TITLE XII

COLLABORATIVE RESEARCH SUPPORT PROGRAM

POND DYNAMICS/AQUACULTURE

CRSP WORK PLAN: FOURTH EXPERIMENTAL CYCLE

September 1, 1987 - August 31, 1989

(Revised January 1989)

Pond Dynamics/Aquaculture CRSP
Program Management Office
Office of International Research & Development
Oregon State University
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Oregon State University
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This work plan describes a standardized set of experiments to be undertaken by the Pond Dynamics/Aquaculture CRSP during the fourth and fifth years of project operation. *Program activities are funded in part by AID Grant No.: DAN-4023-G-SS-7066-00.*

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WP 4

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INTRODUCTION

The fourth work plan for the Pond Dynamics/Aquaculture CRSP was developed by the CRSP Technical Committee at a meeting in Kona, Hawaii on January 11-19, 1988. In response to the recommendation of the External Evaluation Panel, this is the first biennial work plan developed by the CRSP. Previous work plans were developed on an annual basis. This work plan describes research activities to be accomplished during the period from September 1, 1987 through August 31, 1989.

Previous CRSP work plans were directed towards compiling a global baseline of data for aquaculture pond systems. Data were collected at seven research locations in six developing countries (Honduras, Indonesia, Panama, Philippines, Rwanda, and Thailand) in Africa, Asia, and Central America. The data were collected in accordance with a standard protocol; the same experiments were conducted at all locations. The resulting data are compiled in a central CRSP Data Base, which is maintained by the Management Entity.

In the course of completing the earlier work plans, CRSP researchers identified specific questions about the dynamic processes that regulate the productivity of aquaculture ponds. The answers to these questions are essential to improving pond management practices. The fourth work plan outlines the methodology for conducting experiments to answer these questions.

The general objectives of the present work plan are:

(1) to preserve the global nature of the CRSP experiments;
(2) to continue entering standardized data into the global CRSP Data Base;
(3) to conduct experiments to test specific hypotheses about pond dynamics; and
(4) to accelerate the synthesis and translation of CRSP research findings into transferable technologies for aquaculture pond management.

WORK PLAN

This work plan differs from earlier work plans in which the same experiment was conducted at each location. Hypotheses about pond dynamics will be tested in different field experiments at each research location. It is anticipated that this procedure will allow the CRSP to proceed rapidly through the testing process. Otherwise, many years of work would be required to thoroughly evaluate each hypothesis at all sites.

In addition to the division of experiments between the sites, the CRSP global experiment will continue intensive sampling of pond variables during the course of each field experiment. A standard sampling protocol will be used at all locations, and the standardized data will be added to the CRSP Data Base to make the existing information even more comprehensive. Sampling protocol, descriptions of field experiments, and data synthesis activities are presented below.

The CRSP Global Experiment

CRSP researchers should read this section carefully because there have been significant changes from previous work plans. In particular, section headings have been changed to reflect alterations in the sampling protocol, some parameters are no longer required, and the frequency of sampling for the "diurnal" studies (now more appropriately named "diel" studies) has been changed from every six hours to the following: pre-dawn, 1000, 1400, 1600, 1800, 2300, and pre-dawn of the next day. Unless otherwise indicated, the fish cultural and analytical methods are as presented in appropriate appendixes to the third CRSP work plan. Please refer to Standard Methods for the Examination of Water and Wastewater for methods not included in the third work plan. Some methods for new procedures are included in the appendix for easy reference. All research locations will follow the same protocol for daily measurements, intensive sampling measurements, fish measurements, optional measurements, and occasional measurements.

Daily Measurements

(See Table 1 for Materials and Methods.)

The following meteorological and physical pond parameters will be recorded daily:

- Solar Radiation
- Wind Speed (*Note*: Anemometers are to be set at a height of 2 m above the level of the pond banks)
- Air Temperature (maximum and minimum)
- Rainfall
- Evaporation
- Pond Depth
- Pond Inflow

Intensive Sampling Measurements
(See Table 2 for Materials and Methods.)

There will be three intensive sampling periods for each experiment: (1) during the second week; (2) midway through the experiment; and (3) during the final week. A number of parameters will be determined on a whole water column sample for each pond in addition to the studies. Primary productivity will be determined by calculation from data collected during the diel studies (i.e., whole pond determinations). The variables to be observed are:

- Total Kjeldahl Nitrogen
- Ammonia Nitrogen
- Total Phosphorus
- Secchi Disk
- Chlorophyll *a*
- Dark Bottle Respiration
- Total Suspended Solids
- Total Volatile Solids
- Diel Studies (Sampling times: pre-dawn, 1000, 1400 1600, 1800, 2300, and pre-dawn the next day. Sample at three depths (top, middle, bottom) for each pond): Dissolved Oxygen
  Temperature
  pH
  Alkalinity
  Wind (cumulative between sampling times)
  Solar Radiation (cumulative between sampling times)

Fish Measurements
(See Table 3 for Materials and Methods.)

Sex-reversed *Oreochromis niloticus* of an average weight of 25 grams will be stocked at a rate of two fish/m^2 (20,000 fish/hectare). In addition to the specific measurements listed below, a record will be kept of any reproduction that may occur during the experiment.

- Initial Stocking
  - Total Number
  - Group Weight
  - Mean Weight per Individual
  - Mean Length per Individual
- Monthly Sampling
  - Sample Number
  - Group Weight
  - Mean Weight per Individual
  - Mean Length per Individual
- Harvest
  - Total Number
  - Group Weight
  - Mean Weight per Individual
  - Mean Length per Individual
  - Survival (% of initial stocked)

Optional Monthly Measurements
(See Table 4 for Materials and Methods.)

- Phytoplankton Composition
- Zooplankton Composition
- Benthos Composition

Occasional Measurements
(See Table 5 for Materials and Methods.)

- Pond Soil Characteristics at the beginning and end of each experiment
- Liming Requirements
- Pond Morphometric Characteristics
- Seepage (to complete hydrological characteristics, most of which are included under "Daily Measurements" above)
- Chemical Oxygen Demand (COD) of Inputs
- Nutrient Analysis of Inputs

FIELD EXPERIMENTS

Central America - Honduras Project

Cooperating Institutions and Principal Investigators:

Auburn University (lead institution)
Drs. Bryan Duncan and Claude Boyd

CIFAD (University of Hawaii)
Dr. Kevin Hopkins

Pond System: Brackish water or freshwater ponds

September 1, 1987 to August 31, 1988

INTRODUCTION

Research over the past several years at El Carao on nutrient input vs. tilapia production has led to a vastly improved understanding of aquaculture and the economics of aquaculture in Honduras. However, much work still needs to be done on optimizing economic gain by mixing low-cost manure with high-cost commercial feeds. Of greater interest is the point in the growth cycles at which it becomes of biological and economic benefit to start adding feeds in addition to, or in place of, manure. This problem quickly becomes complicated with varying stocking densities, so additional testing is needed.

Previous CRSP research indicates that plankton populations cyclically increase and decrease, and that the frequency and amplitude of this cycling probably is related to quantity of nutrient input. Since oxygen dynamics are integrally bound to plankton dynamics, the intensive study of plankton cycling may contribute to better management for oxygen in ponds. However, the frequency of water sampling to date has not been sufficient to adequately document the cycles. Frequency of water sampling can usually be increased only if fewer ponds are being sampled. Thus, more frequent sampling of fewer ponds may yield a more complete picture of pond dynamics.

GOAL

To more fully understand oxygen dynamics of fish production ponds and to refine production systems to make them more economically productive in Honduras and tropical Central America.

OBJECTIVES

To document cyclical changes in water chemistry and biology and to correlate one with the other; to set up a data logging system on the pond bank; and to economically refine tilapia production systems based on feed and chicken litter inputs.

Materials and Methods

Four treatments, each replicated three times, will be tested in earthen ponds of 1,000 m^2. The treatments will be as follows.

1. Chicken litter added at 500 kg/ha/wk, total solids.

2. Chicken litter added at 500 kg/ha/wk, total solids, and a commercial shrimp diet (25% crude protein) added daily (six days a week) at 0.5% biomass.

3. Chicken litter added at 500 kg/ha/wk, total solids, and a commercial shrimp diet (25% crude protein) added daily (six days a week) at 1% biomass.

4. Chicken litter added at 500 kg/ha/wk, total solids, and a commercial shrimp diet (25% crude protein) added daily (six days a week) at 2% biomass.

Water Quality Measurements

Early morning oxygen and chlorophyll *a* will be measured in all ponds twice and once per week, respectively. Total alkalinity and total hardness will be measured in all ponds once per month. Primary productivity will be measured in all ponds once per week using the 24-hr diurnal oxygen curve method. Oxygen will be measured at 0, 25, 50, and 75 cm depth at 6 a.m., 10 a.m., 2:30 p.m., 6 p.m., and again the following day at 6 a.m. Once a month, diurnal oxygen will be measured every two hours during the day in ponds of treatment 1. Total volatile solids will also be measured in ponds of treatment 1 once per month to coincide with the oxygen diurnal.

Two ponds each from treatments 1 and 4 will be measured twice a week for chlorophyll *a*, filterable orthophosphate, total phosphorus, total ammonia, pH, primary productivity (diurnal oxygen curve method), and zooplankton density. All variables will be analyzed in samples of water combined from four to five 90-cm column subsamples taken from a transection of the pond between 6:43 and 7:30 in the morning. Zooplankton will be filtered from 12 liters of water using an 80-micron mesh net, and will be enumerated by groups of copepoda, cladocera, rotifera, and nauplii. A data logging system will be set up on the pond bank to sample these same four ponds for oxygen, pH, and temperature, every 20 minutes, 24 hours a day.

Climatic Variables

Wind speed, solar radiation, rainfall, and evaporation will be measured daily. During diurnals, wind speed will be measured on the hour that oxygen is measured in the ponds.

Data Analysis

Costs will be assigned to all inputs and outputs of these production systems, and ANOVA and regression analyses will be used to help determine which system gives optimum economic gain. Special attention will be given to times on the growth

curves at which fish growth among treatments becomes different. Effects on primary productivity of adding feed with manure will be analyzed.

Variables measured frequently in the four ponds will be subjected to graphical and correlation analyses to document plankton cycling and to correlate this cycling with water chemistry. Differences in cycle frequency and/or amplitude between ponds receiving high and low quantities of nutrient input will be analyzed.

September 1, 1988 - August 31, 1989

INTRODUCTION

Previous studies indicate that plankton and oxygen dynamics are measureably affected by density of planktivorous fish; however, no known studies have been specifically designed to quantify these effects in ponds. Moreover, planktivorous fish would presumably have more influence on plankton dynamics in organically fertilized ponds where concentrated diets are not being used. Such systems are used more extensively in the tropical world than in the temperate zones, so the influence of fish density on plankton dynamics is more appropriately studied in the tropics. Consequently, this work plan will be dedicated to that end.

GOALS

To more fully understand oxygen dynamics of fish production ponds and to refine production systems to make them more economically productive in Honduras and tropical Central America.

OBJECTIVES

To document cyclical changes in water chemistry and biology and to correlate one with the other; to analyze effects of fish density on the dynamics of plankton populations and oxygen dynamics; to economically refine tilapia production systems based on chicken litter and feed inputs; and to discover whether there is a difference in growth between sex-reversed and normal male tilapia.

Materials and Methods

Three stocking densities of fish, each replicated four times, will be tested in earthen ponds of 1,000 m^2 (see Figure 1). *Oreochromis niloticus* will be stocked in all ponds at 0.5, 1, or 2/m^2; two ponds within each treatment will contain normal males and two will contain sex-reversed fish. This trial will last 20 weeks.

Sex-reversed and normal fish will have been produced by the same parents and handled in an equal manner throughout the hormone treatment and nursery stages. This will culminate in a study of the influence of androgen treatment on tilapia growth from larvae to harvest.

Water Quality Measurements

Chlorophyll *a*, primary production, zooplankton density, Kjeldahl nitrogen, total ammonia nitrogen, pH, total phosphorus, and filterable orthophosphate will be

measured twice a week, and total hardness and total alkalinity will be measured monthly in two ponds (those ponds containing the sex-reversed fish) from each treatment.

Primary production will be measured using the 24-hour diurnal oxygen curve method. Oxygen will be measured at 0, 25, 50, and 75 cm depth at 6 a.m., 10 a.m., 2:30 p.m., 6 p.m. and again the following day at 6 a.m. When the data logging system is functional, oxygen will be measured at two or three depths every 20 minutes, 24 hours a day. Water quality variables will be analyzed in samples of water combined from four to five 90-cm column subsamples taken from a transection of the pond between 6:30 and 7:30 in the morning. Zooplankton will be filtered from 12 liters of water using an 80-micron mesh net, and will be enummerated by groups of copepoda, cladocera, rotifera, and nauplii.

Climatic Variables

Wind speed, solar radiation, rainfall, and evaporation will be measured daily. During diurnals, wind speed will be measured on the hour that oxygen is measured in the ponds. Quantities of water added to ponds will be recorded.

Data Analysis

By use of ANOVA and correlation methods, we intend to answer the following questions:

A. Do hormone-treated fish grow faster than normal fish?

B. Does the growth relationship between hormone-treated and normal fish remain the same under conditions of adequate nutrient availability as well as inadequate nutrient availability (i.e., does stocking density or additions of high quality feeds moderate the influence of hormone treatment on fish growth)?

C. Does the fish density (i.e., cropping intensity) influence primary productivity and oxygen dynamics?

D. Is the cyclical rise and fall of plankton populations or the amplitude of the cycles affected by fish density?

Benefits

This study will evaluate the most cost-effective means for providing nutrient input to fish ponds. It will quantify effects of low dissolved oxygen concentrations on fish growth and yield. It also will evaluate artificial aeration as a means to overcome oxygen limitation. All of these parameters are important in the management of tilapia ponds.

CRSP/Aquaculture Project Design for January 1989 at El Carao, Honduras

Stocking Density	Hormone Treat	Reps	Water Quality
5,000/ha	Reversed	2	2/week in one replicate
	Normal	2	2/week in one replicate
10,000/ha	Reversed	2	2/week in one replicate
	Normal	2	2/week in one replicate
20,000/ha	Reversed	2	2/week in one replicate
	Normal	2	2/week in one replicate

Pond System: **Granjas Marinas San Bernardo Brackish Water Production Experiment**

Project Narrative:

GOALS

The purpose of the proposed first research cycle experiment (Sept.-Dec. 1988) is to compare the brackish water production obtained using alternative management systems to that obtained with the standard management procedures as currently practiced at Granjas Marinas. Twelve 5.0 acre (2.0 ha) ponds will be used in this study, where the following treatments will be tested utilizing a completely randomized design.

A. Standard Procedure
   - Chicken litter applied weekly at one sack ac^{-1} [84 kg ha^{-1} wk^{-1} (75 lb ac^{-1} wk^{-1})] during the first eight weeks.
   - Standard San Bernardo feeding regime from stocking.

B. Feed Only
   - Standard San Bernardo feeding regime from stocking.

C. Fertilize For Four Weeks, Then Feed
   - Chicken litter applied weekly at one sack ac^{-1} [84 kg ha^{-1} wk^{-1} (75 lb ac^{-1} wk^{-1})] during the first four weeks.
   - Standard San Bernardo feeding regime beginning week 5, adjusted for shrimp size at that point.

D. Fertilize For Eight Weeks, Then Feed
   - Chicken litter applied weekly at one sack ac^{-1} [84 kg ha^{-1} wk^{-1} (75 lb ac^{-1} wk^{-1})] during the first eight weeks.
   - Standard San Bernardo feeding regime beginning week 9, adjusted for shrimp size at that point.

Materials and Methods

A. Pond Area and Average Depth
   - Fill all ponds to normal depth in shortest possible time.

- Obtain pond dimensions using 50 m tape.
- Obtain average pond depth by multiple sampling.

B. Stocking
   - Juveniles will be stocked at + 5/m^2 (20,000/acre).
   - All ponds will be stocked on the same day.
   - All juveniles must come from the same nursery pond or a common 'pool' made by mixing the shrimp from more than one pond prior to stocking.
   - Juveniles will be stocked into all ponds in a two-phase operation. All ponds will first receive 50% of their total number. Once all ponds have received this initial stocking, the remaining 50% of the population will be stocked into each pond.
   - Total weight, total number and species composition of juveniles stocked into each pond will be recorded.
   - Sample shrimp in each pond weekly to determine growth using standard San Bernardo protocol.

C. Feed and Manure
   - The daily ration for ponds within each treatment will be determined using the mean individual shrimp weight for that treatment; mean individual shrimp weight is obtained by averaging sample weight of shrimp from replicate ponds within each treatment. Feeding rates will be adjusted weekly based upon sample data.
   - Feed will be weighed to the nearest pound or kilogram.
   - Chicken litter will be broadcast over the pond surface.
   - Quantities of feed and manure added to ponds will be recorded.

D. Dissolved Oxygen (DO)
   - DO will be measured in each pond according to standard San Bernardo protocol.

E. Secchi Disk Visibility (SDV)
   - SDV will be measured in all ponds weekly between 11:00 a.m. and 1:00 p.m. SDV will be measured at two locations per pond; at each location the depth of disappearance and that of reappearance noted, with SDV being the average of the two.

F. Salinity
   - Salinity will be measured in each pond weekly using a refractometer; three 90-cm water column samples will be pooled and used for the salinity determination.

G. Water Exchange
   - Water will be exchanged in ponds according to normal San Bernardo operating procedures.
   - Records of dates and approximate percentage of water exchange will be kept.

H. Harvest
   - All ponds will be harvested the same day.
   - The total weight of shrimp harvested from each pond will be determined.
   - The average individual weight of shrimp, by species, will be determined for each pond.
   - The species composition of the shrimp population in each pond will be determined.
   - The harvest shrimp will be classified by size category.

Africa - Rwanda Project

Cooperating Institutions and Principal Investigators:

CIFAD (Oregon State University - lead institution)
Dr. Wayne Seim

Auburn University
Dr. Tom Popma

National University of Rwanda

Pond System: Cooler freshwater tropical ponds typical of higher elevations

Project Narrative:

INTRODUCTION

Pond culture of *Oreochromis niloticus* and other fish depends upon the addition of natural or artificial fertilizers that increase the abundance of natural food items in the diet of the cultured fish, or the direct feeding of an artificial diet. Food items used to prepare a balanced artificial diet for fish are scarce or expensive in Rwanda and many other developing countries. Manufactured nitrogen and phosphorus fertilizers must be imported from developed countries and are too expensive for pond culture of fish. It appears that the most practical means of increasing fish growth is by developing a compost system for utilizing a mixture of animal manures and vegetation.

GENERAL APPROACH

The relatively low productivity of small ponds can be enhanced greatly by maximizing nutrient inputs. Important changes can occur in physicochemical and biological components of this production-enhanced system. The nature of this response is known to be variable both in terms of the ecological processes involved and in terms of the levels of success in enhancing productivity for the species of interest. Variability in system response can be attributed partially to the chemical form of the nutrient materials added and to the rate of nutrient addition. Molecular form (i.e., whether nitrogen is mineralized or organic, or whether organic carbon is present) and the ratios between components are critical factors in determining relative importance of autotrophic and heterotrophic assemblages of organisms and overall system productivity.

This study will investigate the relation between chemical input and pond system response. Input materials will be biologically derived. Higher elevation portions of Africa's interior are generally densely populated, unindustrialized areas where commercial, inorganic fertilizers are in short supply and expensive. Composting available biological materials may be an efficient and effective way of enhancing annual protein production, conserving valuable nutrients, and increasing the economic productivity of the small farmer.

Investigation of pond-system processes will involve measurements of the following critical compartments of this system: materials dissolved in water; materials suspended as particulates in the water column; and materials settled in a benthic deposit.

Measurements of organic carbon, chlorophyll, organic and total nitrogen, total phosphorus, dry weight and loss on ignition (where applicable) will provide the basis for examining the resource-consumer relationships between these components and the top consumer, tilapia. While nutrient and energy pathways may not be fully separable by these techniques, these measurements in conjunction with food preference studies will allow strong inferences concerning the relative importance of autotrophic and heterotrophic pathways. Relationships will be developed from values of mass or density rather than rates of transformation or utilization, which are much more difficult to measure and which change more rapidly. However, rates can be inferred from changes in mass and from consumer-resource interactions.

This approach will provide insights into pond dynamics as part of the overall CRSP model format while also investigating specific questions relating to the use of composted materials as nutrient sources in African aquaculture.

GENERAL OBJECTIVES

The major objective is to improve tilapia production by studying the relations between nutrient materials and biological components of pond systems receiving variable compost inputs. The purpose is to develop an adequate understanding of the role of plant and manure composts, added at different levels and ratios, in pond productivity. This understanding will contain sufficient generality to be useful in the global context of the CRSP studies. It will also provide insights into pond dynamics that will be useful for evaluating pond management practices.

Operational Objectives to accomplish the general objective:

A. To evaluate the effect of varying the manure:plant ratio on nutrient composition of composts.

B. To determine the relationships of compost characteristics and input rates to tilapia production.

C. To determine the relationships of organic carbon, organic nitrogen, total nitrogen, and total phosphorus in dissolved, particulate, and benthic compartments within the ponds to the production, biomass, and food preferences of tilapia.

D. To determine the relationship between tilapia stocking density and production for optimized compost inputs.

EXPERIMENTAL PROTOCOL

Study 1. Compost Characteristics.

Development of an optimum compost system that provides predictable results for fish culture requires consideration of protein:carbohydrate ratios, particle size and

fiber content of decomposing material, nitrogen:phosphorus ratios, anaerobic versus aerobic systems of decomposition in the pond and on land, and the thermal requirements of the composting materials. Establishing a standard compost mixture for increasing fish production is the first step in our cooperative research project.

Objectives

A. To determine the characteristics of compost formed aerobically and anaerobically on land and anaerobically in water.

B. To characterize these composts at five ratios of manure:plant matter in relation to time of composting. The composition of the nutrient input and the quantity and chemical form of nutrient elements will influence the effect of these materials on pond productivity and on the ecological processes and trophic pathways within the ponds.

Methods

Composting will be done aerobically and anaerobically on land and anaerobically in water. Aerobic compost will be hand-mixed daily. Anaerobic composting will be accomplished in earthen pits. Anaerobic composting in water will occur in small pond enclosures.

Ratios of manure:plant matter tested will be 0:1, 1:1, 1:3, 1:5, 1:9. Initial material volume will be 0.5 m^3. The experiment will continue a maximum of 90 days or until the concentration of organic matter stabilizes. Samples will be taken at 0, 15, 30, 60, and 90 days.

Benefits

This experiment provides two major benefits. First, the composition of materials to be added in our subsequent experiments will be well characterized. Thus, analysis of nutrient pathways and fish production in pond experiments can be related to specific characteristics of the nutrient input. For instance, organic content of nutrient inputs may determine relative importance of autotrophic and heterotrophic pathways.

Secondly, the time course of oxidation and mineralization within the compost will provide the necessary basis for control and prediction of compost quality.

Study 2. Pond Response in Relation to Compost Type

The method of composting is probably an important factor in the speed and efficiency of transferring nutrients into pond ecosystems. Field trials will establish the composting method that produces the greatest growth of fish over 150 days. Each compost type will be standardized for nitrogen, phosphorus, and carbon concentrations before the composts are added to the ponds.

Objectives

A. To determine pond productivity for tilapia in response to composting method and manure:plant ratio.

B. To investigate pond processes in relation to characteristics of nutrient material input. One relatively high input rate will be used in all ponds. Fish production will be analyzed in terms of input quality and in terms of estimated differences in organic carbon, organic nitrogen, total nitrogen, and total phosphorus within pond compartments including benthic deposits, water column particulates, and dissolved matter. Fish-food habit analysis also will provide information on the community impact of various inputs.

Experimental Design

The experiment will be conducted for 150 days, in triplicate, using 21 ponds.

	Aerobic Compost			Anaerobic Compost			Submerged Compost		
	Percent Plant Material								
% Manure	100	80	50	100	80	50	100	80	50
0	X			X			X		
10		X			X			X	
50									X

Compost will be added at 500 kg/ha/wk as dry weight. Submerged compost will be added to pond corners as raw materials during the experiment.

Benefits

This experiment should provide information on pond response in relation to input quality. Total input and ratios of N, P, and C in each compost type can be evaluated in terms of optimizing yield. Stomach sample analysis and measurements of total nutrient present in sampled pond compartments will increase our understanding of pond dynamics and our ability to optimize compost effectiveness.

Study 3. Loading rate, input characteristics, and pond productivity

Objective

To determine the impact of loading rate and manure:plant ratio on pond productivity for tilapia.

To evaluate the impact on nutrient and energy pathways of an optimized compost in comparison to green plants used as a direct feed source added at similar nitrogen levels.

Methods

This will be a 150-day pond experiment comparing combinations of manure and plant matter for a selected compost process (aerobic, anaerobic, in-pond) with broadcast green plants. The experiment will be in triplicate and will require 18 ponds. Green feed will be added at the same total nitrogen level as the compost.

Design for Exp 3

POND INPUTS IN KG/HA/WK AS DRY WEIGHT

Compost	Broadcast green plants	Compost with added inorganic N
1000	TN equivalent of;	
750	750 kg/ha/wk compost	
500	500 kg/ha/wk compost	500 + N equivalent of 250 additional kg/ha/wk compost

In-pond measurements will be the same as those used in Study 2. Each measurement will be reviewed in terms of its usefulness and in terms of improving collection or processing samples.

Benefits

Knowledge of the relationship between compost N-P-C content and pond productivity for fish at different input rates is necessary to optimize application rate and input composition. In-pond measurements will provide insight into nutrient and energy pathways for a relatively long and a short pathway.

Thailand Project

Cooperating Institutions and Principal Investigators:

National Inland Fisheries Institute
Dr. Kitjar Jaiyen

University of Michigan (lead university)
Dr. James S. Diana
Dr. C. Kwei Lin

Michigan State University
Dr. C. D. McNabb
Dr. T. R. Batterson

University of Hawaii
Dr. Kevin Hopkins

Pond System: Freshwater tropical ponds typical of lower elevations

Project Narrative:

INTRODUCTION

Three integrated experiments will be conducted in Cycle IV. Experiments will test fish stocking density, fertilizer quality, and pond depth as management variables that affect dynamic pond processes on which fish yield depends.

EXPERIMENTAL PROTOCOL

Study 1. Fish Stocking Density

The null hypothesis for Experiment I is as follows:

> *fish stocking density neither affects the state (quantity) of the inorganic nutrient and terminal fish components of the pond nor does it affect grazing (as expressed indirectly by stomach analyses and fish yield) in fertilized ponds.*

Earthen ponds with a surface area of 220 m^2 each will be used. *Oreochromis niloticus* fingerling males will be stocked at rates of 0, 1, 2, and 3 fish per square meter. Random selection of ponds will be made to obtain replicates at each density. Each pond will be fertilized with 11 kg chicken manure dry weight per week (500 kg/ha/wk), a rate taken from results of Cycle III experiments in the CRSP as producing maximum fish yield. Study 1 will last 150 days. In order to obtain data for our long-term goals, we will examine the diet of tilapia as the experiment progresses. Study I will be run at Ayutthaya Station.

Study 2. Elemental Ratios in Fertilizer

Study 2 is based on the results of experiments reported in the literature that have shown a weight ratio on the order of 1P:7N is required for normal growth of algae.

In the past, chicken manures have been used as experimental fertilizers at freshwater CRSP sites in southeast Asia. These are approximately 4%P:2.5%N by dry weight, which indicates a clear imbalance in relation to the requirements of algae for growth. Chicken manure is deficient in nitrogen; the literature shows that other animal manures are deficient in nitrogen as well.

The null hypothesis for Study 2 is as follows:

> *a nitrogen supplement added to chicken manure neither affects the state (quantity) of the inorganic nutrient and terminal fish components of the pond dynamics model nor affects the rates of primary production, respiration, or fish grazing (as expressed indirectly by stomach analyses and fish yield).*

Study 2 will consist of two experiments each having three treatments which are replicated (n = 3). In the first experiment, treatment 1 will overlap with a treatment of Study 1:stocking density of two fingerlings per m^2 and 11 kg dry chicken manure per pond per week (500 kg/ha/wk). The other ponds in Study 2 will also be stocked at two fingerlings per m^2. Ponds in treatment 2 will be fertilized with a mixture of chicken manure at the rate of 11 kg per pond per week, plus a nitrogen supplement in the form of urea. The amount of nitrogen supplement used in this treatment will bring the P:N ratio of chicken manure + urea to 1:7. Chicken manure used in these experiments will be assayed for percent composition by weight of P and N. The amount of urea ($CO(NH_2)_2$), which is 46.7% N by weight, required to bring P:N to 1:7 will be calculated. For example, for chicken manure that contains 4.12%P and 2.45%N, ponds in treatment 2 would receive 11 kg dry weight chicken manure + 6.17 kg urea per week.

In the third treatment of experiment 1, ponds will be fertilized with a mixture of chicken manure + urea that contains the amount of N added per pond per week in treatment 1, and an amount of P that brings P:N to a ratio of 1:7. At 2.46% N, and a fertilizer rate of 11 kg chicken manure per pond per week, ponds in treatment 1 will receive 0.27 kgN per week. The amount of P required to balance that N at a weight ratio of 1:7 is 0.04 kg. That amount of P is contained in 0.97 kg of dried chicken manure. An additional 0.54 kg of urea will be added to bring N in urea + chicken manure to a 1P:7N ratio.

The first experiment in Study 2 will test the validity of using 1:7 weight ratios of total-P:total-N in fertilizer applications in ponds at Ayutthaya. Calculations from earlier CRSP data at Ayutthaya show that ponds held about 8.5 g Total Inorganic Carbon/ m^2 for photosynthesis. For fertilizer application rates in experiment 1, calculations show that adequate carbon is available in ponds so that carbon limitation will not likely occur, except for portions of daylight hours in treatment 2. These treatments are designed to test assumptions on efficiencies of conversion of P and N in fertilizers into available forms for algal uptake; proportional conversion and uptake of P and N was assumed in calculations for the experimental design.

Treatments in the second experiment will be determined after results of the first experiment have been analyzed. It is anticipated that both the P:N ratio and the rate of fertilization will be changed for this experiment.

Study 3: Pond Depth

The null hypothesis for Study 3, which will follow Study 2, is:

> *pond depth neither affects the state (quantity) of the inorganic nutrient and terminal fish components of the pond nor affects thermal regimes and rates of primary production and respiration in fertilized ponds.*

The experimental design involves three replicated treatments. Pond depths of 0.5, 1.0, and 1.5 m will be used as treatments. Sex-reversed Nile tilapia fingerlings will be stocked in all ponds at two fish per square meter. Each pond will be fertilized with chicken manure at a rate of 20 kg per week (Study 1).

Benefits

The effect of three management manipulations on pond dynamics and fish yield will be tested in Cycle IV: fish stocking rate, fertilizer quality, and pond depth. The long-term goal of these experiments is to optimize the efficiency of fertilizer conversion to fish yield and to achieve this optimization economically.

DATA SYNTHESIS ACTIVITY

The Data Synthesis Team is a fully collaborative unit and each team member will participate in all aspects of the activity. However, each member was appointed on the basis of bringing specific skills to the Team. Dr. Chang is a biometrician experienced in data processing and modeling. Dr. Lannan is an experienced fish and shellfish culturist and specializes in synthesizing technical information of a multidisciplinary nature. Dr. Piedrahita is an agricultural engineer specializing in ecosystems modeling. Each member has been assigned the principal responsibility for one or more areas of the work plan. In phase one, Dr. Lannan will supervise the development of a conceptual framework for presentation of the synthesis, Dr. Chang will determine the statistical relationships among and between variables in the data base, and Dr. Piedrahita will conduct preliminary model testing and exploratory data analysis. In phase two, Dr. Chang and Dr. Piedrahita will accomplish simulations, and Dr. Lannan will be responsible for translation of model outputs into pond management practices. In phase three, Dr. Lannan will serve as principal editor while Dr. Chang will provide statistical interpretation and presentation of data, and Dr. Piedrahita will provide interpretation and presentation of the models.

The fourth CRSP work plan will assign the highest priority to the analysis and synthesis of research data from the first three work plans, and the translation of the resulting information into the first manual of pond management practices. The work plan involves three progressive phases leading to completion of the manual:

1. The first phase was initiated during the first grant period and involves preparation for conducting simulations of pond processes using various types of models. Statistical analysis of the data has been carried out, and significant relationships between variables have been identified. This analysis will continue to be carried out on all data collected by the CRSP. Variations between ponds, and differences between sites will be studied.

2. The second phase involves the development of computer models to simulate pond processes. Two types of models will be developed: descriptive and mechanistic. Statistical relationships will be used to develop descriptive models from the data available for the first three experimental cycles. These models tend to be accurate predictors of conditions under which data were collected. The models will be validated using data collected from the first three cycles and those collected in the later cycles as they become available. Validated descriptive models will be used to study the possible effect of management practices on pond processes. These results will be used to design new field experiments in collaboration with the field research investigators, and to develop pond management guidelines for farm use.

   Mechanistic mathematical models will be developed from theoretical relationships, and will be based on conceptual models. These types of models are general in nature, have greater data requirements than the descriptive models for execution, and tend to be more complex. These models provide useful insights into the mechanisms and processes taking place in the system being modeled. Most useful models are neither purely empirical nor mechanistic but include characteristics of both to provide empirical accuracy and mechanistic

insights. After the initial mathematical implementation of the mechanistic models, parameter calibration will be carried out using the data from the first three experimental work plans. Model validation will be performed with data obtained primarily in the second year of the continuation grant.

3. The third phase is the preparation of the manual. The calibrated and validated models will be used to provide a guide in the preparation of the manual. The models are then to be used to predict responses of the pond ecosystems to certain management actions. This information is useful in generating guidelines for managing ponds under different production regimes. The guidelines will be compiled in a manual describing pond management procedures for optimizing yields under various sets of constraints.

It is important to note that the predictive quality of the models has to be established by carrying out the calibration and validation processes as described above, prior to being able to use them for evaluating and proposing management practices.

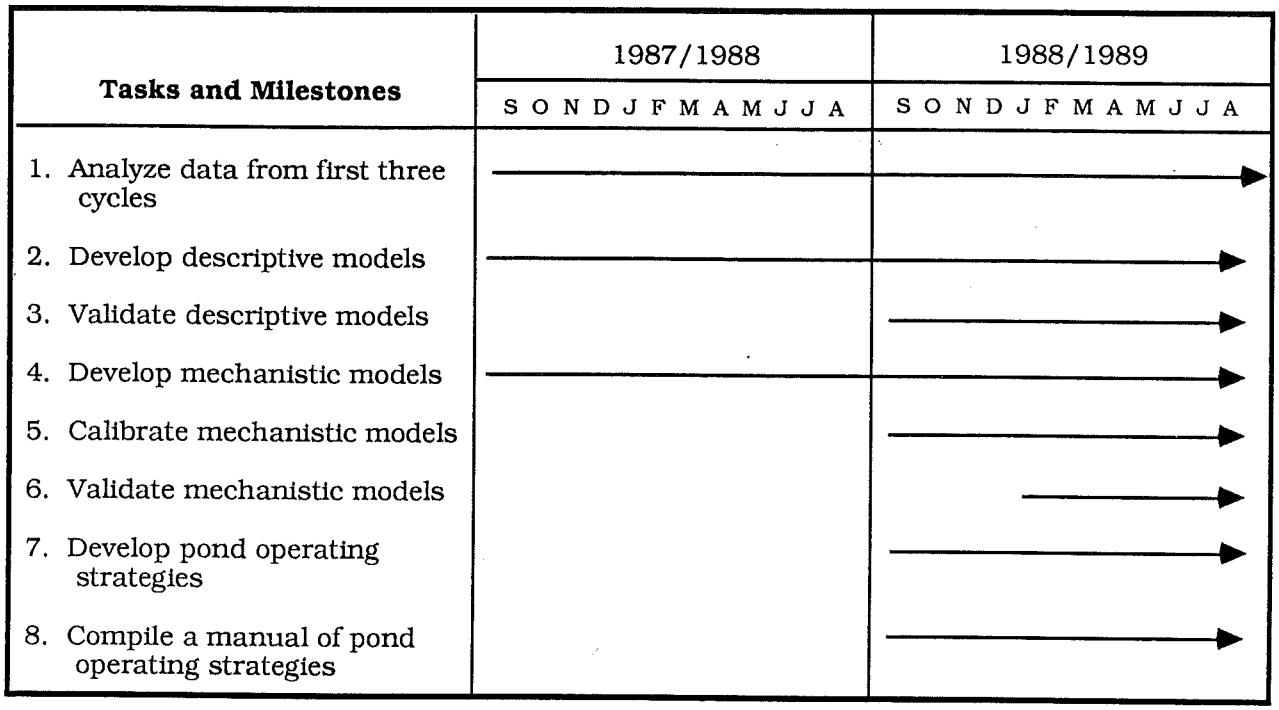

Figure 1. Time line for activities to be undertaken by the Data Synthesis Team.

TABLE 1
DAILY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Solar Radiation	Install Solar Monitor and Quantum Sensor at study site and read at 24-hour intervals.	LI-COR Solor Monitor Model LI-1776 and Quantum Sensor Model LI-190SB.	- -	$E/m^2/day$
Rainfall	Install three rain gauges at study site; read and empty at 24-hour intervals; report average of three readings.	No type specified. Recommended gauge from Grassroot Co., Wisconsin.	- -	cm/day
Wind Speed	If instantaneous windspeed and direction meter already in use, read at appropriate intervals to correlate with thermal and oxygen stratification of ponds with preferred totalizing anemometer, read between 8:00-9:00am and calculate average hourly wind speed.	Instantaneous windspeed and direction meter comparable to Taylor Model 110930 acceptable if already in use. For new purchase, recommend totalizing anemometer comparable to WEATHERtronics Model 2510. The instrument should be located in the pond complex 2 m above the level of the pond banks.	- -	km/hour
Air Temperature	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

TABLE 1 (Continued)
DAILY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Pond Depth	Install staff gauge in each pond and read to nearest 0.5 cm at same time each day. (Maintain 0.9 m average depth on daily basis.)	No type specified	- -	m
Evaporation and Inflow	In the course of each pond experiment, a water budget should be made for each pond. Surface Inflow/Outflow and Evaporation should be determined using procedures described in Wood, 1974 (Wood, J.W. 1974. Diseases of Pacific Salmon: Their Prevention and Treatment. State of Washington. pp. 71-77) or comparable approaches. The contribution of precipitation should be calculated using rainfall data, while seepage must be estimated based on rainflow, inlet water, and evaporation.	- -	- -	m^3/day

TABLE 2
INTENSIVE SAMPLING AND
DIEL STUDIES
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Dissolved Oxygen*	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 (American Public Health Association, 1980)	mg/L
Pond Temperature*	Near center of each pond, take readings at 25 cm below the water surface and 25 cm above the bottom. Take readings as part of diel study at 7 different times. If a probe is used, calibrate using a precision thermometer.	YSI Model 57 Dissolved Oxygen Meter with Temperature Indicator.	- -	C
Solar Radiation*	Install Solar Monitor and Quantum Sensor at study site and read at each of the 7 time intervals for the diel study.			E/m^2

* Indicates parameters to be measured as part of diel studies at the following sampling times: pre-dawn, 1000, 1400, 1600, 1800, 2300, and pre-dawn the next day.

TABLE 2 (continued)
INTENSIVE SAMPLING AND
DIEL STUDIES
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Wind Speed*	Use totalizing anemometer and read at each of the 7 time intervals for the diel study.			km
pH*	Near center of each pond, take readings at 25 cm below the water surface and 25 cm above the bottom. Take readings as part of diel study at 7 different times. If a probe is used, calibrate using a precision thermometer. Meter should be calibrated with standard buffers at pH 7 and pH 4.	pH Meter with Combination Electrode comparable to Orion 2000 Series with Ross Model 81-55 Electrode.	- -	pH units
Total Kjeldahl Nitrogen	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); or in-country analysis by qualified laboratory.	mg/L

* Indicates parameters to be measured as part of diel studies at the following sampling times: pre-dawn, 1000, 1400, 1600, 1800, 2300, and pre-dawn the next day.

TABLE 2 (continued)
INTENSIVE SAMPLING AND
DIEL STUDIES
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Secchi Disk Visibility	At 2 locations in each pond, calculate Secchi Disk Visibility using procedure described by Lind (1974).		- -	cm
Chlorophyll a (corrected and uncorrected)	Collect one sample per pond by pooling three 90 cm column samples. Take samples with diel studies.		Spectrophotometric Determination (American Public Health Association, 1980).	mg/m^3 → $\mu g/L$
Alkalinity*	Near center of each pond, take readings at 25 cm below the water surface and 25 cm above the bottom. Take readings as part of diel study at 7 different times. Keep samples cool in refrigeration unit or ice chest, and analyze within 24 hours.	Hach Digital Titrator Test Kit/ Alkalinity (optional).	Low or High Standard Alkalinity Method (as appropriate) (American Public Health Association, 1980), or Hach Test Kit.	mg $CaCO_3$/L

* Indicates parameters to be measured as part of diel monthly studies.

TABLE 2 (continued)
INTENSIVE SAMPLING AND
DIEL STUDIES
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
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.	Nesslerization Method (Michigan State University Limnological Research Laboratory, 1984).	mg/L
Total Phosphorus	Collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours.	- -	Persulfate Digestion and Ascorbic Acid/ Colorimetric Method (American Public Health Association, 1980).	mg/L
Dark Bottle Respiration	Collect one sample (by pooling three 90 cm column samples) from each pond. Incubate for four hours in dark bottles suspended at mid-depth in ponds.		Oxygen method, adapted from the American Public Health Association (1980).	mg carbon fixed/m^3/ day
Total Suspended Solids	Please refer to the appendix.		In: Standard Methods for the Examination of Water and Wastewater Amer. Public Health	

TABLE 2 (continued)
INTENSIVE SAMPLING AND
DIEL STUDIES
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Total Volatile Solids	Please refer to the appendix.		In: Standard Methods for the Examination of Water and Wastewater Amer. Public Health	

TABLE 3
FISH PRODUCTION MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
Fish/Shrimp Group Weight	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 grab sample.*	- -	- -	kg/# individuals
Fish/Shrimp Mean Weight per Individual	For a representative 10% sub-sample of the grab sample referenced above, weigh and count individuals. Express as mean weight per individual.	- -	- -	g
Fish/Shrimp Mean Length per Individual	For a representative 10% sub-sample referenced above, determine total length of each individual and express as mean length per individual.	- -	- -	cm
Tilapia Reproduction	Concurrent with measurement of fish growth, note the number and collective weight of any fry collected during monthly sampling.	- -	- -	g/# individual

* NOTE: If substantial variation is observed or if reproduction is suspected, divide sample into centimeter groups; count and weigh each group. Any female tilapia observed should be removed and replaced with a male of similar weight. Any animals collected other than those stocked should be counted, weighed, measured and discarded. Record observations on reproduction of fish health.

TABLE 4
OPTIONAL MONTHLY MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	INSTRUMENTATION	ANALYTICAL METHOD	REPORTING UNIT
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 appropriate references to identify major groups (green, blue-green, or diatom) and relative abundance of each group (abundant, common, rare).	- -	- -	group/ relative abundance
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 note relative abundance (abundant, common, rare).	- -	- -	order/ relative abundance
Benthos Composition**	Monthly and when changes in the community are observed, 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. Identify at the order level and note relative abundance (abundant, common, rare).	- -	- -	order/ relative abundance

** Indicates analyses that are recommended but not required.

TABLE 5
OCCASIONAL MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	REPORTING UNIT
Pond Soil Characteristics: pH, Phosphorus, Extractable Bases (Ca, Mg, K, Na), Organic Matter, Total Nitrogen, Nitrate Nitrogen, Ammonium Nitrogen, Cation Exchange Capacity, Soluble Salts, Metals (Al, Fe, Zn, Mn, Cu), Sulfate Sulfur, Lime Requirement, Free $CaCO_3$ or $CaCO_3$ Equivalent, Exchangeable H, Exchangeable Na.	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. Take an appropriate subsample for each pond and analyze using either a qualified local laboratory or U.S. laboratory.	As appropriate
Morphometric Characteristics: Maximum Length, Maximum Width, Area, Depth, Volume	At project initiation and subsequently whenever pond facilities are altered, map ponds as described in Appendix F. Note inflow out outflow locations, pertinent surrounding elevations and buildings and structures on the site. Measure or calculate the listed morphometric parameters.	m, m^2, m^3 (as appropriate)
Hydrologic Characteristics: Surface Inflow, Precipitation, Outflow, Evaporation, Seepage (calculated)	In the course of each pond experiment, a water budget will be determined for each pond. Surface Inflow/Outflow and Evaporation should be determined using procedures described in Appendix F or comparable approaches. The contribution of precipitation should be calculated using rainfall data, while seepage must be estimated based on measurement of the other parameters.	m^3/day

TABLE 5 (Continued)
OCCASIONAL MEASUREMENTS
MATERIALS AND METHODS

PARAMETER	PROCEDURE	REPORTING UNIT
Chemical Oxygen Demand of Organic Inputs	Please refer to the appendix (from: Standard Methods for the Examination of Water and Wastewater. APHA, 1980).	
Fish/Shrimp Production:		
Initial Stocking - number stocked - group weight - mean weight per individual - mean length per individual	Initial stock will be weighed as a group and counted. Tilapia will be sexed individually (Appendix F). A 10% sample will be weighed and measured (use total length for tilapia measurements). Refer to sections on stocking in Chapter 2.	kg/# individual g cm
Termination of Experiments: - mean weight per individual - total number harvested - group weight (calculated) - survival (% of initial number stocked)	All fish/shrimp will be removed from each pond 150 days (90-120 days for shrimp) after stocking. A random sample equivalent to 10% of the initial stocking will be weighed and measured. The total number of fish/shrimp from each pond will be determined and the total biomass per pond will be calculated. Any fish other than tilapia will be counted by species, weighed and measured.	# individuals g kg %

APPENDIX

TITLE

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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-

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

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/cm^2 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.

$$\text{Normality of FAS solution} = \frac{\text{Volume } 0.25N\ K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.25$$

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:I 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-

*Corning 5000 or equivalent.
†Corning 2360, 91548, or equivalent.
‡G. F. Smith Chemical Co., Columbus, Ohio.

TABLE 508:I. 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
40.0	20.0	60	0.8	0.20	280
50.0	25.0	75	1.0	0.25	350

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

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3. MEDALIA, A.I. 1951. Test for traces of organic matter in water. *Anal. Chem.* 23:1318.
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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.

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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 1*N* 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

1. General Discussion

Filtrable residue is material that passes through a standard glass fiber filter and remains after evaporation and drying to constant weight at 180 C.[1] The determined values may not check with the theoretical value for solids calculated from chemical analysis of water. Approximate methods for correlating chemical analysis with residue are available.[2]

The filtrate from the total nonfiltrable residue (Section 209D) may be used for determination 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.

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

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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.