysis. Bull. 167 (2nd edition). Fish. Res. Bd. Canada, Ottawa, 310 p.

Wood, E. E., F.A.J. Armstrong and F.A. Richards, 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. 47:23-31.

*a. Reduction column:* Purchase or construct the column (Figure 418:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing.

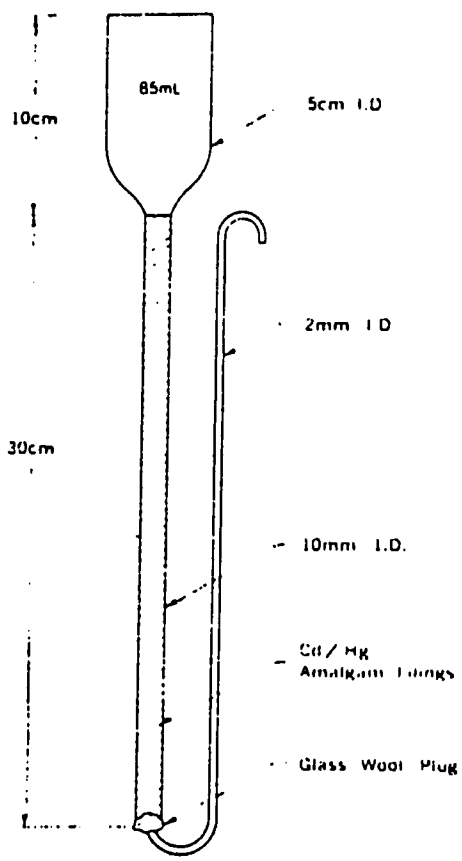


**Figure 418:1: Reduction column.**

ANALYTICAL METHOD

CHLOROPHYLL a, b, and c

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 950-954.

## 1002 G. Chlorophyll

The characteristic algal pigments are chlorophylls, xanthophylls, and carotenes. The three chlorophylls commonly found in planktonic algae are chlorophylls *a*, *b*, and *c*. Chlorophyll *a* constitutes approximately 1 to 2% of the dry weight of organic material in all planktonic algae and is the preferred indicator for algal biomass estimates. Chlorophyll content of cells varies with species or taxonomic groups and is affected by age, growth rate, light, and nutrient conditions.[11]

Two methods for determining chlorophyll *a* in phytoplankton are available, the spectrophotometric[9,12,13] and fluorometric.[9,14,15,16] The latter is more sensitive, requires less sample, and has been adapted for in vivo measurements.[17] A specific method for chlorophyll *c*, more sensitive than the trichromatic method described below, especially for samples of low pigment content, is available[18] but is not included here.

Pheophytin *a*, a common degradation product of chlorophyll *a*, can interfere with the determination of chlorophyll *a* because it absorbs light and fluoresces in the same region of the spectrum as chlorophyll *a* and, if present, may cause errors in chlorophyll *a* values.[19,20] When measuring chlorophyll *a* measure also the concentration of pheophytin *a*. The ratio of chlorophyll *a* to pheophytin *a* serves as a good indicator of physiological condition of phytoplankton. Another useful water quality indicator is the ratio of biomass to chlorophyll *a* (Autotrophic Index). In unpolluted waters the plankton population is composed largely of autotrophic (food-producing), chlorophyllous algae. As waters become organically enriched, the proportion of heterotrophic (consuming), nonchlorophyllous organisms, such as the filamentous bacteria and stalked protozoa, increases. The Autotrophic Index (AI) is a means of relating changes in plankton species composition to changes in water quality.[21] Calculate as:

$$\text{AI} = \frac{\text{Biomass (ash-free wt of organic matter), mg/m}^3}{\text{Chlorophyll } a\text{, mg/m}^3}$$

Normal AI values range from 50 to 200. Larger AI values (above 200) indicate poor water quality.

### 1. Spectrophotometric Determination of Chlorophyll *a*, *b*, and *c* (Trichromatic Method)

The pigments are extracted from the plankton concentrate with aqueous acetone and the optical density (absorbance)

CHLOROPHYLL a, b, AND c (Continued)



of the extract is determined with a spectrophotometer. When immediate pigment extraction is not possible (as described below), the samples may be stored frozen for as long as 30 days if kept in the dark. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve complete extraction of the pigments, it is necessary usually to disrupt the cells mechanically with a tissue grinder.

*a. Equipment and reagents:*

1) *Spectrophotometer*, with a narrow band (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow. At a spectral band width of 20 nm the chlorophyll *a* concentration may be underestimated by as much as 40%.

2) *Cuvettes* with 1 cm, 4 cm, and 10 cm path length.

3) *Clinical centrifuge*.

4) *Tissue grinder*.* Successfully macerating glass fiber filters in tissue grinders with grinding tube and pestle of conical design may be difficult. Preferably use grinding tubes and pestles with rounded bottoms.

5) *Centrifuge tubes*, 15 mL, graduated, screw-cap.

6) *Filtration equipment*, filters, membrane (0.45 μm porosity, 47-mm diam) or glass fiber (GF/C or GF/A, 4.5-cm diam); vacuum pump.

7) *Magnesium carbonate suspension:* Add 1.0 g finely powdered $MgCO_3$ to 100 mL distilled water.

8) *Aqueous acetone solution:* Mix 90 parts acetone (reagent grade BP 56 C) with 10 parts water (v/v).

*b. Procedure:*

1) Concentrate the sample by centrifuging or filtering (membrane or glass fiber filter). Add 0.2 mL $MgCO_3$ suspension before centrifuging or during the final phase of filtering. Store concentrated samples frozen in a desiccator in the dark if extraction is delayed. Use glassware and cuvettes that are clean and acid-free.

2) Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, and macerate. Use TFE/glass grinder for a glass-fiber filter and glass/glass grinder for a membrane filter.

3) Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to a constant level, 5 to 10 mL, with 90% aqueous acetone. Use solvent sparingly and avoid excessive dilution of pigments. Steep samples overnight at 4 C in the dark.

4) Clarify extract by centrifuging in closed tubes for 20 min at 500 *g*. Decant the clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure the total volume of extract.

5) Transfer extract to a 1-cm cuvette and measure optical density (OD) at 750, 663, 645, and 630 nm. Choose a cell path length or dilution to provide an OD663 greater than 0.2 and less than 1.0.

*c. Calculations:* Use the optical density readings at 663, 645, and 630 nm for the determination of chlorophyll *a*, *b*, and *c*, respectively. The OD reading at 750 nm serves as a correction for turbidity. Subtract this reading from each of the pigment OD values of the other wavelengths before using them in the equations below. Because the OD of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, adhere rigidly to the 90 parts acetone: 10 parts water (v/v) formula for pigment extraction.

To avoid using the 750-nm reading, clear the pigment solution by centrifuging for 20 min at 1,000 *g* and use a light path limited to 1 cm. However, when the possibility of resuspending sediment exists, make the 750-nm reading. This is commonly a problem when using glass fiber filters

*Kontes Glass Company, Vineland, N.J. 08360: Glass/glass grinder, Model No. 885500; Glass/teflon grinder, Model No. 886000; or equivalent.

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# CHLOROPHYLL a, b, AND c (Continued)



and a centrifuge with a slant head. To reduce this difficulty use a swing-out centrifuge head and additional amounts of $MgCO_3$ added immediately before centrifuging.

1) Calculate the concentrations of chlorophyll *a*, *b*, and *c* in the extract by inserting the corrected optical densities in following equations:

a) Chl *a*, mg/L = 11.64 (OD663) − 2.16 (OD645) + 0.10 (OD630)

b) Chl *b*, mg/L = 20.97 (OD645) − 3.94 (OD663) − 3.66 (OD630)

c) Chl *c*, mg/L = 54.22 (OD630) − 14.81 (OD645) − 5.53 (OD663)

where:

OD663, OD645, and OD630 = corrected optical densities (with a 1 cm light path) at the respective wavelengths.

2) After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = \frac{\text{Chl } a \times \text{extract volume, L}}{\text{Volume of sample, m}^3}$$

where:

Chl *a* = chlorophyll concentration in the extract determined by Equation a) above.

## 2. Fluorometric Method for Chlorophyll a

The fluorometric method for chlorophyll *a* is more sensitive than the spectrophotometric method, requires a smaller sample, and does not require the wavelength resolution needed for the spectrophotometric method. Optimum sensitivity for in vitro chlorophyll *a* measurements is obtained at an excitation wavelength of 430 nm and an emission wavelength of 663 nm. A method for continuous measurement of chlorophyll *a* in vivo is available,[17] but is reported to be less efficient than the in vitro method given here, yielding about one-tenth as much fluorescence per unit weight as the same amount in solution. Pheophytin *a* also can be determined fluorometrically.[7]

*a. Equipment and reagents:*

1) *Fluorometer*, equipped with a high-intensity F4T.5 blue lamp, photomultiplier tube R-136 (red sensitive), sliding window orifices 1×, 3×, 10×, and 30×, and filters for light emission (CS-2-64) and excitation (CS-5-60), and a high-sensitivity door.[†]

2) Other equipment and reagents as specified for the Spectrophotometric Determination of Chlorophyll, above.

*b. Procedure:*

1) Calibrate fluorometer with a chlorophyll solution of known concentration as follows:

a) Prepare chlorophyll extract and analyze spectrophotometrically.

b) Prepare serial dilutions of the extract to provide concentrations of approximately 2, 6, 20, and 60 µg chlorophyll *a*/L.

c) Make readings for each solution at each sensitivity setting (sliding window orifice): 1×, 3×, 10×, and 30×.

d) Using the values obtained above, derive calibration factors to convert fluorometric readings in each sensitivity level to concentrations chlorophyll *a*, as follows:

$$F_s = \frac{C_a}{R_s}$$

where:

$F_s$ = calibration factor for sensitivity setting $S$,

$R_s$ = reading of the fluorometer for sensitivity setting $S$, and,

$C_a$ = concentration of chlorophyll *a* determined spectrophotometrically, µg/L

2) Measure sample fluorescence at sensitivity settings that will provide a mid

[†]Model 111, Turner Assoc., 2524 Pulgas Ave., Palo Alto, Calif., or equivalent

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# CHLOROPHYLL a, b, AND c (Continued)



scale reading. Convert fluorescence readings to concentrations of chlorophyll *a* by multiplying the readings by the appropriate calibration factor.

3) Avoid using the 1× window because of quenching effects.

### 3. Spectrophotometric Determination of Chlorophyll *a* in the Presence of Pheophytin *a*

Chlorophyll *a* may be overestimated by including pheopigments that absorb near the same wavelength as chlorophyll *a*. Chlorophyll *a*, acidified with dilute acid, degrades to pheophytin *a*, which has maximum absorption at wavelengths of 410 and 665 (667) nm. Additional acidification with more concentrated acid results in further degradation to pheophorbide-like compounds.[41] Addition of acid to chlorophyll *a* results in loss of the magnesium atom, converting it to pheophytin *a*. When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption peak is reduced to approximately 60% of its original value and shifts from 663 nm to 665 nm. This results in a before-to-after acidification absorption-peak-ratio (OD663/OD665) of 1.70 and is used in correcting the apparent chlorophyll *a* concentration for pheophytin *a*.

Samples with an OD663 before/OD665 after acidification ratio ($663_b/665_a$) of 1.70 are considered to contain little if any pheophytin *a* and to be in excellent physiological condition. Solutions of pure pheophytin show no reduction in OD665 upon acidification and have a $663_b/665_a$ ratio of 1.0. Thus, mixtures of chlorophyll *a* and pheophytin *a* have absorption peak ratios ranging between 1.0 and 1.7. These ratios are based on the use of 90% acetone as solvent. Using 100% acetone as solvent results in a chlorophyll *a* before-to-after acidification ratio of about 2.0.[31,42]

*a. Equipment and reagents:*

1) See Section 1002G.1*a*.

2) *Hydrochloric acid*, HCl, 1*N*.

*b. Procedure:*

1) Extract the pigment with 90% acetone (v/v), clarify by centrifuging (see Section 1002G.1*b*), and read OD at 750 nm and 663 nm.

2) Acidify extract in a 1-cm cuvette with 2 drops 1*N* HCl. If a larger cell is used add a proportionately larger volume of acid. Gently agitate the acidified extract and read OD at 750 nm and at 665 nm not sooner than 1 min or later than 2 min after acidification. Treat all samples identically.

3) Subtract the 750-nm OD value from the readings before (OD663 nm) and after acidification (OD665 nm).

*c. Calculations:* Using the corrected values calculate chlorophyll *a* (*C*) and pheophytin *a* (*P*) per cubic meter as follows:

$$1)\ C,\ \text{mg/m}^3 = \frac{26.73\,(663_b - 665_a) \times V_1}{V_2 \times L}$$

$$2)\ P,\ \text{mg/m}^3 = \frac{26.73\,[1.7\,(665_a) - 663_b] \times V_1}{V_2 \times L}$$

where:

$V_1$ = volume of extract, L,

$V_2$ = volume of sample, m³,

$L$ = light path length or width of cuvette, cm, and

$663_b$, $665_a$ = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.73 is the absorbance correction and equals $A \times K$

where:

$A$ = absorbance coefficient for chlorophyll *a* at 663 nm = 11.0, and

$K$ = ratio expressing correction for acidification.

$$= \frac{\left(\frac{663_b}{665_a}\right)_{\text{pure chlorophyll } a}}{\left(\frac{663_b}{665_a}\right)_{\text{pure chlorophyll } a} - \left(\frac{663_b}{665_a}\right)_{\text{pure pheophytin } a}}$$

$$= \frac{1.7}{1.7 - 1.0} = 2.43$$

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CHLOROPHYLL a, b, AND c (Continued)



4. Fluorometric Determination of Chlorophyll *a* in the Presence of Pheophytin *a*

To determine fluorometrically the concentration of pheophytin *a* requires the measurement of the fluorescence of acetone extracts before and after acidification. Acidification of acetone extracts of chlorophyll *a* and the resultant conversion of chlorophyll *a* to pheophytin *a* causes a reduction in fluorescence, which can be used to determine the concentration of pheophytin *a* in the extract.

*a. Equipment and reagents:*

1) See Section 1002G.2*a*.

2) *Hydrochloric acid*, HCl, 1*N*.

3) *Pure chlorophyll a*‡ (or a plankton chlorophyll extract with before-and-after acidification ratio of 1.70).

*b. Procedure:* Calibrate fluorometer as in Section 1002G.2*b*. Determine extract fluorescence at each sensitivity setting before and after acidification. Calculate calibration factors ($F_s$) and before-and-after acidification fluorescence ratio by dividing the fluorescence reading obtained before acidification by the reading obtained after acidification. Avoid readings on the 1 × scale and those outside the range of 20 to 80 fluorometric units.

‡ Purified chlorophyll *a*, Sigma Chemical Company, St Louis, Mo., or equivalent

*c. Calculations:* Determine the "corrected" chlorophyll *a* and pheophytin *a* in extracts of plankton samples, using the following equations:[7,16]

$$\text{Chlorophyll } a, \text{ mg m}^{-3} = F_s \frac{V}{V_s} \frac{r}{1} (R_b - R_a)$$

$$\text{Pheophytin } a, \text{ mg m}^{-3} = F_s \frac{V}{V_s} \frac{1}{1} (rR_a - R_b)$$

where:

$F_s$ = conversion factor for sensitivity setting "S" (see 1002G.2*b*),

$R_b$ = fluorescence of extract before acidification,

$R_a$ = fluorescence of extract after acidification, and

$r$ = $R_b/R_a$, as determined with pure chlorophyll *a* for the instrument. Redetermine $r$ if filters or light source are changed.

ANALYTICAL METHOD

PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE)

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 409-426.

For phosphorus forms, the methods presented in the previous workplan represented an adaptation of "Method 365.2 (Colorimetric, Ascorbic Acid, Single Reagent)" published by the U.S. Environmental Protection Agency (1979). The [illegible] in principle with the procedures reproduced below that were outlined by the American Public Health Association (1980). To facilitate cross referencing, relevant sections from both sources are as follows:

| Parameter | Procedure | American Public Health Association (1980) | U.S. Environmental Protection Agency (1979) |
|---|---|---|---|
| Total Phosphorus | Persulfate digestion | 424 C (III) | 8.1 |
| | Ascorbic acid/ colorimetric method | 424 F | 8.3.2 |
| Dissolved Orthophosphate (Filterable Reactive Phosphorus) | Preliminary filtration | 424 A | 4.2 |
| | Ascorbic acid/ colorimetric method | 424 F | 8.3 |

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# 424 PHOSPHORUS

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phophate arise from a variety of sources. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

## 1. Definition of Terms

Phosphorus analyses embody two general procedural steps: *(a)* conversion of the phosphorus form of interest to dissolved orthophosphate, and *(b)* colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45-$\mu$m membrane filter separates "filtrable" from "nonfiltrable" forms of phosphorus. No claim is made that filtration through 0.45-$\mu$m filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technic designed to make a gross separation. This is reflected in the use of the term "filtrable" (rather than dissolved) to describe the phosphorus forms determined in the filtrate that passes the 0.45-$\mu$m filter.

Membrane filtration is selected over depth filtration because of the greater likelihood of obtaining a consistent separation of particle sizes. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE) (Continued)



Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both filtrable and nonfiltrable forms.

Acid hydrolysis at boiling-water temperature converts filtrable and particulate condensed phosphates to filtrable orthophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term "acid-hydrolyzable phosphorus" is preferred over "condensed phosphate" for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidative destruction of the organic matter present are considered "organic" or "organically bound" phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the filtrable and nonfiltrable fractions. With minor variations, the filtrable and nonfiltrable fractions of a sample correspond to dissolved and particulate phosphates, respectively.

The total phosphorus as well as the filtrable and nonfiltrable phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid-hydrolyzable, and organic phosphorus. Figure 424:1 shows the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Nonfiltrable fractions generally are determined by difference.

## 2. Selection of Method

*a. Digestion methods:* Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given. The perchloric acid method, the most drastic and time-consuming method, is recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technic. It is recommended that this method be checked against one or more of the more drastic digestion technics and be adopted if identical recoveries are obtained.

*b. Colorimetric methods:* Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdic acid method (D) is most useful for routine analyses in the range of 1 to 20 mg P/L. The stannous chloride method (E) or the ascorbic acid method (F) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. An automated version of the ascorbic acid method also is presented.

## 3. Precision and Accuracy

To aid in method selection, Table 424:I presents the results of various combinations of digestion, hydrolysis, and colorimetric technics for three synthetic samples of the following compositions:

Sample 1: 100 μg orthophosphate phosphorus ($PO_4$-P)/L, 80 μg condensed phosphate phosphorus/L (sodium hexametaphosphate), 30 μg organic phosphorus/L (adenylic acid), 1.5 mg $NH_3$-N/L, 0.5 mg $NO_3$-N/L, and 400 mg chloride/L.

Sample 2: 600 μg $PO_4$-P/L, 300 μg con-

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PHOSPHORUS (TOTAL, DISSOLVED ORTHOPHOSPHATE) (Continued)



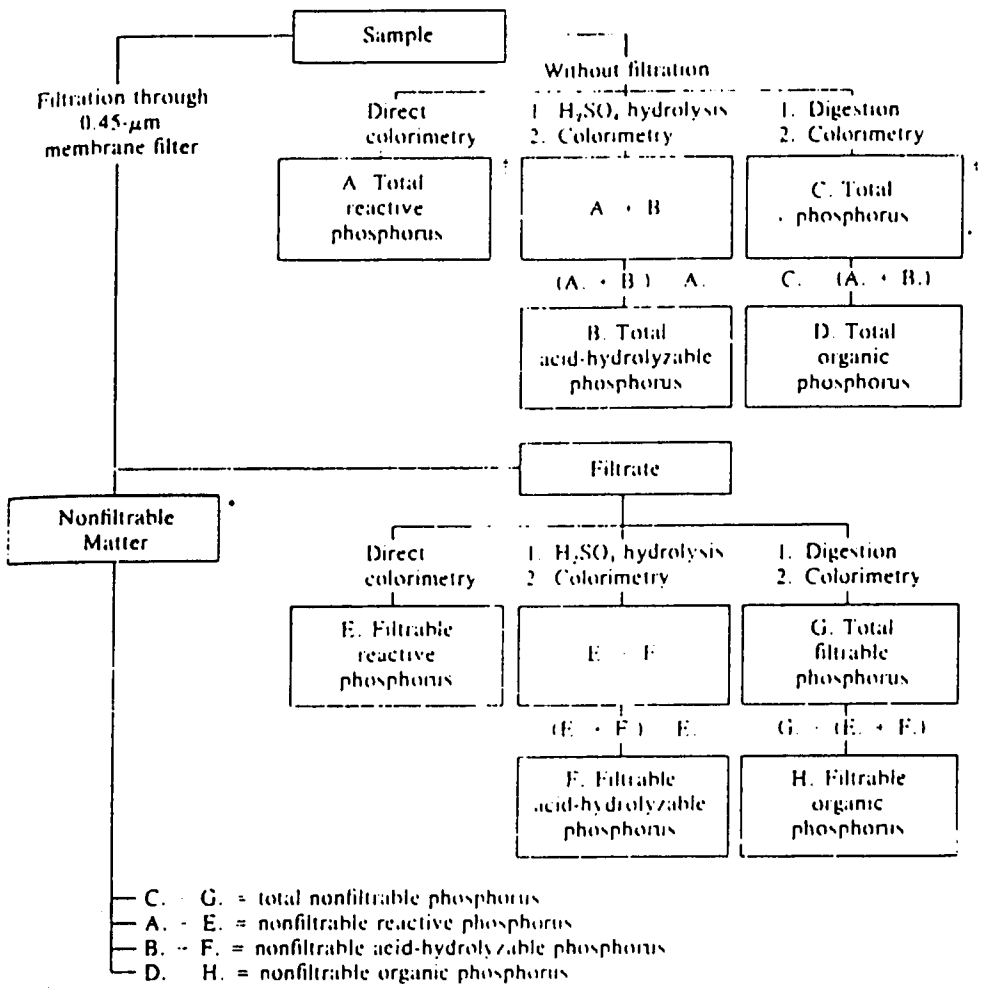


Figure 424:1. Steps for analysis of phosphate fractions.

*Direct determination of phosphorus on the membrane filter containing nonfiltrable matter will be required where greater precision than that obtained by difference is desired. Digest filter with $HNO_3$ and follow by perchloric acid. Then perform colorimetry.

†Total phosphorus measurements on highly saline samples may be difficult because of precipitation of large quantities of salt as a result of digestion techniques that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total filtrable phosphorus and total nonfiltrable phosphorus and add the results.

‡When determining total filtrable or total nonfiltrable reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time dependent desorption of orthophosphate from the suspended particles.

densed phosphate phosphorus/L (sodium hexametaphosphate), 90 μg organic phosphorus/L (adenylic acid), 0.8 mg $NH_3$-N/L, 5.0 mg $NO_3$-N/L, and 400 mg chloride/L.

Sample 3: 7.00 mg $PO_4$-P/L, 3.00 mg condensed phosphate phosphorus/L (sodium hexametaphosphate), 0.230 mg organic phosphorus/L (adenylic acid), 0.20 mg $NH_3$-N/L, 0.05 mg $NO_3$-N/L, and 400 mg chloride/L.

4. Sampling and Storage

If phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below -10 C. Add 40 mg $HgCl_2$/L to the samples, especially when they are to be stored for long periods. Do not add either acid or $CHCl_3$ as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add 1 mL conc HCl/L or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl, then rinse several times in distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

## 424 A. Preliminary Filtration Step

Filter samples for determination of filtrable reactive phosphorus, filtrable acid-hydrolyzable phosphorus, and total filtrable phosphorus through 0.45-μm membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use one of two washing technics: *(a)* soak 50 filters in 2 L distilled water for 24 hr; *(b)* soak 50 filters in 2 L distilled water for 1 hr, change distilled water, and soak filters an additional 3 hr. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

## 424 B. Preliminary Acid Hydrolysis Step for Acid-Hydrolyzable Phosphorus

1. Discussion

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phos-

phate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (D, E, or F). Interferences, precision, accuracy, and sensitivity will depend on the colorimetric method used.

### 2. Apparatus

*Autoclave or pressure cooker*, capable of operating at 98 to 137 kPa.

### 3. Reagents

*a. Phenolphthalein indicator aqueous solution.*

*b. Strong acid solution:* Slowly add 300 mL conc $H_2SO_4$ to about 600 mL distilled water. When cool, add 4.0 mL conc $HNO_3$ and dilute to 1 L.

*c. Sodium hydroxide*, NaOH, 6N.

### 4. Procedure

To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method D, E, or F) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method D, E, or F. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

## 424 C. Preliminary Digestion Steps for Total Phosphorus

Total phosphorus includes all orthophosphates and condensed phosphates, both dissolved and particulate, organic and inorganic. To release phosphorus from combination with organic matter, digest and oxidize. The rigor of digestion required depends on the type of sample. The three digestion technics presented, in order of decreasing rigor, are perchloric acid digestion, sulfuric acid-nitric acid digestion, and persulfate digestion. Compare phosphorus recovery by each digestion technic for the specific type of sample being tested; if the less tedious persulfate method gives good phosphorus recovery, use this method.

After digestion, determine liberated orthophosphate by Method D, E, or F. The colorimetric method used, rather than the digestion procedure, governs in matters of interference and minimum detectable concentration.

orimetric method used). Add 1 mL conc $H_2SO_4$ and 5 mL conc $HNO_3$.

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove $HNO_3$.

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1*N* NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method D, E, or F, for which a separate calibration curve has been constructed by carrying standards through the acid digestion pro-

300 mL conc $H_2SO_4$ to approximately 600 mL distilled water and dilute to 1 L with distilled water.

*c. Ammonium persulfate,* $(NH_4)_2S_2O_8$, solid, or potassium persulfate, $K_2S_2O_8$, solid.

*d. Sodium hydroxide,* NaOH, 1*N*.

3. Procedure

Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add $H_2SO_4$ solution dropwise to just discharge the color. Then add 1 mL $H_2SO_4$ solution and either 0.4 g solid $(NH_4)_2S_2O_8$ or 0.5 g solid $K_2S_2O_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Cool, dilute to 30 mL with

nolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method D, E, or F, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

## III—PERSULFATE DIGESTION METHOD

1. Apparatus

*a. Hot plate:* A 30- × 50-cm heating surface is adequate.

*b. Autoclave:* An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.

*c. Glass scoop,* to hold required amounts of persulfate crystals.

2. Reagents

*a. Phenolphthalein indicator aqueous solution.*

*b. Sulfuric acid solution:* Carefully add

## 424 D. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

*a. Principle:* In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the

Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic $H_2SO_4$ solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic $H_2SO_4$. Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

*a. Direct procedure:*

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

*b. Extraction procedure:*

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

6. Precision and Accuracy

See Table 424:1.

## 424 F. Ascorbic Acid Method

1. General Discussion

*a. Principle.* Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

*b. Interference:* Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg arsenic/L interfere with the phosphate determination. Hexavalent chromium and nitrite interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide ($Na_2S$) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

*c. Minimum detectable concentration:* Approximately 10 µg P/L. P ranges are as follows:

| Approximate P Range *mg/L* | Light Path *cm* |
|---|---|
| 0.30-2.0 | 0.5 |
| 0.15-1.30 | 1.0 |
| 0.01-0.25 | 5.0 |

2. Apparatus

*a. Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer,* with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer,* equipped with a red color filter and a light path of 0.5 cm or longer.

*b. Acid-washed glassware:* See Method D, ¶ 2*b* above.

3. Reagents

*a. Sulfuric acid, $H_2SO_4$, 5N:* Dilute 70 mL conc $H_2SO_4$ to 500 mL with distilled water.

*b. Potassium antimonyl tartrate solution:* Dissolve 1.3715 g $K(SbO)C_4H_4O \cdot \frac{1}{2}H_2O$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

*c. Ammonium molybdate solution:* Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle.

*d. Ascorbic acid, 0.01M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4 C.

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*e. Combined reagent:* Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5*N* $H_2SO_4$, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hr.

*f. Stock phosphate solution:* See Method D, ¶ 3*e*.

*g. Standard phosphate solution:* Dilute 50.0 mL stock phosphate solution to 1,000 mL with distilled water; 1.00 mL = 2.50 μg P.

4. Procedure

*a. Treatment of sample:* Pipet 50.0 mL sample into a clean, dry test tube or 125 mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5*N* $H_2SO_4$ solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

*b. Correction for turbidity or interfering color.* Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimonyl potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

*c. Preparation of calibration curve:* Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in Section 424F.1*c*. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1{,}000}{\text{mL sample}}$$

6. Precision and Accuracy

The precision and accuracy values given in Table 424:I are for a single-solution procedure given in the 13th edition. Procedure 424F differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and accuracy to the previous technic in the analysis of both distilled water and river water at the 228 μg P/L level (Table 424:II).

TABLE 424:II. COMPARISON OF PRECISION AND ACCURACY OF ASCORBIC ACID METHODS

| Ascorbic Acid Method | Phosphorus Concentration, Filtrable Orthophosphate μg/L | No. of Laboratories | Relative Standard Deviation % | | Relative Error % | |
|---|---|---|---|---|---|---|
| | | | Distilled Water | River Water | Distilled Water | River Water |
| 13th Edition (Edwards, Molof, and Schneeman) | 228 | 8 | 3.87 | 2.17 | 4.01 | 2.08 |
| Current method (Murphy and Riley) | 228 | 8 | 3.03 | 1.75 | 2.38 | 1.39 |

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## 424 H. Bibliography


KITSON, R.E. & M.G. MELLON. 1944. Colorimetric determination of phosphorus as molydovanadophosphoric acid. *Ind. Eng. Chem.*, Anal. Ed. 16:379.

BOLTZ, D.F. & M.G. MELLON. 1947. Determination of phosphorus, germanium, silicon, and arsenic by the heteropoly blue method. *Ind. Eng. Chem.*, Anal. Ed. 19:873.

GREENBERG, A.E., L.W. WEINBERGER & C.N. SAWYER. 1950. Control of nitrite interference in colorimetric determination of phosphorus. *Anal. Chem.* 22:499.

YOUNG, R.S. & A. GOLEDGE. 1950. Determination of hexametaphosphate in water after threshold treatment. *Ind. Chem.* 26:13.

GRISWOLD, B.L., F.L. HUMOLLER & A.R. MCINTYRE. 1951. Inorganic phosphates and phosphate esters in tissue extracts. *Anal. Chem.* 23:192.

BOLTZ, D.F., ed. 1958. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.

AMERICAN WATER WORKS ASSOCIATION. 1958. Committee report. Determination of orthophosphate, hydrolyzable phosphate, and total phosphate in surface waters. *J. Amer. Water Works Ass.* 50:1563.

JACKSON, M.L. 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, N.J.

SLEEPEN, O. & C.M. BACH. 1961. Modified stannous chloride reagent for orthophosphate determination. *J. Amer. Water Works Ass.* 53:1031.

MURPHY, J. & J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta* 27:31.

ABBOT, D.C., G.E. EMSDEN & J.R. HARRIS. 1963. A method for determining orthophosphate in water. *Analyst* 88:814.

GOTTFRIED, P. 1964. Determination of total phosphorus in water and wastewater as molybdovanadophosphoric acid. *Limnologica* 2:407.

STRICKLAND, J.D.H. & T.R. PARSONS. 1965. A Manual of Sea Water Analysis, 2nd ed. Fish. Res. Board, Ottawa, Canada.

BLACK, C.A., D.D. EVANS, J.L. WHITE, L.E. ENSMINGER & F.E. CLARK, eds. 1965. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. American Society for Agronomy, Madison, Wisc.

EDWARDS, G.P., A.H. MOLOF & R.W. SCHNEEMAN. 1965. Determination of orthophosphate in fresh and saline waters. *J. Amer. Water Works Ass.* 57:917.

LEE, G.F., N.L. CLESCERI & G.P. FITZGERALD. 1965. Studies on the analysis of phosphates in algal cultures. *J. Air Water Pollut.* 9:715.

JENKINS, D. 1965. A study of methods suitable for the analysis and preservation of phosphorus forms in an estuarine environment. SERL Report No. 65-18, Sanitary Engineering Research Laboratory, Univ. of California, Berkeley.

HENRIKSEN, A. 1966. An automatic method for determining orthophosphate in sewage and highly polluted waters. *Analyst* 91:652.

SHANSON, J.E. & G.F. LEE. 1966. Hydrolysis of condensed phosphates in natural waters. *J. Air Water Pollut.* 10:735.

GALES, M.E., JR., E.C. JULIAN & R.C. KRONER. 1966. Method for quantitative determination of total phosphorus in water. *J. Amer. Water Works Ass.* 58:1363.

LEE, G.F. 1967. Analytical chemistry of plant nutrients. *In* Proc. Int. Conf. Eutrophication, Madison, Wisc.

FITZGERALD, G.P. & S.L. FAUST. 1967. Effect of water sample preservation methods on the release of phosphorus from algae. *Limnol. Oceanogr.* 12:332.

*Automated Ascorbic Acid Reduction Method*

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1971. Methods for Chemical Analysis of Water and Wastes. National Environmental Research Center, Cincinnati, Ohio.

LOHRING, L.B. & R.L. BOOTH. 1973. Evaluation of the AutoAnalyzer II; A progress report. *In* Advances in Automated Analysis: 1972 Technicon International Congress. Vol. 8, p. 7. Mediad, Inc., Tarrytown, N.Y.

U.S. ENVIRONMENTAL PROTECTION AGENCY. MDQARL Method Study 4, Automated Methods. National Environmental Research Center, Cincinnati, Ohio (in preparation).

ANALYTICAL METHOD

PRIMARY PRODUCTIVITY

REFERENCE

American Public Health Association, 1980. Standard methods for the examination of water and waste water, 15th ed. APHA, Washington, D.C. pp. 957-959.

## 1002 I. Metabolic Rate Measurements

The physiological condition of the aquatic community and the spectrum of biological interactions must be considered. Earlier, numbers, species composition, and biomass were the prime considerations. Recognition of the limitations of this approach, however, led to the measurement of rates of metabolic processes such as photosynthesis (productivity), nitrogen fixation, respiration, and electron transport. These provide a better understanding of the complex nature of the aquatic ecosystem. An indication of photosynthetic efficiency can be determined by the productivity index (mg C fixed/unit chlorophyll *a*).[49]

### 1. Nitrogen Fixation

The ability of an organism to fix nitrogen is a great competitive advantage and plays a major role in population dynamics. Two reliable methods for estimating nitrogen fixation rates in the laboratory are the $^{15}N$ isotope tracer method[50,51] and the acetylene reduction method.[52] Because the rate of nitrogen fixation varies greatly with different organisms and with the concentration of combined nitrogen, nitrogen fixation rates cannot be used to estimate biomass of nitrogen-fixing organisms. However, the acetylene reduction method is useful in measuring nitrogen budgets and in algal assay work.[51]

### 2. Productivity, Oxygen Method

Productivity is defined as the rate at which inorganic carbon is converted to an organic form. Cholorphyll-bearing plants (phytoplankton, periphyton, macrophytes) serve as primary producers in the aquatic food chain. Photosynthesis results in the formation of a wide range of organic compounds, release of oxygen, and depletion of carbon dioxide ($CO_2$) in the surrounding waters. Primary productivity[54] can be determined by measuring the changes in oxygen and $CO_2$ concentrations.[55] In poorly buffered waters, pH can be a sensitive property for detecting variations in the system. As $CO_2$ is removed during photosynthesis, the pH rises. This shift can be used to estimate both photosynthesis and respiration.[56] The sea and many fresh waters are too highly buffered to make this useful, but it has been applied successfully to productivity studies in some lake waters.

Two methods of measuring the rate of carbon uptake and net photosynthesis in situ are: (*a*) the oxygen method[57] and (*b*) the carbon 14 method.[58] In both methods, clear (light) and darkened (dark) bottles are filled with water samples and suspended at regular depth intervals for an incubation period of several hours or samples are incubated under controlled conditions in environmental growth chambers

## PRIMARY PRODUCTIVITY (Continued)

in the laboratory.

The basic reactions in algal photosynthesis involve uptake of inorganic carbon and release of oxygen, summarized by the relationship:

$$CO_2 + H_2O \rightarrow (CH_2O)_x + O_2$$

The chief advantages of the oxygen method are that it provides estimates of gross and net productivity and respiration and that analyses can be performed with inexpensive laboratory equipment and common reagents. The DO concentration is determined at the beginning and end of the incubation period. Productivity is calculated on the assumption that one atom of carbon is assimilated for each molecule of oxygen released.

*a. Equipment:*

1) *BOD bottles*, numbered, 300-mL, clear borosilicate glass, with ground-glass stopper and flared mouth, for sample incubation. Acid-clean the bottles, rinse thoroughly with distilled water, and just before use, rinse with the water being tested. Do not use phosphorus-containing detergents.

If suitable opaque bottles are not available, make clear BOD bottles opaque by painting them black and wrapping with black waterproof tape. As a further precaution, wrap the entire bottle in aluminum foil or place in a light-excluding container during incubation.

2) *Supporting line or rack* that does not shade the suspended bottles.

3) *Nonmetallic opaque acrylic Van Dorn sampler* or equivalent, of 3- to 5-L capacity.

4) *Equipment and reagents for dissolved oxygen determinations* (see Section 421).

5) *Pyrheliometer.*

6) *Submarine photometer.*

*b. Procedure:*

1) Obtain a profile of the input of solar radiation for the photoperiod with a pyrheliometer.

2) Determine depth of euphotic zone (the region that receives 1% or more of surface illumination) with a submarine photometer. Select depth intervals for bottle placement. The photosynthesis-depth curve will be closely approximated by placing samples at intervals equal to one-tenth the depth of the euphotic zone. Estimate productivity in relatively shallow water with fewer depth intervals.

3) Introduce samples taken from each preselected depth into duplicate clear, darkened, and initial-analysis bottles. Insert delivery tube of sampler to bottom of sample bottle and fill so that three volumes of water are allowed to overflow. Remove tube slowly and close bottle. Use water from the same grab sample to fill a "set" (one light, one dark, and one initial bottle).

4) Immediately treat (fix) samples taken for the chemical determination of initial dissolved oxygen (see Dissolved Oxygen, Section 421) with manganous sulfate ($MnSO_4$), alkaline iodide, and sulfuric acid ($H_2SO_4$) or check with an oxygen probe. Analyses may be delayed several hours if necessary, if samples are fixed or iced and stored in the dark.

5) Suspend duplicate paired clear and darkened bottles at the depth from which the samples were taken and incubate for at least 2 hr, but never longer than it takes for oxygen-gas bubbles to form in the clear bottles or DO to be depleted in the dark bottles.

6) At the end of the exposure period, immediately determine DO as described above.

*c. Calculations:* The increase in oxygen concentration in the light bottle during incubation is a measure of net production which, because of the concurrent use of oxygen in respiration, is somewhat less than the total (or gross) production. The loss of oxygen in the dark bottle is used as an estimate of respiration. Thus:

**Note: Instead of using a pyrheliometer, researchers should take solar monitor readings at the start and end of a 10:00 AM - 2:00 PM incubation period and for the entire photoperiod. If a submarine photometer is unavailable, suspend bottles at mid-depth (45 cm).**

## PRIMARY PRODUCTIVITY (Continued)

Net photosynthesis = light bottle DO − initial DO
Respiration = initial DO − dark bottle DO
Gross photosynthesis = light bottle DO − dark bottle DO

Average results from duplicates.

1) Calculate the gross or net production for each incubation depth and plot:

mg carbon fixed/$m^3$
= mg oxygen released/L × 12/32 × 1,000

Use the factor 12/32 to convert oxygen to carbon; 1 mole of $O_2$ (32 g) is released for each mole of carbon (12 g) fixed.

2) Productivity is defined as the rate of production and generally is reported in grams carbon fixed per square meter per day. Determine the productivity of a vertical column of water 1 m square by plotting productivity for each exposure depth and graphically integrating the area under the curve.

3) Using the solar radiation profile and photosynthesis rate during incubation adjust the data to represent phytoplankton productivity for the entire photoperiod. Because photosynthetic rates vary widely during the daily cycle.[39,60] do not attempt to convert data to other test circumstances.

ANALYTICAL METHOD

PRIMARY PRODUCTIVITY, MODIFIED DIURNAL CURVE METHOD

REFERENCE

McConnell, William J. 1962. Productivity relations in carboy microcasms. Limnol. Oceanogr. 7:35-43.

Welch, Harold E. 1968. Use of modified diurnal curves for the measurement of metabolism in standing water. Limnol. Oceanogr. 13:679-687.

Measurements of primary production can be made either in some sort of enclosure or the open water. The main disadvantage of enclosures is that they may not be representative of events that may be happening throughout the entire water mass. For this reason, free-water techniques should be superior to enclosures provided that other errors can be eliminated.

The flux of oxygen has been used to measure community metabolism but has usually been limited to well-mixed waters where one to two determinations are representative of the entire water mass. This restriction to turbulent water has magnified the chief drawback to oxygen free-water methods - the problem of diffusion. Unlike bottle methods, free-water techniques allow interchange at the air-water interface. Since oxygen diffuses readily, diffusion rates often may be as high as the metabolism one is trying to measure.

Another difficulty in applying free-water techniques to limnetic situation is in obtaining representative samples, since the system will most likely be physically and biologically stratified. This difficulty may be overcome by redefining the sampling techniques to suit the stratified nature of the water mass.

One free-water technique for determining community metabolism is by the diurnal oxygen curve technique. The basic procedure is to sample oxygen and temperature every three hours for a period of 24 hours. Dissolved oxygen is measured either by the Winkler iodometric procedure or by a galvanic probe. Community metabolism (gross photosynthesis and respiration) is obtained from the 24 hour oxygen curves by graphing a rate of change oxygen curve that has been corrected for diffusion, extrapolating the night respiration rate over 24 hours and then integrating different areas under the curve [see either Odum and Haskins (1958) or Hall and Moll (1975) for a detailed description of this technique. Hall and Moll (1975) give a step by step example for computing one point on the corrected rate of change curve that they present in their article]. An alternative to this rather laborious method has been developed by McConnell (1962) and incorporated by Welch (1968) in which only three points of a diurnal curve are used: one after dark and one before dawn to determine the rate of respiration, and one the same time the following evening to determine whether there has been a net change in the total oxygen content of the system. It should be noted that the times that are suggested by McConnell (1962) and Welch (1968) may not be optimal for all situations. It is recommended that daily maximum and minimum oxygen concentrations first be determined and those times used for the calculations. Respiration and gross

PRIMARY PRODUCTIVITY, MODIFIED DIURNAL CURVE METHOD (Continued)

photosynthesis rates can be determined by graphing the three oxygen measurements and calculating the slopes of the 24 hour respiration line and the daylight photosynthesis line [see Figure 1 which was modified from McConnell (1962)]. The extrapolation of the night respiration line through the following day involves the same assumption that one is forced to make in light and dark bottle experiments: that daytime respiration rate is about equal to that measured during the dark hours. However, studies have shown that many times daytime respiration may be considerably higher than nighttime respiration but until some adequate means for measuring photorespiration in the field becomes available, the method of connecting the pre-dawn point by a straight line to the post-sunset point is at least objective and is probably a minimal, but reasonable, estimate of all community respiration (Hall and Moll 1975).

LITERATURE CITED

Hall, Charles A.S. and Russell Moll. 1975. Methods of assessing aquatic primary productivity, pp. 1953. In: Helmut Lieth and Robert H. Whittaker (Eds.). Primary Productivity of the Biosphere. Springer-Verlag, New York.

McConnell, William J. 1962. Productivity relations in carboy microcosms. Limnol. Oceanogr. 7:35-43.

Odum, Howard T. and Charles M. Hoskin. 1958. Comparative studies on the metabolism of marine waters. Publ. Inst. Mar. Sci. Univ. Tex. 5:16-46.

Welch, Harold E. 1968. Use of modified diurnal curves for the measurement of metabolism in standing water. Limnol. Oceanogr. 13:679-687.

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PRIMARY PRODUCTIVITY, MODIFIED DIURNAL CURVE METHOD (Continued)

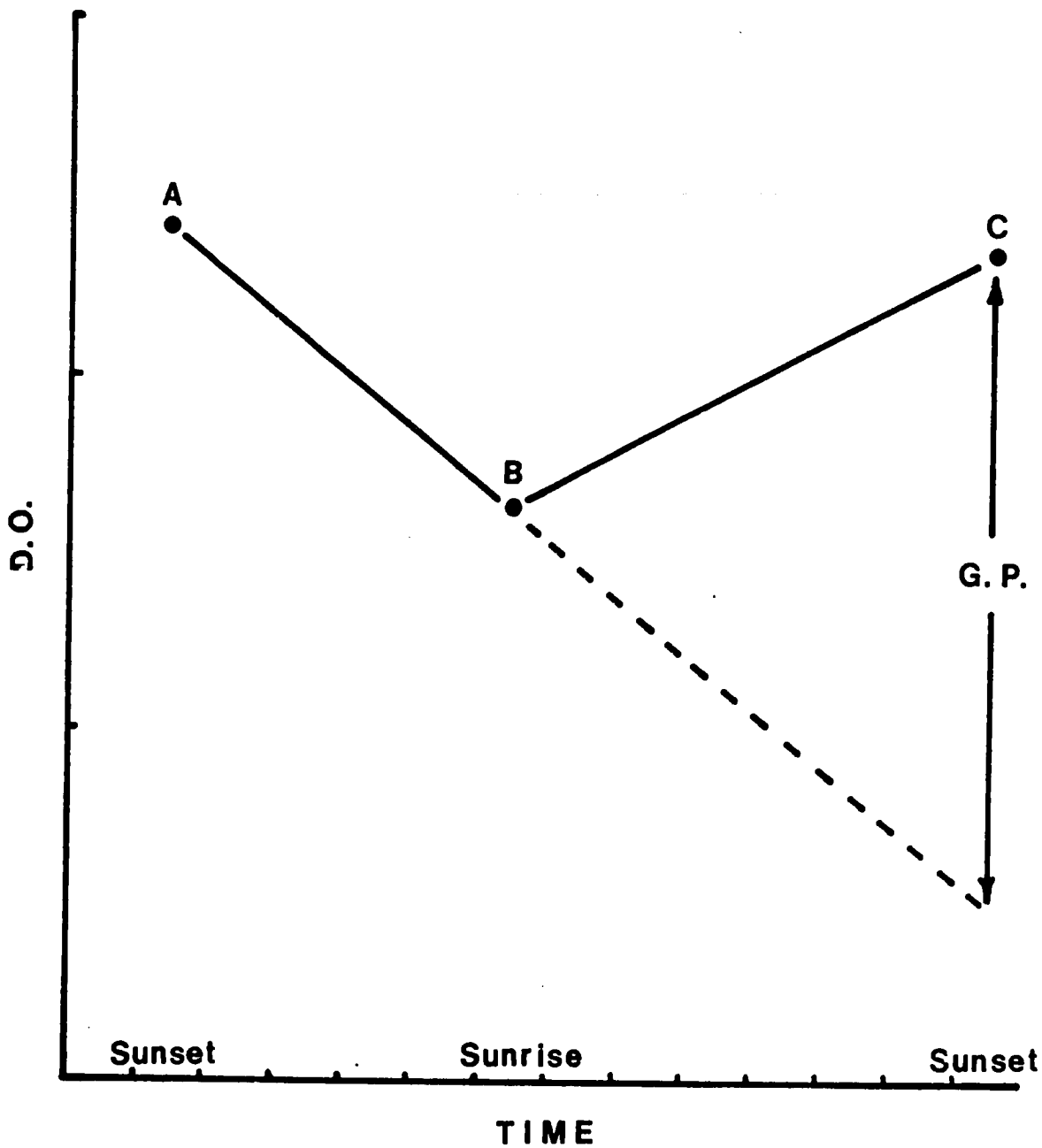


Figure 1. Graphic determination of gross photosynthesis (G.P.) from dissolved oxygen (D.O.) concentrations at sunset, sunrise, and sunset during a 24 hour period. Measured D.O. concentrations indicated by filled circles. The slope of line AB equals the rate of respiration and the slope of line BC equals the rate of gross daylight photosynthesis. Values not corrected for diffusion. Modified from McConnell (1962).

APPENDIX from Work Plan IV

TITLE

Standard Methods for the Examination of Water and Wastewater
15th Edition, 1980 APHA-AWWA-WPCF pages 489-493

## 508 OXYGEN DEMAND (CHEMICAL)

The chemical oxygen demand (COD) is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. For samples from a specific source, COD can be related empirically to BOD, organic carbon, or organic matter content.

### 1. Selection of Method

The dichromate reflux method is preferred over other methods using oxidants because of superior oxidizability, applicability to a wide variety of samples, and ease of manipulation. The test is most useful for monitoring and control, especially after correlations with constituents[1,2] such as BOD and organic carbon have been developed. For most organic compounds oxidation is 95 to 100% of the theoretical value.[2,3] Pyridine is not oxidized.[2] Benzene and other volatile organics are oxi-



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dized if they have sufficient contact with the oxidants.[2] While the carbonaceous portion of nitrogen-containing organic matter is oxidized, no oxidation of ammonia, either present in a waste or liberated from the nitrogen-containing organic matter, takes place in the absence of significant chloride concentrations.

2. Sampling and Storage

Test unstable samples without delay. Homogenize samples containing settleable solids in a blender to permit representative sampling. If there is to be a delay before analysis, preserve the sample by acidification to pH 2 or lower with conc sulfuric acid ($H_2SO_4$). Make preliminary dilutions for wastes containing a high COD to reduce the error inherent in measuring small volumes of sample.

## 508 A. Dichromate Reflux Method

1. General Discussion

*a. Principle:* Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate (FAS), the amount of $K_2Cr_2O_7$ consumed is determined, and the amount of oxidizable organic matter is calculated in terms of oxygen equivalent.

*b. Interferences and limitations:* Volatile straight-chain aliphatic compounds are not oxidized to any appreciable extent. This failure occurs partly because volatile organics are present in the vapor space and do not come in contact with the oxidizing liquid. Straight-chain aliphatic compounds are oxidized more effectively when silver sulfate ($Ag_2SO_4$) is added as a catalyst. However, $Ag_2SO_4$ reacts with chloride, bromide, and iodide to produce precipitates that are oxidized only partially. The difficulties caused by the presence of halides can be largely, though not completely, overcome by complexing with mercuric sulfate ($HgSO_4$) before the refluxing procedure.[4] Do not use the test for samples containing more than 2,000 mg chloride/L.

Nitrite ($NO_2^-$) exerts a COD of 1.1 mg $O_2$/mg $NO_2^-$-N. Because concentrations of $NO_2^-$ in polluted waters rarely exceed 1 or 2 mg $NO_2^-$-N/L the interference is considered insignificant and usually is ignored. To eliminate a significant interference due to $NO_2^-$, add 10 mg sulfamic acid/mg $NO_2^-$-N present in the refluxing flask. Also add the same amount of sulfamic acid to the reflux flask containing the distilled water blank.

Reduced inorganic species such as ferrous iron, sulfide, manganous manganese, etc., are oxidized quantitatively under the test conditions. For samples containing significant levels of these species, stoichiometric oxidation can be assumed from known initial concentration of the interfering species and corrections can be made to the COD value obtained.

*c. Minimum detectable concentration:* Determine COD values of >50 mg/L using 0.250*N* $K_2Cr_2O_7$. With 0.025*N* $K_2Cr_2O_7$, COD values from 5 to 50 mg/L can be determined but with lesser accuracy.[5]

2. Apparatus

*Reflux apparatus*, consisting of 500-mL



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or 250-mL erlenmeyer flasks with ground-glass 24/40 neck* and 300-mm jacket Liebig, West, or equivalent condensers,† with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm² of heating surface, or equivalent.

3. Reagents

*a. Standard potassium dichromate solution*, 0.250*N*: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103 C for 2 hr, in distilled water and dilute to 1,000 mL.

*b. Silver sulfate*, $Ag_2SO_4$, reagent or technical grade, crystals or powder.

*c. Sulfuric acid reagent:* Add $Ag_2SO_4$ to conc $H_2SO_4$ at the rate of 22 g $Ag_2SO_4$/4 kg bottle. Let stand 1 to 2 days to dissolve $Ag_2SO_4$.

*d. Sulfuric acid*, $H_2SO_4$, conc.

*e. Ferroin indicator solution:* Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.‡

*f. Standard ferrous ammonium sulfate titrant*, approximately 0.25*N*: Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (FAS) in distilled water. Add 20 mL conc $H_2SO_4$, cool, and dilute to 1,000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution, as follows:

Dilute 10.0 mL standard $K_2Cr_2O_7$ solution to about 100 mL. Add 30 mL conc $H_2SO_4$ and cool. Titrate with FAS titrant, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

Normality of FAS solution

$$= \frac{\text{Volume 0.25}N\ K_2Cr_2O_7\ \text{solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.25$$

*Corning 5000 or equivalent.

†Corning 2360, 91548, or equivalent.

‡G. F. Smith Chemical Co., Columbus, Ohio.

*g. Mercuric sulfate:* $HgSO_4$, crystals or powder.

*h. Sulfamic acid:* Required only if the interference of nitrites is to be eliminated (see ¶ 1*b* above).

*i. Potassium hydrogen phthalate standard:* Lightly crush and then dry potassium acid phthalate ($HOOCC_6H_4COOK$) to constant weight at 120 C, dissolve 425 mg in distilled water, and dilute to 1,000 mL. Potassium hydrogen phthalate has a theoretical COD of 1.176 g $O_2$/g and this solution has a theoretical COD of 500 mg $O_2$/L. Prepare fresh for each use.

4. Procedure

*a. Treatment of samples with ≤50 mg COD/L:* Place 50.0 mL sample (for samples with COD >900 mg COD/L, use a smaller sample portion diluted to 50.0 mL) in the 500-mL refluxing flask. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$. Cool while mixing to avoid possible loss of volatile materials. Add 25.0 mL 0.250*N* $K_2Cr_2O_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent. CAUTION: *Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents.* If sample volumes other than 50 mL are used, keep ratios of reagent weights, volumes, and strengths constant. See Table 508:1 for examples of applicable ratios. Maintain these ratios and follow the procedure as outlined above.

Use 1 g $HgSO_4$ with a 50.0-mL sample to complex up to a maximum of 100 mg chloride (2,000 mg/L) For smaller samples use less $HgSO_4$, according to the chloride concentration: maintain a 10:1 ratio of $HgSO_4$:Cl. A slight precipitate does not affect the determination adversely. Gener-



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TABLE 508:1. REAGENT QUANTITIES AND NORMALITIES FOR VARIOUS SAMPLE SIZES

| Sample Size *mL* | 0.25*N* Standard Dichromate *mL* | Sulfuric Acid Reagent *mL* | $HgSO_4$ *g* | Normality of FAS | Final Volume before Titration *mL* |
|---|---|---|---|---|---|
| 10.0 | 5.0 | 15 | 0.2 | 0.05 | 70 |
| 20.0 | 10.0 | 30 | 0.4 | 0.10 | 140 |
| 30.0 | 15.0 | 45 | 0.6 | 0.15 | 210 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |

ally, COD cannot be measured accurately in samples containing more than 2,000 mg chloride/L.

Reflux mixture for 2 hr. Use a shorter period for particular wastes if it has been shown that the shorter period yields the same COD as that found by 2-hr refluxing. Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture. Cool and wash down condenser with distilled water.

Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same volume for all titrations. Take as the end point of the titration the first sharp color change from blue-green to reddish brown. The blue-green may reappear.

Reflux and titrate in the same manner a blank containing the reagents and a volume of distilled water equal to that of sample.

*b. Alternate procedure for low-COD samples:* Follow the above procedure, ¶ 4*a*, with two exceptions: (*i*) Use standard 0.025*N* $K_2Cr_2O_7$, and (*ii*) titrate with 0.025*N* FAS. Exercise extreme care with this procedure because even a trace of organic matter on glassware or from the atmosphere may cause gross errors.

If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux as follows: Add all reagents to a sample larger than 50 mL and reduce total volume to 150 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached. Compute amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4$:Cl, using the amount of chloride present in the original volume of sample. Carry a blank reagent through the same procedure.

This technic has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost, but an improvement is gained over ordinary evaporative concentration methods.

*c. Determination of standard solution:* Evaluate the technic and quality of reagents by testing a standard potassium hydrogen phthalate solution.

5. Calculation

$$\text{mg COD/L} = \frac{(A - B) \times N \times 8{,}000}{\text{mL sample}}$$

where:

$A$ = volume FAS used for blank, mL,
$B$ = volume FAS used for sample, mL, and
$N$ = normality of FAS.

### 6. Precision and Accuracy

A set of synthetic samples containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories.[5] At 200 mg COD/L in the absence of chloride, the standard deviation was ± 13 mg/L (coefficient of variation, 6.5%). At 160 mg COD/L and 100 mg chloride/L the standard deviation was ± 14 mg/L (coefficient of variation, 10.8%).

## 508 B. References


1. MOORE, W.A., R. C. KRONER & C.C. RUCHHOFT. 1949. Dichromate reflux method for determination of oxygen consumed. *Anal. Chem.* 21:953.
2. MOORE, W.A., F. J. LUDZACK & C.C. RUCHHOFT. 1951. Determination of oxygen-consumed values of organic wastes. *Anal. Chem.* 23:1297.
3. MEDALIA, A.I. 1951. Test for traces of organic matter in water. *Anal. Chem.* 23:1318.
4. DOBBS, R.A. & R.T. WILLIAMS. 1963. Elimination of chloride interference in the chemical oxygen demand test. *Anal. Chem* 35:1064.
5. ANALYTICAL REFERENCE SERVICE, USHEW-PHS. 1965. Oxygen Demand No. 2. Study No. 21. Environmental Health Ser., Water. PHS Publ. No. 999-WP-26.




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# 209 RESIDUE

The term "residue" refers to solid matter suspended or dissolved in water or wastewater. Residue may affect water or effluent quality adversely in a number of ways. Waters with high residue generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer. Highly mineralized waters also are unsuitable for many industrial applications. For these reasons, a limit of 500 mg residue/L is desirable for drinking waters. Waters with very high levels of nonfiltrable residues may be esthetically unsatisfactory for such purposes as bathing.

## 1. Definitions

"Total residue" is the term applied to the material left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total residue includes "nonfiltrable residue," that is, the portion of total residue retained by a filter, and "filtrable residue," the portion of total residue that passes through the filter.

The earlier-used terms "suspended" and "dissolved" (residue) correspond to nonfiltrable and filtrable residue, respectively. The chemical and physical nature of the material in suspension, the pore size of the filter, the area and thickness of the filter mat, and the amount and physical state of the materials deposited on it are the principal factors affecting separation of nonfiltrable from filtrable residue. A



method designed to control all variables affecting filtration would be too cumbersome for practical use. It must be recognized, therefore, that residue determinations are not subject to the usual criteria of accuracy. The types of residue are defined arbitrarily by the methods used for their determination, and these in turn represent practical approaches to what otherwise would be exceedingly complex operations.

## 2. Sources of Error and Variability

Analyses performed for some special purposes may demand deviation from the stated procedures to include an unusual constituent with the measured residue. Whenever such variations of technic are introduced, record and present them with the results.

In interpreting results, recognize the following sources of error: Results for total, volatile, and fixed residues are subject to considerable error because of losses of volatile compounds during evaporation and of carbon dioxide ($CO_2$) and volatile minerals during ignition; results for residues high in oil or grease content may be questionable because of the difficulty of drying to constant weight in a reasonable time.

The temperature at which the residue is dried has an important bearing on results, because weight losses due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition, as well as weight gains due to oxidation, depend on temperature and time of heating. A choice of two drying temperatures is provided and the analyst should be familiar with the probable effects of each.

"Fixed residue"—the residue remaining after ignition for 1 hr at 550 ± 50 C—does not distinguish precisely between organic and inorganic residue because the loss on ignition is not confined to organic matter. It includes losses due to decomposition or volatilization of certain mineral salts. A better characterization of the organic matter in water can be made by methods such as total organic carbon, BOD, or COD, described in Sections 505, 507, and 508, respectively.

Conductivity measurements are approximately proportional to the filtrable residue and may be used in selecting proper sample size for residue determinations. However, close correlation of results of the two tests is not obtained always.

An additional possibility for checking fixed filtrable residue is by use of ion-exchange procedures described in the Introduction, Section 106.

*Selection of drying temperature:* The methods described are gravimetric and permit a choice of drying temperature.

Residues dried at 103 to 105 C may retain not only water of crystallization but also some mechanically occluded water. Loss of $CO_2$ will result in conversion of bicarbonate to carbonate. Loss of organic matter by volatilization usually will be very slight at this temperature. Because removal of occluded water is marginal at 105 C, attainment of constant weight is very slow.

Residues dried at 180 ± 2 C will lose almost all mechanically occluded water. Some water of crystallization may remain, especially if sulfates are present. Organic matter is lost by volatilization but is not completely destroyed. Bicarbonates are converted to carbonates and carbonates may be decomposed partially to oxides or basic salts. Some chloride and nitrate salts may be lost. In general, evaporating and drying water samples at 180 C yields values for total residue closer to those obtained through summation of individually determined mineral species than the values for total residue secured through drying at a lower temperature.

Select drying temperature best suited to the sample. Examine waters low in organ-

ic matter and total mineral content and intended for human consumption at either temperature, but dry waters containing considerable mineral salts or those with pH over 9.0 at the higher temperature. In any case, report drying temperature.

### 3. Sample Handling and Preservation

Begin analysis as soon as possible because of the impracticality of preserving the sample. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample in Methods A, D, and E.

Water has considerable solvent action on glass. Use resistant-glass bottles or plastic bottles provided that the material in suspension does not adhere to container walls. Analyze samples likely to contain iron or manganese promptly to minimize the possibility of chemical or physical change during storage.

### 4. Selection of Method

Methods A through F are suitable for the determination of residue in potable, surface, and saline waters, as well as domestic and industrial wastewaters in the range up to 20,000 mg/L.

Historically, Method C, determining total filtrable residue dried at 103 to 105 C has been used by most laboratories. Because of problems discussed above, Method B, specifying that the residue be dried at 180 C, is preferable for drinking waters, waters low in organic matter, and waters with high mineral content.

Method G is applicable to determining volatile and fixed fractions in sediments, suspended matter, and solid and semisolid materials produced during water and wastewater treatment.

The amount and type of suspended matter, the purpose of the analysis, and the relative ease of making the determination will dictate whether the nonfiltrable residue is obtained directly or by calculation of the difference between total and filtrable residues.

## 209 A. Total Residue Dried at 103-105 C

### 1. General Discussion

*a. Principle:* A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105 C. The increase in weight over that of the empty dish represents the total residue. Although the results may not represent the weight of actual dissolved and suspended solids in wastewater samples, the determination is useful for plant control. In some instances, correlation may be improved by adding 1N sodium hydroxide (NaOH) to wastewater samples with a pH below 4.3 and maintaining the pH of 4.3 during evaporation. Correct final calculation for added sodium.

*b. Interferences:* Exclude large, floating particles or submerged agglomerates of nonhomogeneous materials from the sample. Disperse visible floating oil and grease with a blender before withdrawing a sample portion for analysis.

### 2. Apparatus

*a. Evaporating dishes:* Dishes of 100-mL capacity made of the following materials:

1) Porcelain, 90-mm diam.
2) Platinum—Generally satisfactory for all purposes.
3) High-silica glass.*

*Vycor, product of Corning Glass Works, Corning, N.Y., or equivalent.



*b. Muffle furnace* for operation at 550 ± 50 C.

*c. Steam bath.*

*d. Drying oven,* for operation at 103 to 105 C.

*e. Desiccator,* provided with a desiccant containing a color indicator of moisture concentration.

*f. Analytical balance,* 200-g capacity, capable of weighing to 0.1 mg.

### 3. Procedure

*a.* Ignite clean evaporating dish at 550 ± 50 C for 1 hr in a muffle furnace.

*b.* Cool, desiccate, weigh, and store dish in desiccator until ready for use.

*c.* Transfer a measured volume of sample to preweighed dish and evaporate to dryness on a steam bath or in a drying oven. Choose a sample volume that will yield a residue between 2.5 mg and 200 mg. Volume required may be estimated from conductivity. If necessary, add successive sample portions to the same dish. When evaporating in a drying oven, lower temperature to approximately 2 C below boiling to prevent splattering.

*d.* Dry evaporated sample for at least 1 hr at 103 to 105 C.

*e.* Cool dish in desiccator to balance temperature and weigh.

*f.* Repeat cycle of drying at 103 to 105 C, cooling, desiccating, and weighing until a constant weight is obtained, or until weight loss is less than 4% of previous weight.

### 4. Calculation

$$\text{mg total residue/L} = \frac{(A - B) \times 1{,}000}{\text{sample volume, mL}}$$

where:

$A$ = weight of sample + dish, mg, and
$B$ = weight of dish, mg.

### 5. Precision and Accuracy

Precision is about ±4 mg or ±5%. When the residue from a 50- to 100-mL sample of raw sewage was weighed, the standard deviation of the weighing was 1.9 mg ($n$ = 3; 60 × 10), but the data are considered statistically unreliable because of sampling errors. On settled effluents a statistically reliable standard deviation of 0.9 mg ($n$ = 1; 5 × 20) was found.

## 209 B. Total Filtrable Residue Dried at 180 C

### . General Discussion

Filtrable residue is material that passes rough a standard glass fiber filter and re- ains after evaporation and drying to con- ant weight at 180 C.[1] The determined lues may not check with the theoretical lue for solids calculated from chemical alysis of water. Approximate methods r correlating chemical analysis with resi- e are available.[2]

The filtrate from the total nonfiltrable sidue (Section 209D) may be used for termination of total filtrable residue.

*Interferences:* Highly mineralized waters with a considerable calcium, magnesium, chloride, and/or sulfate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Samples high in bicarbonate require careful and possibly prolonged drying at 180 C to insure complete conversion of bicarbonate to carbonate.

### 2. Apparatus

All of the apparatus listed in Section 209A.2 is required and in addition:

*a. Glass-fiber filters**, circular, without organic binder.

*b. Filtration apparatus* suitable for filter selected:

1) *Filter holder:* Gooch crucible adapter or membrane filter funnel.

2) *Gooch crucible*, 25-mL to 40-mL capacity, suitable for filter size selected.

*c. Suction flask*, 500-mL capacity.

3. Procedure

*a. Preparation of glass-fiber filter:* Place filter either on membrane filter apparatus or bottom of a suitable Gooch crucible. Apply vacuum and wash filter with three successive 20-mL volumes of distilled water. Continue suction to remove all traces of water. Discard washings.

*b. Preparation of evaporating dish:* Ignite cleaned evaporating dish at 550 ± 50 C for 1 hr in a muffle furnace. Cool and store in desiccator until needed. Weigh immediately before use.

*c. Sample analysis:* Because excessive residue in the evaporating dish may form a water-entrapping crust, use a sample yielding between 2.5 mg and 200 mg total filtrable residue. If sample contains less than 10 mg filtrable residue/L, use 250 mL. Under vacuum, filter well-mixed sample through glass-fiber filter, wash with three successive 10-mL volumes of distilled water, and continue suction for about 3 min after filtration is complete. Transfer filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath. Dry for at least 1 hr in an oven at 180 ± 2 C, cool in a desiccator to balance temperature, and weigh. Repeat drying cycle until a constant weight is obtained or until weight loss is less than 4% of previous weight or 0.5 mg, whichever is less. Base calculation on original sample volume because all filtrate is evaporated.

4. Calculation

$$\text{mg total filtrable residue at 180 C/L} = \frac{(A - B) \times 1{,}000}{\text{sample volume, mL}}$$

where:

$A$ = weight of dried residue + dish, mg, and

$B$ = weight of dish, mg.

*Whatman grade 934AH and 984H; Gelman type A/E; Millipore type AP40; or equivalent. Available in diameters of 2.2-cm to 4.7-cm.

## 209 C. Total Filtrable Residue Dried at 103-105 C

Follow procedure described in Section 209B. Dry filtrate at 103 to 105 C instead of 180 C.

*Precision and accuracy:* In 18 laboratories, a synthetic sample containing 134 mg filtrable residue/L was analyzed at a drying temperature of 103 to 105 C with a standard deviation of 13 mg/L.

## 209 D. Total Nonfiltrable Residue Dried at 103-105 C (Total Suspended Matter)

1. General Discussion

Total nonfiltrable residue is the retained material on a standard glass-fiber filter after filtration of a well-mixed sample. The residue is dried at 103 to 105 C. If the suspended material clogs the filter and prolongs filtration, the difference between the total residue and the total filtrable residue provides an estimate of the total nonfiltrable residue.

Volatile nonfiltrable residue and fixed nonfiltrable residue can be determined on the material retained on the glass-fiber filters in the Gooch crucibles on completion of the drying at 103 to 105 C.

2. Apparatus

Apparatus listed in Sections 209A.2 and 209B.2 is required.

3. Procedure

*a. Preparation of glass-fiber filter:* Place filter either on membrane filter apparatus or the bottom of a suitable Gooch crucible. Apply vacuum and wash filter with three successive 20-mL portions of distilled water. Continue suction to remove all traces of water, and discard washings. Remove filter from membrane filter apparatus and transfer to an aluminum or stainless steel planchet as a support. Remove crucible and filter combination if a Gooch crucible is used. Dry in an oven at 103 to 105 C for 1 hr. Store in desiccator until needed. Weigh immediately before use.

*b. Sample treatment:* Because excessive residue on the filter may entrap water and extend drying time, take for analysis a sample volume that will yield between 2.5 mg and 200 mg total nonfiltrable residue. As a practical limit, filter 100 mL of well-mixed sample under vacuum. Wash filter with three successive 10-mL portions of distilled water. Carefully remove filter from membrane filter funnel assembly and transfer to an aluminum or stainless steel planchet as a support. Alternatively remove crucible and filter combination from crucible adapter if a Gooch crucible is used. Dry for at least 1 hr at 103 to 105 C, cool in a desiccator to balance temperature, and weigh. Repeat drying cycle until a constant weight is attained or until weight loss is less than 4% of previous weight, or 0.5 mg, whichever is less.

*c.* The dried residue in the Gooch crucible may be used for determining volatile and fixed matter at 550 C in Section 209G.3*b*4).

4. Calculation

$$\text{mg total nonfiltrable residue/L} = \frac{(A - B) \times 1{,}000}{\text{sample volume, mL}}$$

where:

$A$ = weight of filter + residue, mg, and

$B$ = weight of filter, mg.

5. Precision and Accuracy

The precision of the determination varies directly with the concentration of suspended matter. The standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1,707 mg/L ($n$ = 2; 4 × 10). There is no satisfactory procedure for obtaining the accuracy of the method on wastewater samples because the true concentration of suspended matter is unknown. See Section 209A.5 for other comments.

## 209 E. Total Volatile and Fixed Residue at 550 C

1. General Discussion

The volatile and fixed components in the total residue of Method A may be determined by igniting the sample at 550 ± 50 C. The determination is useful in con-

trol of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

### 2. Apparatus

See Sections 209A.2 and 209B.2.

### 3. Procedure

Ignite residue produced by Method A to constant weight in a muffle furnace at a temperature of 550 ± 50 C. Constant weight has been reached when two successive weighings do not differ by more than 4%. Have furnace up to temperature before inserting sample. Usually, 15 to 20 min ignition are required. Let dish cool partially in air until most of the heat has been dissipated. Transfer to a desiccator for final cooling in a dry atmosphere. Do not overload desiccator. Weigh dish as soon as it has cooled completely. Report loss of weight on ignition as total volatile residue and weighed residue as total fixed residue.

### 4. Calculation

$$\text{mg volatile residue/L} = \frac{(A - B) \times 1{,}000}{\text{sample volume, mL}}$$

$$\text{mg fixed residue/L} = \frac{(B - C) \times 1{,}000}{\text{sample volume, mL}}$$

where:

$A$ = weight of residue + dish before ignition, mg,
$B$ = weight of residue + dish after ignition, mg, and
$C$ = weight of dish, mg.

### 5. Precision and Accuracy

Three laboratories examined four samples by means of 10 replicates with a standard deviation of 11 mg/L at 170 mg/L volatile residue concentration.

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## 209 F. Settleable Matter

### 1. General Discussion

Settleable matter in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (milliliters per liter) or a weight (milligrams per liter) basis.

### 2. Apparatus

The apparatus listed under Sections 209A.2 and 209B.2, and an Imhoff cone, are required for a gravimetric test. The volumetric test requires only an Imhoff cone.

### 3. Procedure

*a. By volume:* Fill an Imhoff cone to the 1-L mark with a thoroughly mixed sample. Settle for 45 min, gently stir sides of cone with a rod or by spinning, settle 15 min longer, and record volume of settleable matter in the cone as milliliters per liter. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled matter. The practical lower limit of measurement is about 1 mL/L. Where a separation of settleable and floating materials occurs, do not estimate the floating material as settleable matter.

*b. By weight:*

1) Determine total nonfiltrable residue of well-mixed sample (Section 209D).

2) Pour a well-mixed sample into a glass vessel of not less than 9 cm diam. Use a sample of not less than 1 L and sufficient to give a depth of 20 cm. Alternatively use a glass vessel of greater diameter and a larger volume of sample. Let stand quiescent for 1 hr and, without disturbing the settled or floating material, siphon 250 mL from center of container at a point halfway between the surface of the settled sludge and the liquid surface. Determine nonfiltrable residue (milligrams per liter) of this supernatant liquor (Section 209D). This is the nonsettling matter.

### 4. Calculation

mg settleable matter/L
= mg suspended matter/L
− mg nonsettleable matter/L



## 209 G. Volatile and Fixed Matter in Nonfiltrable Residue and in Solid and Semisolid Samples

### 1. General Discussion

This method is applicable to the determination of total residue on evaporation and its fixed and volatile fractions in such solid and semisolid samples as river and lake sediments, sludges separated from water and wastewater treatment processes, and sludge cakes from vacuum filtration, centrifugation, or other sludge dewatering processes.

The determination of both total and volatile residue in these materials is subject to negative error due to loss of ammonium carbonate [$(NH_4)_2CO_3$] and volatile organic matter while drying. Although this is true also for wastewater, the effect tends to be more pronounced with sediments, and especially with sludges and sludge cakes.

The mass of organic matter recovered from sludge and sediment requires a longer ignition time than that specified for residue from wastewaters, effluents, or polluted waters. Carefully observe specified ignition time and temperature to control losses of volatile inorganic salts.

Make all weighings quickly because wet samples tend to lose weight by evaporation. After drying or ignition, residues often are very hygroscopic and rapidly absorb moisture from the air.

### 2. Apparatus

See Sections 209A.2 and 209B.2.

### 3. Procedure

*a. Solid and semisolid samples:*

1) Total residue and moisture—

a) Preparation of evaporating dish—Ignite a clean evaporating dish at 550 ± 50 C for 1 hr in a muffle furnace. Cool in a desiccator, weigh, and store in a desiccator until ready for use.

b) Fluid samples—If the sample contains enough moisture to flow more or less readily, stir to homogenize, place 25 to 50 g in a prepared evaporating dish, and weigh to the nearest 10 mg. Evaporate to dryness on a water bath, dry at 103 C for 1 hr, cool in an individual desiccator containing fresh desiccant, and weigh.

c) Solid samples—If the sample consists of discrete pieces of solid material (dewatered sludge, for example), take cores from each piece with a No. 7 cork borer or pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves. Place 25 to 50 g in a prepared evaporating dish and weigh to the nearest 10 mg. Place in an oven at 103 C overnight. Cool in an individual desiccator containing fresh desiccant and weigh. Prolonged heating may result in a loss of volatile organic matter and $(NH_4)_2CO_3$, but it usually is necessary to dry samples thoroughly.

2) Volatile residue—Determine volatile residue, including organic matter and volatile inorganic salts, on the total residue

obtained in 1) above. Avoid loss of solids by decrepitation by placing dish in a cool muffle furnace, heating furnace to 550 C, and igniting for 60 min. (First ignite samples containing large amounts of organic matter over a gas burner and under an exhaust hood in the presence of adequate air to lessen losses due to reducing conditions and to avoid odors in the laboratory.) Cool in a desiccator and reweigh. Report results as fixed residue (percent ash) and volatile residue.

*b. Nonfiltrable residue (suspended matter):*

1) Preparation of glass-fiber filter—Place a glass-fiber filter in a membrane filter holder, Hirsch funnel, or Buchner funnel, with wrinkled surface of filter facing upward. Apply vacuum to the assembled apparatus to seat filter. With vacuum applied, wash filter with three successive 20-mL portions of distilled water. After the water has filtered through, disconnect vacuum, remove filter, transfer to an aluminum or stainless steel planchet as a support, and dry in an oven at 103 C for 1 hr (30 min in a mechanical convection oven). If volatile matter is not to be determined, cool filter in a desiccator to balance temperature and weigh. If volatile matter is to be determined, transfer filter to a muffle furnace and ignite at 550 C for 15 min. Remove filter from furnace, place in a desiccator until cooled to balance temperature, and weigh.

2) Treatment of sample—Except for samples that contain high concentrations of filtrable matter, or that filter very slowly, select a sample volume ≥14 mL/cm² filter area.

Place prepared filter in membrane filter holder, Hirsch funnel, or Buchner funnel, with wrinkled surface upward. With vacuum applied, wet filter with distilled water to seat it against holder or funnel. Measure well-mixed sample with a wide-tip pipet or graduated cylinder. Filter sample through filter using suction. Leaving suction on, wash apparatus three times with 10-mL portions of distilled water, allowing complete drainage between washings. Discontinue suction, remove filter and dry to constant weight (see 209B.3c) at 103 C for 1 hr in an oven (30 min in a mechanical convection oven). After drying, cool filter in a desiccator to balance temperature and weigh.

3) Filtration with Gooch crucibles—Alternatively, use glass-fiber filters of 2.2 or 2.4 cm diam with Gooch crucibles and follow the procedure in Section 209D.3*b*.

4) Ignition—Ignite filter with its nonfiltrable residue (total suspended matter) for 15 min at 550 ± 50 C, transfer to a desiccator, cool to balance temperature, and weigh.

## 4. Calculation

*a. Solid and semisolid samples:*

$$\%\ \text{total residue} = \frac{A \times 100}{B}$$

$$\%\ \text{volatile residue} = \frac{(A - C) \times 100}{A}$$

$$\%\ \text{fixed residue} = \frac{C \times 100}{A}$$

*b. Nonfiltrable residue (suspended matter):*

$$\text{mg nonfiltrable volatile residue/L} = \frac{(D - E) \times 1{,}000}{\text{sample volume, mL}}$$

$$\text{mg nonfiltrable fixed residue/L} = \frac{C \times 1{,}000}{\text{sample volume, mL}}$$

where:

$A$ = weight of dried solids, mg,
$B$ = weight of wet sample, mg,
$C$ = weight of ash, mg,
$D$ = weight of residue before ignition, mg, and
$E$ = weight of residue after ignition, mg.

## 5. Precision and Accuracy

See Section 209D.5.



# 209 H. References


1. Methods for Chemical Analysis of Water and Wastes. 1974. U.S. EPA, Technology Transfer, 625-/6-74-003, pp. 266-267.
2. SOKOLOFF, V.P. 1933. Water of crystallization in total solids of water analysis. *Ind. Eng. Chem.*, Anal. Ed. 5:336.


# 209 I. Bibliography


THERIAULT, E.J. & H.H. WAGENHALS. 1923. Studies of representative sewage plants. *Pub. Health Bull.* No. 132.

HOWARD, C.S. 1933. Determination of total dissolved solids in water analysis. *Ind. Eng. Chem.*, Anal. Ed. 5:4.

SYMONS, G.E. & B. MOREY. 1941. The effect of drying time on the determination of solids in sewage and sewage sludges. *Sewage Works J.* 13:936.

FISCHER, A.J. & G.E. SYMONS. 1944. The determination of settleable sewage solids by weight. *Water Works Sewage* 91:37.

DEGEN, J. & F.E. NUSSBERGER. 1956. Notes on the determination of suspended solids. *Sewage Ind. Wastes* 28:237.

CHANIN, G., E.H. CHOW, R.B. ALEXANDER & J. POWERS. 1958. Use of glass fiber filter medium in the suspended solids determination. *Sewage Ind. Wastes* 30:1062.

NUSBAUM, I. 1958. New method for determination of suspended solids. *Sewage Ind. Wastes* 30:1066.

SMITH, A.L. & A.E. GREENBERG. 1963. Evaluation of methods for determining suspended solids in wastewater. *J. Water Pollut. Control Fed.* 35:940.

GOODMAN, B.L. 1964. Processing thickened sludge with chemical conditioners. Pages 78 et seq *in* Sludge Concentration, Filtration and Incineration. Univ. Michigan Continued Education Ser. No. 113, Ann Arbor.

WYCKOFF, B.M. 1964. Rapid solids determination using glass fiber filters. *Water Sewage Works* 111:277.


# 210 SALINITY

Salinity is an important measurement in the analysis of certain industrial wastes and seawater. It is defined as the total solids in water after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized. It is numerically smaller than the filtrable residue and usually is reported as grams per kilogram or parts per thousand (‰).

Associated terms are chlorinity, which includes chloride, bromide, and iodide, all reported as chloride, and chlorosity, which is the chlorinity multiplied by the water density at 20 C. An empirical relationship[1] between salinity and chlorinity often is used:

$$\text{Salinity, ‰} = 0.03 + 1.805\ (\text{chlorinity, ‰})$$

*Selection of method:* Three procedures are presented. The electrical conductivity (A) and hydrometric (B) methods are suited for field use along a shoreline or in a small boat. For laboratory or field analysis of estuarine or coastal inlet waters the argentometric method (C) is recommended.