FACTORS CONTROLLING OXYGEN DEPLETION IN THE HYPOLIMNION

by

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ABSTRACT

The purpose of this research was to determine the factors controlling oxygen depletion in the water column (more specifically, the hypolimnion).

Five factors were hypothesized to be important:

1. Soluble organic carbon concentration
2. Temperature
3. Nutrient concentration
4. Initial oxygen concentration
5. Algal respiration

These factors were related by a two-level Factorial Experimental Design. Two separate but related Designs were used to "block" and "average" the effects, allowing for differentiation between main effects (for example, temperature) and two-factor interactions (for example, the combined effect of soluble organic carbon concentration and temperature).

Four bodies of water (Hamilton Harbour, Guelph Reservoir, Red Chalk Lake, and Chub Lake) were tested. The results show that in all cases, except Chub Lake, temperature is the most important factor controlling oxygen depletion. Soluble organic carbon concentration is the most important variable in Chub Lake. The second most important variables are: soluble organic carbon concentration in Hamilton Harbour and Red Chalk Lake, temperature in Chub Lake and the initial oxygen concentration in the Guelph Reservoir. The remain-

ing factors have varying degrees of importance depending on the water body. This suggests that biodegradation is not limited by nutrients and that algal respiration is a small component of oxygen consumption.

In addition to these main studies, Temperature and Oxygen Kinetics were also investigated. The Temperature Kinetic experiments showed that higher temperatures produce higher oxygen uptake rates. The Oxygen Kinetic experiments showed no trends except when Hamilton Harbour water was tested. In Hamilton Harbour, higher initial oxygen concentrations produced higher oxygen uptake rates.

It is proposed that the Experimental Design represents a bioassay on oxygen consumption. It represents quantification of biodegradation under standard conditions. The relative magnitudes of oxygen consumption under these standard conditions correlate with empirical ideas of degradability of organics in the different water bodies. More work is required to refine the scaling of factors selected, seasonal variability, etc.

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DEDICATION

This book is dedicated to my parents without whom this work would have been impossible. I greatly appreciate the kindness and encouragement they gave to me throughout some extremely difficult times that arose, although at the time these feelings were not expressed. I can never repay them for their understanding.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Chemicals

ATP	Adenosine Triphosphate
CO_2	Carbon dioxide
N_2	Nitrogen
NH_3	Ammonia
NO_2	Nitrite
NO_3	Nitrate
O_2	Oxygen
TKN	Total Kjeldahl Nitrogen

General

BOD	Biochemical oxygen demand
CI	Confidence interval (statistics)
C:N	Carbon to nitrogen ratio
C:N:P	Carbon to nitrogen to phosphorous ratio
df	Degrees of freedom
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
f_i	Factor interaction (statistics)
F:M	Food to microorganism ratio

pH	Negative logarithm of the hydrogen ion concentration
POC	Particulate organic carbon
SOC	Soluble organic carbon
SOD	Sediment oxygen demand
UV	Ultra-violet

Units

day^{-1}	Per day (reciprocal time)
°C	Degrees Celsius (temperature)
g	Gram (mass)
g/m^2-day	Gram per metre squared day (areal uptake rate)
g/m^3-day	Gram per metre cubed day (volumetric uptake rate)
ℓ	Litre (volume)
mg/ℓ	Milligram per litre, 10^{-3} g/ℓ (concentration)
mg/ℓ-day	Milligram per litre day, identical to g/m^3-day (volumetric uptake rate)
mℓ	Millilitre, 10^{-3} litre (volume)
µg/ℓ	Microgram per litre, 10^{-6} g/ℓ (concentration)

CHAPTER 1

INTRODUCTION

The depletion of the oxygen in the hypolimnion of lakes has been recognized as a natural phenomena as well as a man-induced problem. Observations on many Canadian Shield lakes, relatively uninfluenced by man's activities, show that oxygen concentrations reach 1 to 3 mg/ℓ near the end of summer stratification. These concentrations are at 10-12 mg/ℓ after fall overturn, and decrease slowly through the winter and summer. If spring circulation occurs, this continuous decline is interrupted, some oxygen is replenished. Observations on reservoirs in agricultural areas (Grand River Reservoirs) and on the Great Lakes (Lake Erie, Burns and Ross, 1970; Hamilton Harbour, Ministry of the Environment, Ontario, 1974) show that the depletion is often nearly complete within one month. Birge and Juday (1927) are considered by limnologists to be the people who introduced the concept of two basic lake types: autotrophic and allotropic. Autotrophic lakes are those dependent on internal sources, such as planktonic production, for the accumulation of organic matter while allotrophic lakes are those which receive a major supply of organic matter from external sources.

Within limits, these terms may be used to describe the energy source for bacterial growth and oxygen consumption. In lakes situated in forested drainage basins, and with little algal production, the main energy source is organics derived from forest runoff. In agricultural areas, significant export of organics from crops occur; however, the

associated export of nutrients permits algal growth to occur in reservoirs. In the lower Great Lakes, both land-based export of organics and in situ formation of algae represent energy sources for bacterial growth. In water bodies like Hamilton Harbour which are subjected to stormwater runoff and wastewater treatment plant discharges, inputs of ammonia represent energy sources in addition to algal growth and inputs of organics.

Except for small lakes dominated by urban discharges and those in forested watersheds in which algal growth is minimal, it is probable in most lakes influenced by man, the in situ formation of algae is a prime energy source for bacteria. Naumann (1919) is considered to be the person who first introduced the terms oligotrophic and eutrophic into limnology. The terms were used to describe lakes of poorly-nourished and well-nourished conditions, respectively. He postulated a positive relationship between nutrient concentrations and plankton productivity, suggesting that phosphorous and nitrogen were the dominant factors in determining plankton quantity and composition. The term eutrophication has in the past decade been applied to describe the well-nourished or excessively well-nourished condition of lakes and then associated physical, biological, and chemical properties.

Eutrophication is a biological process which acts to shorten the life span of lakes by natural or artificial nutrient enrichment. Biological activity in nutrient poor (oligotrophic) lakes is relatively low. As the nutrients retained in these water bodies increase with time, microbial populations will also increase. These increases result in deposition of detritus on the lake bottom through new cell formation. If the lake is unable to shift the balance back to oligotrophy, it will gradually fill with suspended solids and organic materials over geological

time scales such that rooted aquatic plants dominate. As this process continues, it will be converted to marsh land and lose its value as a viable open water resource (Oswald and Golueke, 1966).

Eutrophication is associated with an unbalanced input of organic material to the water system. When planktonic growth and bacterial degradation are balanced, the rate of eutrophication is minimized (Oswald and Golueke, 1966). An unbalanced situation can cause excess planktonic growth and bacterial deoxygenation tending towards anaerobic conditions hence creating nuisances. Such an imbalance in the ecosystem may result from either an extraordinarily large organic input to the system and/or thermal stratification (Fruh, 1967).

In temperate climates, rapid solar heating, coupled with low mechanical mixing cause lakes to stratify in the summer. A warm layer is formed in the surface waters overlying and separated from colder bottom waters by a zone of high temperature gradient. These three layers are respectively called the epilimnion, the hypolimnion, and the metalimnion. Little vertical transport of soluble materials, including oxygen, occurs through the metalimnion. Thus, the initial oxygen content of the hypolimnion found at the time of stratification is not augmented in an appreciable fashion by physical processes.

Thermal stratification in water bodies causes the two biological processes to occur in separate zones; planktonic activity in the epilimnion and bacterial activity in the hypolimnion. Bacterial activity consumes oxygen in the hypolimnion, resulting in its decrease without significant replenishment. If bacterial oxidation is sufficiently fast, hypolimnetic anoxia may result.

Investigations into the factors affecting oxygen depletion have used one of three approaches: field observations, mathematical modelling,

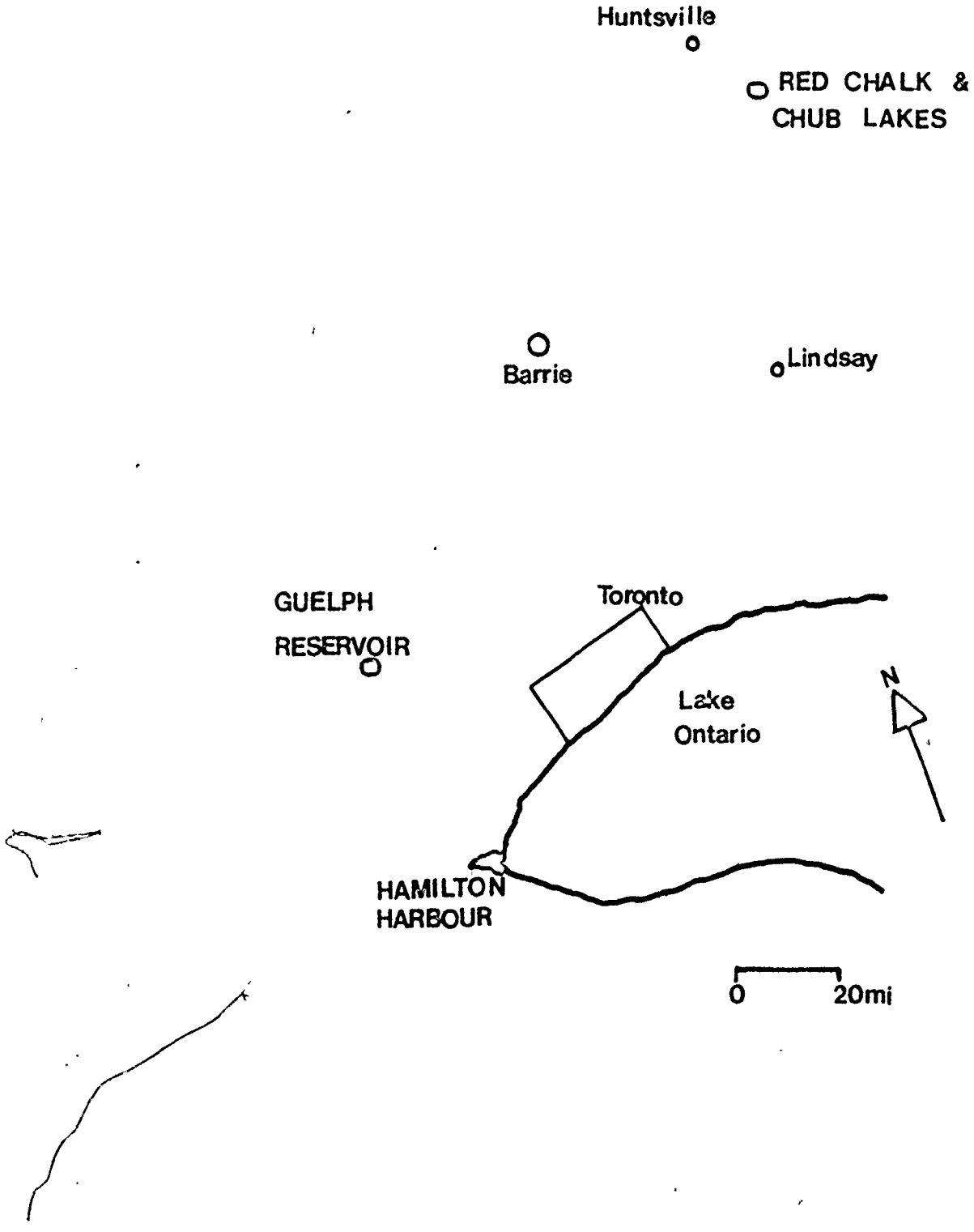
and laboratory experiments. Some have found water column consumption is more important than sediment uptake (Polak and Haffner, 1978 for Hamilton Harbour). Some have found regions of great oxygen demand in the metalimnion and attempted to correlate it with observations on algae, bacteria, and organic carbon (Verhoff and DePinto, 1977). Models of the relationships between algal growth and nutrient cycles have been constructed (DiToro et al, 1972; Dalrymple, 1977; Chen and Smith, 1979); few have examined oxygen consumption aspects. Experimental investigations of the multi-dimensional relationships affecting oxygen consumption are likewise limited in number and scope. The role of bacteria has received too little attention (Great Lakes Basin Commission, 1976).

The purpose of this work is to attempt to assess experimentally the principal factors affecting oxygen consumption in the water column of lakes and to scale their importance. The objectives of this work, thus, are (i) to scale the relative importance of five factors hypothesized to be the main ones controlling oxygen consumption, and (ii) to determine the interactions between these variables. The variables are temperature, soluble organic carbon (SOC) concentration, nutrient concentration, initial oxygen concentration, and the presence or absence of algal and zooplankton respiration. All have been shown to be important in aerobic microbial communities. Temperature affects the metabolic rate of bacteria. SOC is an energy source of organics for bacteria, although its utility as a model compound for simulating the types of organics available in particulate form in lakes can be challenged. Nutrients in minimum amounts are required for bacterial growth. Oxygen concentrations can limit bacterial respiration where the bacteria are in organic assemblages. Algal and zooplankton respiration may be as important as bacteria

in the upper portions of a hypolimnion.

Four water bodies were chosen for the experimental program: two which are relatively nutrient rich (Hamilton Harbour and Guelph Reservoir) and two which are relatively nutrient poor (Red Chalk Lake and Chub Lake). Hamilton Harbour and the Guelph Reservoir are situated in watersheds dominated by calcareous rocks and soils and are substantially buffered by the carbonate system; Red Chalk and Chub Lakes are small Canadian Shield lakes situated in watersheds dominated by igneous bedrock. Hamilton Harbour is heavily influenced by urban sanitary and stormwater discharges. The Guelph Reservoir is situated on an agricultural drainage basin. Red Chalk and Chub Lakes are located in forested watersheds with the latter being a coloured (dystrophic) lake. The choice of these water bodies allows for comparison between and within the two groups. Fig. 1.1 shows the locations of all four water bodies in Ontario. Fig. 1.2 shows the location of Red Chalk and Chub Lakes with respect to each other.

FIG. 1.1
Location of Water Bodies



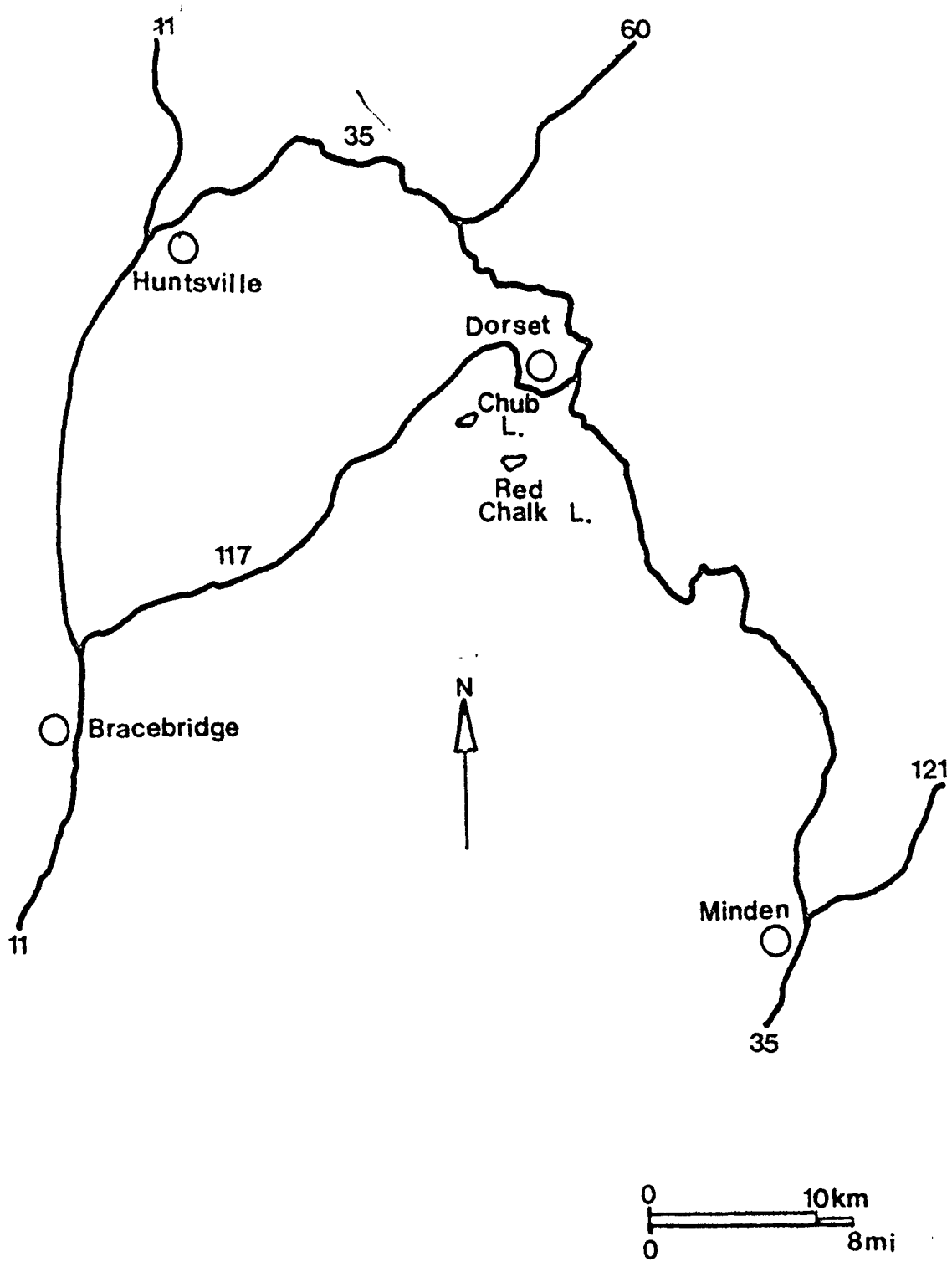


FIG.1.2
Location of Northern Lakes

CHAPTER 2

LITERATURE REVIEW

The prime goal of this work is to determine the factors influencing oxygen consumption in the water column of lakes.

To examine factors controlling biodegradation in the water column, the following sequence is used. Firstly, the cycling of organic material in a water column is examined and then the flux of matter into the hypolimnion is isolated upon. Secondly, the mechanisms involving the transformation of material, and their rates in the hypolimnion are considered. Thirdly, the nitrogen component of oxygen demand is analysed. Fourthly, the effects of temperature on microbial growth are addressed. Finally, some models that incorporate biodegradation and oxygen consumption are examined.

There are three main processes involving the transformation of particulate organic materials to their inorganic forms in the water column of lakes. They are (i) zooplankton metabolism and conversion, (ii) respiration and cellular release by viable algal cells and (iii) active degradation by microbial decomposers (DePinto, 1979). Zooplankton processes have been demonstrated by such investigations as Pomeroy, et al (1963) and Peters (1975) because of the relative ease of physically separating zooplankton from other microorganisms. The separation of algal respiration and active degradation in the laboratory is more difficult. Phytoplankton respiration represents the auto-oxidation of photoplankton organic carbon to carbon dioxide, contributes to a

reduction in the size of algal biomass and augments the pools of soluble and particulate organic carbon through excretion. Heterotrophic decomposers not only process the remains of dead algae and land-based discharges, but also appear to increase the rate of algal death in a parasite-type of interaction under certain conditions such as when algae are physiologically weak. This chapter will concentrate upon bacterially-mediated degradation. The complexity of the symbiotic relationships between algal death and bacterial processes will, of necessity, be dealt with further.

In the literature, various terms including decay, decomposition, degradation and respiration are used to describe oxygen demand. An enumeration of their differences is useful for the selection of terminology for this research. Consider the cycling of matter in very general terms. Primary producers take the major elements in inorganic forms, and through the use of solar energy, convert them into organic forms. The resultant organic compounds then pass through the food chains of the consumers, providing energy and matter for the formation of cellular material. Before the elements can again become available to the producers, they must be returned to the inorganic state in a conversion celled degradation (Grady and Lim, 1977). The term respiration is defined as that part of decomposition that involves the transfer of electrons from reduced compounds to electron acceptors; electron acceptors are oxygen in aerobic systems, nitrate in anoxic systems and other organic molecules in anaerobic systems. Good examples of aerobic respiration can be seen in the pyruvic acid cycle or the tricarboxylic acid (TCA) cycle.

Hence, the overall step of degradation or conversion of a unit mass of organics from a particulate form to its final inorganic end products

can be viewed as being composed of a series of respiration steps in which some mass is converted into CO_2 and into a particulate form and oxygen is consumed. Each new particulate formed can become an energy source for subsequent attack by bacteria or other micro-organisms. The complexity of bacterial communities in the food web necessitate the lumping of these steps in some form in order to permit their characterization. In this research, algal and zooplankton respiration will be differentiated from the series of steps involving bacterial respiration; the latter will be referred to as degradation.

2.1 Cycling of Material in a Lake's Water Column

The cycling of material in the water column of a lake is a simple or complex phenomena depending upon the level of complexity chosen to describe it. Two alternative levels are shown in Figure 2.1. The size of compartments and fluxes are those predicted by the model of Scavia, et al (1976). In the simpler model (Figure 2.1(c)), all the material flow between particulate compartments, such as herbivores-detritus, are disregarded in the aggregation.

Algae promote the cycling of matter due to their ability to convert inorganic forms of elements to organic forms (inorganic carbon to organic carbon). This is demonstrated by the Redfield Equation for oceans (Stumm and Morgan, 1970):

$$C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 \underset{P}{\overset{R}{\rightleftharpoons}} 106\ CO_2 + 122\ H_2O + 16\ NO_3^- + 19\ H^+ + PO_4^{\equiv}$$

(1 mole of algae)

Upon formation of an algal cell, one of three things will happen. It may die and decompose; it may be consumed as part of the grazer food chain; it may settle from the water column. For simplicity, this

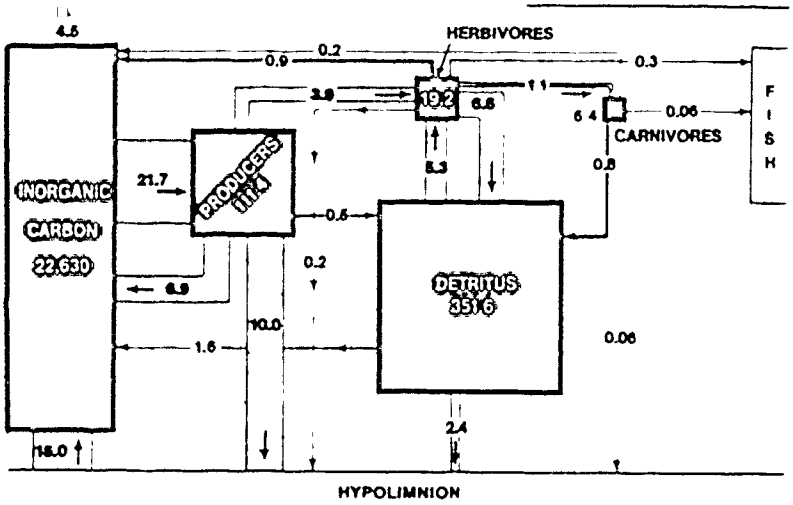
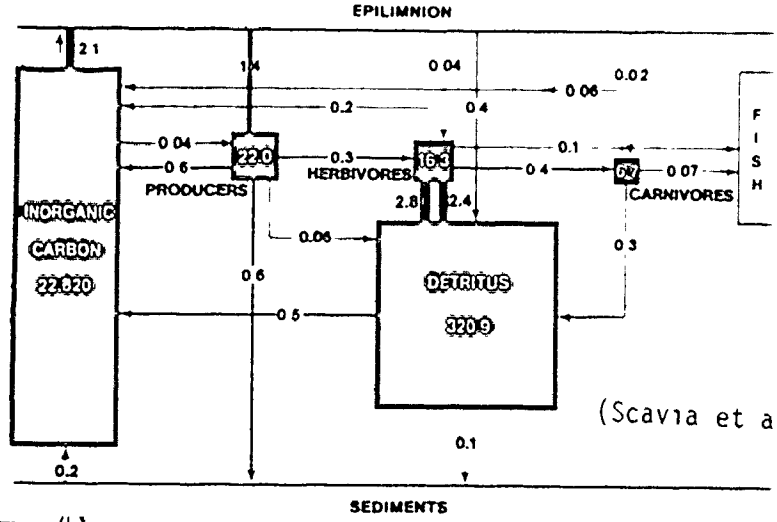


Figure (a) A cycling diagram of the yearly average concentrations of carbon ($\mu g\ l^{-1}$) and yearly average flows of carbon ($\mu g\ l^{-1}\ day^{-1}$) in the epilimnion of Lake Ontario. (Scavia et al, 1976)



(Scavia et al, 1976)

Figure (b) A cycling diagram of the yearly average concentrations of carbon ($\mu g\ l^{-1}$) and yearly average flows of carbon ($\mu g\ l^{-1}\ day^{-1}$) in the hypolimnion of Lake Ontario.

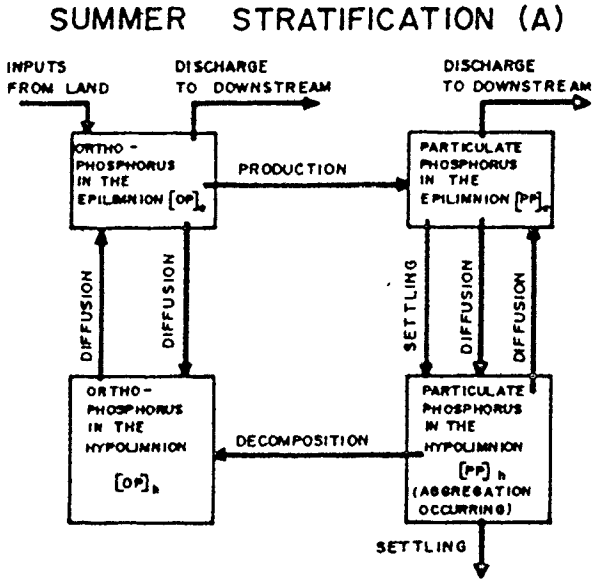


Figure (c) Compartment model for P during summer stratification (after Snodgrass and O'Melia, 1975).

FIG. 2.1

Two Alternate Cycling Models

discussion will lump the complexity and the intricacies of the lake's food web and its dynamics by focusing primarily on a model based on cycling from soluble carbon to particulate carbon and the reverse.

Consider the organic molecule in the Redfield Equation. The organic carbon and nutrient (nitrogen and phosphorous) portions can both decompose and settle. Much of the organic carbon particle which decomposes will be oxidized to CO_2. The CO_2 will either be discharged to the atmosphere or remain in solution, becoming available for photosynthesis. The nutrient particulate portion can either settle out of the column into the sediments or it can solubilize and become available to resupply microbial growth. That portion entering the sediments may either decompose or be ultimately buried. That portion decomposing may either remain in the water column or form a mineral such as an apatite.

In the euphotic zone, oxygen can be either produced, through photosynthesis, or consumed through respiration or decay processes. Below the euphotic zone, oxygen is only consumed. The euphotic zone may be confined to the epilimnion. In this study, the euphotic zone did not extend into the hypolimnion in any of the water bodies tested. Unless otherwise stated, all subsequent discussion will assume that the euphotic zone does not extend below the epilimnion.

The main sources of material fluxes into the hypolimnion are inputs from the epilimnion, land based sources which enter directly via gravity currents, sediments and other mixing and diffusional effects. First consider inputs from the epilimnion to the hypolimnion. Algae can settle from the epilimnion to the hypolimnion in a live state. There are two methods which nutrient-depleted algae can leave the epilimnion (Titman and Kilham, 1976); one is by settling and the other is by Langmuir cell motion. When older less motile algae get caught in Langmuir

motion, these algae get pushed farther from the centre of the Langmuir cell (due to centrifugal force). These nutrient deplete algae then sink to the thermocline region where they encounter nutrient rich water. Because of revitalization due to the new environment causing the algae to control their settling or the increased density of water slowing the cells, their sinking rate is slowed, allowing oxygen consumption to increase in this region. Titman and Kilham also observed that the sinking rate of a culture in stationary phase was greater than a culture in the exponential growth phase. This tends to indicate one of two occurances. Either younger cells may be more motile than older cells or younger cells do not floc together as much as older cells; a general rule in the operation of biological sewage treatment plants is to aim at a 5% viability of the biological solids to achieve good final settling. In other words, a good sewage treatment plant operates in the decay (or possibly stationary growth) phase.

Gordon and Skelton (1977) suggested that one direct result of eutrophication in deep reservoirs is an increase in the settling of particles and the thickening of the oxygen depleted metalimnion. Previous investigators had suggested that the metalimnion could accumulate organic material and coupled with the small amount of light penetration for photosynthesis, would result in trapped microbes either respiring or decomposing. In the field, they observed a relationship between the settling rate of particles and an increased concentration of natural oxygen demanding material in the thermocline region. Using a modelling study, they showed that decreases in vertical settling rates caused by an increase in density (decrease in temperature) with depth can account for this increased concentration of oxygen demanding materials in the metalimnetic waters.

Algae undergo considerable decomposition in the water column before reaching the hypolimnion or the sediments. Kuznetsov (1968) found that between 90% to 98% decomposition of organic matter occurred in the water column while Verhoff and DePinto (1977) determined at least 70% of algae and algae-derived organics decay before reaching the sediments. Although the term water column in the above values includes both the epilimnion and the hypolimnion, it is reasonable to assume that the algae entering the hypolimnion are in various states of decomposition and that a significant amount of this decomposition occurs in the hypolimnion.

The extent of degradation before particles enter the hypolimnion is important because it provides a measure of the energy left for bacterial growth and the ease with which it may be utilized. Only in the past decade have studies concentrated upon water column carbon balances with the view of determining the amount utilized in different portions of the water column. Representative studies include the work of Kajak, et al (1970) on several Polish lakes. They estimate that 63% of the total primary production was decomposed in the epilimnion. Of the remaining 37% which reached the hypolimnion, about half was decomposed before reaching the sediments.

Certain zones in the hypolimnion appear to undergo higher rates of oxygen consumption than others. Verhoff and DePinto (1977) observed a significant but unspecified amount of oxygen consumption in the thermocline area. Similar observations have also been noted by Titman and Kilham (1976) and Gordon and Skelton (1977). Sediments also have zones of high oxygen consumption. This takes the form of oxygen consumption by sediments and entrainment of sediment-associated organics in the bottom waters by currents. Some controversy exists as to the importance of sediment oxygen consumption. Lasenby (1975) determined that only

27% of the total oxygen consumption in the hypolimnion of a shield lake could be attributed to the sediments. Polak and Haffner's (1978) data indicate that sediments consume 30% of the total oxygen consumed in Hamilton Harbour while the studies detailed by Burns and Ross (1970) suggest that 50% may be due to sediments in the thin hypolimnion (2 metres) of Lake Erie.

Significant build-up of nutrients also occurs in the metalimnion of lakes (Verhoff and DePinto, 1977; Titman and Kilham, 1976; Gordon and Skelton, 1977). The build-up of nutrients in this area is attributed to the high rates of microbial degradation of algal biomass and hence nutrient regeneration.

Perhaps the one aspect of decomposition and nutrient regeneration which has received the least attention in research concerns the relative importance of the algal community and the microbial decomposer community. Parsons and Strickland (1962) found that algae are capable of heterotrophic activity but do not elaborate as to the mechanisms or the extent of this activity. From an extensive literature review and field studies, Kuznetsov (1968) concluded that bacterial biomass production was greater than algal biomass production; this is possible only if bacteria use products of photosynthesis as well as land based organic material carried into the lake for growth. From their results, Jewell and McCarty (1971) stated that algal endogenous respiration accounts for no greater than 20% to 30% of the total oxygen consumption required to completely decompose all algal cellular material. From these studies, the importance of heterotrophs (bacteria) in decomposition of algal biomass in the water column is clearly demonstrated.

Such studies have led to the hypothesis that the microbial community is more important than the algal characteristics in promoting

recycling and decomposition. Such hypotheses have been extended in the analysis of currently available models for phytoplankton growth. A sensitivity analysis of a total phosphorous model (Snodgrass, 1974) and of a photoplankton model (Thomann, et al. 1975) suggests that the rate of recycle is more strongly influenced by the rate of decomposition than the rate of formation of particulate organics. Larsen, et al. (1973) performed mass balances on nitrogen and phosphorous in Lake Shagawa in Minnesota. With this information, they hoped to predict observed algal population dynamics but could not account for large algal blooms in late summer and fall without arbitrarily inputting nutrient doses at these times into the model. Verhoff and DePinto (1977) hypothesized from their own work that had Larsen, et al. accounted for nutrient regeneration and recycle, the late summer and fall algal blooms could have been adequately modelled.

Predatory activity (eg. zooplankton consuming algae, fish or zooplankton feeding upon zooplankton) is another recycle mechanism of significance in lakes. Grazing of zooplankton by and subsequent excretion of undigested food or fecal pellets can account for some oxygen consumption and recycling of organic carbon and nutrients within the epilimnion as well as losses to the hypolimnion.

Algae can be a cause of cycling directly through the excretion of organic matter. Berman (1976) concluded that the amount of organic matter excreted could not be quantified and hence the amount of organic matter leaving the hypolimnion could not be quantified. Further, Owens and Esaias (1976) found that urea-N is assimilated in great amounts and that this stresses the importance of nitrogen compounds (NH_3 and urea) excreted by animals for maintaining productivity, especially under stratified conditions where the NO_3 supply in deep waters is unavailable.

Sediments can also contribute nutrients to the hypolimnion while

consuming oxygen from it. Such inputs as ammonia and methane are energy sources for bacterial growth. Where sediments are anaerobic or oxygen is in limiting concentrations, there can be a significant flux of nutrients into the water column. It has long been noted since Mortimer's (1941, 1942) studies that fluxes of nutrients, particularly of soluble phosphorous and ammonia, from sediments without an oxidized surface layer, are higher by up to an order of magnitude than sediments with the oxidized zone. The presence of macroinvertebrates also aids this flux. Fillos and Molof (1972) conducted sludge bottom experiments with the flow over the sediments close to scour velocity. From their work, they concluded that it is probably not uncommon to find pockets of anaerobic sediments and waters where small zones of nutrient diffusion into the water column exist.

The cycling of matter is complex involving the input of material and energy into the lake, its utilization within the lake, the transformation of material from particulate forms to soluble forms and vise versa, and its removal via settling, diffusion and outflows. The review given above provides a perspective for some of the inter-relationships. Since these relationships are amenable to mathematical description, extensive modelling efforts have evolved for describing these inter-relationships. Such models are usually developed from either a mass balance or an energy balance perspective. A few such models are subsequently described hereinafter the consideration of mechanisms of transformation, factors influencing oxygen consumption, and descriptions of the mathematics of these transformations.

2.2 Oxygen Consumption as a Function of the Degradability of Organic Carbon Fractions and Oxygen Concentrations

The previous section dealt with the concepts of cycling and recycling of biological material in the epilimnion and how biological material enters and leaves the hypolimnion. This section will examine the transformations of matter in the hypolimnion, principally the degradation of organic material and the associated transformation of inorganic substances such as ammonia, phosphorous and oxygen. Particular attention will be given to those factors that may limit oxygen consumption including substrate concentration, nutrient concentration, oxygen concentration, and temperature.

Various researchers have attempted to measure the lability of different size fractions as a method for estimating the rate of decomposition of particulate matter. Jewell and McCarty (1971), in experiments lasting in the order of 100 days, observed that algal endogenous respiration only accounts for between 20% and 30% of the total oxygen required to decompose the algal biomass; hence, for 80% to 90% net decay to occur, bacterial oxygen consumption must account for the remainder, as no zooplankton were present. They also found that after an initially fast rate, algal derived organic matter decomposes much slower, but can be described by first-order kinetics. A significant fraction of the initial organic matter remained in their system after oxygen consumption apparently ceased or became too slow to measure. This cessation was unrelated to nutrient limitation.

From these results and others, they postulated that algae and algae-derived organic matter consist of three fractions. The first fraction consists of highly degradable storage products that disappear within a few hours after the organisms are placed in the dark. The

oxygen demand of this material is probably insignificant in long-term studies but could be important in determining diurnal oxygen variations in natural waters. The second fraction is biodegradable material, and constituted between 30% to 70% of the total mass of their algal-derived organic material. It degraded at a fairly constant, intermediate rate, with a first-order rate coefficient between 0.01 to 0.06 day^{-1}. This material would, therefore, be consumed within a year at normal temperatures. Because of its relatively slow rate of decomposition, it would significantly affect oxygen concentrations where it is present in sufficient concentrations, like bottom sediments or the hypolimnion of lakes and where the time scale of resupply of oxygen is long such as during stratification.

The third fraction is the refractory fraction. Refractory material is defined as organic material that still has energy available for microbial use in its bonds but it is so complex that it is difficult to break down and that the actual rate of oxygen consumption is very slow. Jewell and McCarty hypothesized that no more than a few percent per year of this material decomposes. Refractory material may have a significant effect on oxygen resources of natural waters only if it accumulates over many years in sediments and only if the products of decomposition can diffuse up through overlying layers of sediment for subsequent aerobic oxidation through such processes as methane formation.

Zobell (1943) conducted experiments on natural waters by spiking them with organic carbon (glucose). After 50% to 60% of the carbon as COD was used, the rate of oxygen consumption decreased sharply. Since glucose is a compound which is readily metabolized by bacteria, this suggests that glucose had been consumed and replaced by cellular remains which are quite refractory and whose oxygen consumption rate

is quite low.

Applying this to Jewell and McCarty's size fractions suggests that the lability, or availability of organic material can be used to qualitatively differentiate oxygen consumption rates. For example, their first fraction, organic material consisting of degradable algal storage products, would have a high rate of oxygen consumption, their second fraction, the biodegradable material, would have a lower rate of oxygen consumption, while the third fraction, the refactory material, would have the lowest rate of oxygen consumption.

These data of Jewell and McCarty and Zobell suggest that internally-formed organic material would primarily consist of material from the first and second fractions of Jewell and McCarty as it enters the hypolimnion. On the other hand, organic material derived from woodland or soil runoff would primarily consist of material in the second fraction of Jewell and McCarty with no material from the first fraction due to the time frames and probable degradation patterns involved in soils. In fact, some soil derived organic material may belong to the third fraction.

Unlike the work of Jewell and McCarty which examined mainly particulate organic matter and its derivatives, Ogura (1975) examined dissolved organic matter (DOM). He found that there are two labile fractions and one refractory fraction in dissolved organic matter (DOM). Despite being DOM, this fraction and their relative reactivities are similar to that of Jewell and McCarty. The objective of these experiments was to determine the rate and extent of DOM decomposition in coastal waters and compare these results to those of the open sea. After ten days, the slowest rate of breakdown of DOM was achieved. Ogura attributed this either to the DOM being refractory to biochemical decomposition or to

the activity of the microorganisms which was hindered due to the dissolved oxygen (DO) decrease. Further experiments were conducted to distinguish these effects. Experiments were run for sixty days in closed containers. After the sixty day period, the containers were reaerated. The rate constant for DOM decomposition was found to be the same before and after reaeration. Ogura then concluded that the rate of DOM decrease was due to refractory buildup and not oxygen depletion.

There is general agreement that organic colour is derived from material similar or identical to humic acids (refractory material) which originate from plant decomposition or from aggregation of simpler molecules into complex molecules in soils and sediments. Generally, heavily coloured waters are unproductive; this may be due to adsorption of phosphorous onto the humus. Such investigators as Novak, et al (1975) have postulated that colour is bioresistant and thus persists in the water. Some work on biodecomposability of colour has been carried out but more is required.

Gibson (1975) conducted experiments on carbohydrate pools in algae and in the water column of a shallow, eutrophic, Irish lake, which undergoes transient summer stratification. A direct relationship was observed between carbohydrate content and respiration rate. Also, labile pools of carbohydrates were found in algal cells. This indicated that there are two forms of carbon in the cells: structural and labile. Gibson postulated that there is a luxury carbon uptake by the cells under certain unspecified conditions. The cell would take a complex organic molecule, break it down to a more labile form, and store it for use when conditions are not conducive for growth and/or maintenance. If an algal cell containing these labile carbohydrate pools lysed, the labile material would be available for bacterial growth, thus contributing to

oxygen consumption.

Gibson also found that the respiration of blue-green algae was an important part of the total oxygen demand. In fact, bacterial oxygen consumption could only account for about 20% of the total oxygen consumption in his shallow unstratified lake. This suggests that his lake was biologically in balance; the excess of produced carbon probably rapidly settles to the sediments. Therefore, in stratified conditions when the lake is biologically out of balance, bacteria would be expected to account for more than 20% of the total oxygen demand in the hypolimnion. It is probable that the main source of labile carbohydrate in the hypolimnion is algal cell lysing and excretion of uningested algae by zooplankton.

In the mathematical description of organic carbon consumption, account is taken indirectly of the refractory nature of remaining organic carbon. Conventional BOD theory, the theory developed to describe the mathematics of oxygen consumption in wastewater treatment, assumes that oxygen consumption is directly proportional to the substrate concentration remaining at any time. On the surface, this appears to suggest that it disregards the degree of refractory character of the carbon. However, by expressing substrate concentration in terms of remaining potential oxygen demand, account is taken of the biodegradability of the remaining carbon. Such extensive work has however meant that examination of the carbon fractions has been most intensively studied only in the past decade.

Zobell and Grant (1943) observed that bacteria could grow on as little as 0.1 mg/ℓ of organic carbon. They concluded that bacteria could probably grow at concentrations of organic carbon so low that it could not be measured. This infers that bacteria can use all types of organic

carbon at all concentration levels. This is probably true only for labile fractions of organic carbon.

Most researchers found a plateauing of oxygen consumption at some time after an initial spike of organic carbon. Bhatla and Gaudy (1964) conducted batch experiments with sewage and acclimated seed spiked with glucose. In all cases, about 12 hours after the lag phase was over, the initial glucose spike was virtually used and the rate of oxygen consumption approached zero. Depending on conditions, the rate of oxygen consumption was nearly zero for between 10 to 25 hours before increasing again. From their experiments, Bhatla and Gaudy concluded that the best possible explanation for this occurance is as follows. There are a few cells at time zero which are suddenly exposed to an environment excessively rich in food. This causes cells to follow a classical S-curve. After reaching a maximum population, the cells may not die off but lose their ability to replicate. An acclimation period may be required before the cells can metabolize certain cellular compounds which were synthesized in the replicating process; that is, an acclimation period may be required before certain cellular compounds can be endogenously metabolized or respired. This oxygen consumption behaviour with time is not described by BOD theory.

The actual behaviour observed by Bhatla and Gaudy and that implied by BOD theory is sketched in Figure 2.2. There is a significant lack of agreement between theory and observation at about one day. However, in later work, Gaudy's research has shown that different carbon components experience different times at which they initially plateau. In a water containing a variety of substrates, the individual times for plateauing become so mixed that the curve given by BOD theory describes the average behaviour of the degradation of all compounds in an adequate fashion.

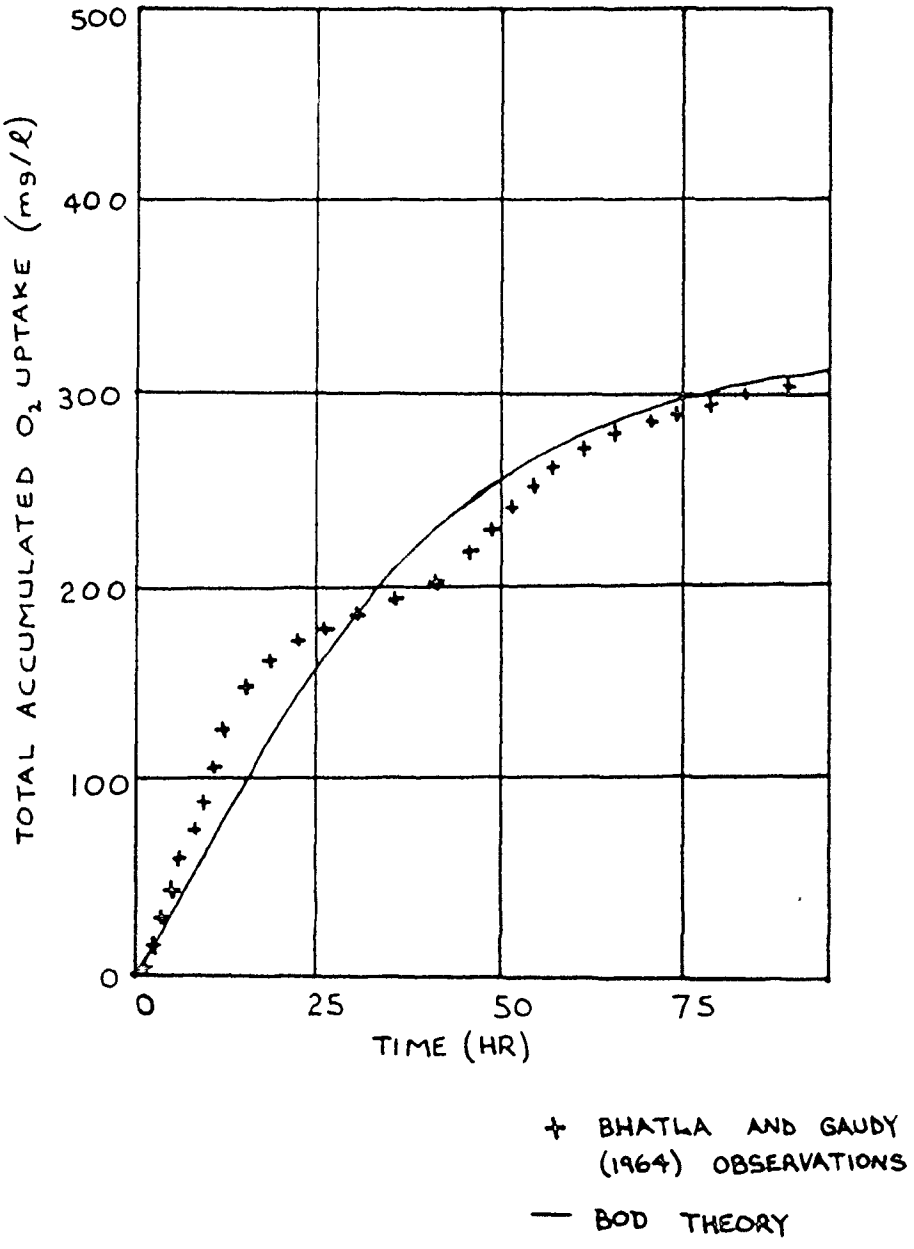


FIG. 2.2

SKETCH OF TIME PATTERN OF BOD

AND CARBON FRACTIONS

The question arises: does oxygen concentration by itself limit bacterial growth rates. Zobell (1943) observed no oxygen limitation to microbial growth until the DO concentration was less than 0.3 mg/ℓ. Gibson (1975) found that oxygen consumption was linear with time in an unspiked natural water incubated in unstirred batch reactors for 160 hours. Overall, the governing idea appears to be that bacteria are not limited by oxygen concentration above 0.3 to 0.5 mg/ℓ if well-dispersed. Hence, above this concentration, oxygen transfer through cell walls is sufficiently fast that it is not the limiting step in the electron transport system of respiration. Below this concentration, transport through cell walls may be the limiting step.

However, in films and flocs, an oxygen limitation has been noted. Hargrave and Philips (1977) observed that oxygen consumption was not reproducible in unstirred samples. This result could be due to a buildup of different sized flocs which cause different rates of diffusion of oxygen into the flocs under different conditions of stirring. They also conducted experiments in which the majority of bacteria were attached to surfaces. They observed that since the thickness of an attached community (supported growth) exceeds the limiting thickness of oxygen supply (21μ in bacterial films) the reduced oxygen supply may limit aerobic respiration even in the presence of adequate nutrients. In other words, agitation limits the thickness of the film enhancing the diffusion of oxygen to the cell located deep in the film or in an agglomeration of cells. They did not, however, specify the amount of stirring or agitation used. Oxygen uptake for a biological film was found to be a function of the surface area of the film. They were able to separate between the effects of the surfaces available for attachment (rocks, pebbles, glass) and the type of communities that

attach to these surfaces (phytoplankton, zooplankton, protozoa).

That bacteria prefer to attach to surfaces rather than be dispersed has been demonstrated by various research. Zobell (1943) observed that bacterial populations in water samples increased during storage and that the magnitude of this increase was often related to the size of the vessel. More oxygen was consumed in water exposed to the largest solid surface. In dilute solutions (less than 5 mg/ℓ of organic nutrients), more bacteria were found attached to the walls than in suspension in the water itself (Fig. 2.3). Zobell and Anderson (1936) observed that increases in solid surface area did not have an effect on bacterial multiplication or metabolism in solutions containing over 5 to 10 mg/ℓ organic carbon. From their experiments, Hargrave and Philips (1977) determined that inert and organically available substrates provide a stimulatory effect for microbial attachment and growth. They observed that an initial sparse microbial population on clean glass or recolonized on rocks and pebbles have a high oxygen consumption per gram organic matter. During succession, as community biomass increases, oxygen consumption per unit organic matter decreases to a lower level that may indicate physical-chemical limitations of diffusion. They concluded that while the presence of surfaces stimulate formation of attached communities, the long term effect is one of regulation and stabilization of both community biomass and oxygen consumption.

In summary these researchers suggest that there are two relatively labile fractions and one less labile fraction of organic carbon in the water column of lakes. Their work suggests that a compound like a carbohydrate may be appropriate for simulating the effects of organic carbon on growth. Oxygen consumption is limited by the concentration of labile carbon but independent of oxygen concentration down to 0.5 mg/ℓ

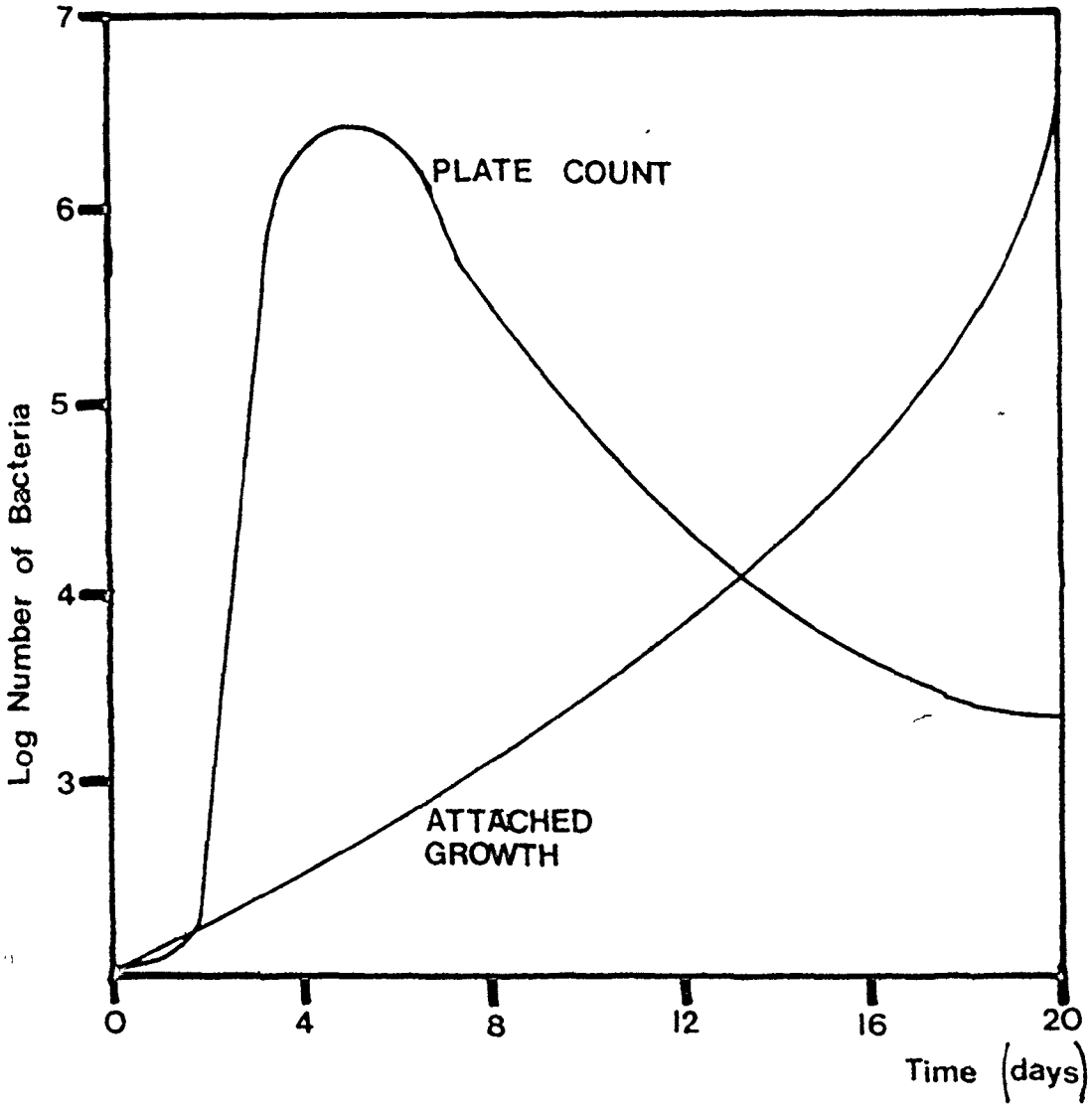


FIG. 2.3

Number of Bacteria Versus Time
(after Zobell)

in monodispersed solution. In flocs and films, oxygen concentration can become a limitation at higher concentrations; such a limitation is a function of turbulence and mixing. Whether wall effects of laboratory apparati are important is dependent upon the relative amount of bacteria attached to suspended particulates and to the walls.

2.3 Nitrogen Effects Upon Degradation

Nitrogen has two principal effects on degradation (i) a requirement as an essential nutrient for structural compounds during bacterial growth, and (ii) an energy source for degradation by nitrifiers in the form of ammonia.

DeMarco, et al. (1967), found that nitrogenous oxygen demand could be three times the oxygen demand exerted by organic matter. The generation time of the autotrophic populations was long compared to that of most heterotrophs. Indications of simultaneous oxidation of carbonaceous and nitrogenous compounds were observed. The presence of high concentrations of ammonia caused a greater oxygen demand than low concentrations but, the rate of oxygen demand was dependent on the rate of microbial utilization of ammonia. Ammonia can result from waste water inputs or from the hydrolysis of organic particulates. Golterman (1964) observed that the efficiency of organic nitrogen conversion to ammonia was concentration dependent. At higher concentrations of organic nitrogen (protein), the conversion to ammonia was less complete. Whether this is a time problem due to slow growth rates of bacteria is not clear from his data.

Nitrogen cycling plays an important role in algal blooms, in algal succession, and in the dynamics of other water quality phenomena. The high turnover rates can be maintained only by nitrogen recycling in the water column, not from land based inputs or other sources (Verhoff and

DePinto, 1977). Many factors control the reaction kinetics of this cycling. Varma, et al. (1975) hypothesized that pH, temperature, composition and complexity of substrate, age of microorganisms, and the carbon to nitrogen (C:N) ratio are the most important. They determined that the ratio of five day BOD to ultimate BOD increased as the soluble C:N ratio increased; the rate of oxygen consumption was highest at the lowest nitrogen concentrations. This implies that when there is more nitrogen in the particulate form than the soluble form, or more microorganisms, the result is higher rates of respiration. When the soluble nitrogen concentration was increased, the rate of respiration decreased but a further increase in the nitrogen concentration beyond a plateau level did not change the rate of respiration.

Jones (1971) observed an increase in the C:N ratio of particulate matter with depth and thus determined that increases in the particulate C:N ratio may affect bacterial populations through the release of nitrogenous compounds. That is since the soluble nitrogen is released with depth, nitrifiers as well as heterotrophs have opportunities to be important components of oxygen consumption. In later work, Jones (1976) concluded that as old and lysed algae settled out of the water column, nitrogen was solubilized more rapidly than phosphorous. Jones' work suggests that some lakes may have a strategy to maintain as much nitrogen as possible in the water column available for microbial growth.

Several researchers have experimentally determined the appropriate rate factors controlling kinetic expressions of nitrogen uptake and release by bacteria and the associated mechanisms. Owens and Esaias (1976) experimentally considered the kinetics of nitrogen uptake. They concluded that the rate of nitrogen uptake is concentration dependent and can be expressed in the form of Michaelis-Menton kinetics. The

total nitrogenper cell was higher when cells were grown with nitrate and ammonia than with ammonia alone. They also observed luxury uptake of nitrate, when present in large amounts, in excess of requirements for growth, resulting in internal pools of unreduced nitrate.

Brown, et al. (1975) and Chan and Campbell (1978), have examined the mechanisms of nitrogen transformations. Brown, et al. showed that nitrate may be used physiologically by bacteria in two ways. One is as an electron acceptor and the other is as a nitrogen source for synthesis. In algae and fungi, ammonia uptake is preferred to nitrate uptake. They stated that this observation has not been proven yet for bacteria. Comparing ammonia effects on nitrate uptake and vise versa. Brown, et al. (1975) observed that ammonia inhibited nitrate uptake when in sufficiently large quantities with respect to nitrate while DeMarco, et al. (1967), observed that nitrate had no effect on ammonia oxidation.

Much more work on nitrate uptake has been carried out for algae than for bacteria. Representative work is that of Chan and Campbell (1978) who observed variations in nitrate uptake kinetics and attributed these variations to species composition and environmental factors (such as temperature, light, and available nutrients). In the dark, they observed that nitrate uptake was small compared to uptake in the light. Dark uptake was thought to be due to endogenous respiration and light uptake was thought to be due to electron transport as a source for synthesis. They also found that nitrate was cycled to a low-molecular weight fraction of dissolved organic nitrogen of which 20% to 45% was due to excretion. Whether this cycling represents significant information for determining and quantifying factors controlling degradation awaits further work.

In summary, whether nitrogen as ammonia or nitrate is the preferred form of the essential nutrient for structural compounds is not known. Levels of nitrate and ammonia sufficient for growth have, however, been determined experimentally. As ammonia, nitrogen represents an oxygen sink whose magnitude can be greater than that caused by heterotrophs. The potential for nitrification exists in hypolimnia if release of organic nitrogen occurs from settling particles.

2.4 Temperature Effects

Temperature has a strong influence upon the rate of microbial activity. In their laboratory experiments, Jewell and McCarty (1971) observed that with some algal cultures, a decrease in the refractory organic fraction with increasing temperature resulted, but in most cases, the refractory fraction was essentially the same at temperatures of 20°C or above. They concluded that the rate but not the extent, of decomposition increased with increasing temperatures. Varma and Nepal (1972) determined that as the temperature increased, acclimation time decreased. Also, the rate of bacterial growth in the log-phase increased with temperature.

Temperature effects on bacterial numbers were considered by Cherry, et al. (1974). At 20°C, total plate counts were high but species diversity was low. This may result from competition effects among bacteria with different growth rates. Temperature was thought to be the dominating effect when compared with oxygen concentration. But this observation may be questioned because it is based upon empirical observations and because temperatures between 16°C and 19°C may favour diversification in community structure as well as greater stability of population growth.

In later work, Jones (1977) observed that under different laboratory conditions, one can obtain the same numbers of viable counts, but that these counts may have a different species composition in a given number range. From field observations on bacterial populations (viable and/or total) he suggested that the interrelationships between the microorganisms are complex and change from year to year. This must make interpretations based on temperature and on oxygen tentative despite the fact that a large effect due to stratification and deoxygenation was observed in his work. If further studies were required on bacterial populations, then the parameters to be measured should be carefully chosen. Jones recommended that for examining controls on the growth rate of microorganisms, DOM, grazing-predation, changes in water body detention time, cell division and death rate, and removal of microbes due to sedimentation or turbulence be examined.

A few of these recommendations will be adapted to this work. However due to the grosser description of bacteria all as one group rather than as specific species, the level of detail suggested by Jones (1977) is inappropriate.

2.5 Mathematical Modelling of Aquatic Systems

Many models for predicting the response of water bodies to various environmental changes or stimulations have been developed during the past decade. A few selected models will be discussed in this section. These models consider water column demand and vertical transport.

In 1970, Bella examined the relative importance of physical and biological processes as controls upon DO variations in stratified lakes. A one-dimensional depth dependent model over time was constructed as it was assumed that horizontal DO variations were small when compared

to vertical variations. Water column respiration was described as a constant zero order rate independent of temperature or substrate concentration. A sensitivity analysis showed that the sediment oxygen demand could explain the shape of the hypolimnetic DO, but reaeration had a negligible effect compared to vertical mixing, even in the thermocline, upon the profile. The model's time base was in the order of weeks to months rather than hours to days. As the model was not extensively compared to field observations, Bella cautioned that the acceptability of the model in one situation does not guarantee the acceptability in others.

More closely related to this research are models such as those of Thomann et al. 1975, Scavia et al. 1976 and Chen and Smith, 1979. These models generally describe phytoplankton growth, phosphorous and nitrogen concentrations, and their interactions as components of eutrophication. The models are based on the concept of conservation of mass, as opposed to conservation of energy, because the details of mass transfer are better understood than energy transfer within biological systems. Their models consider many processes and biochemical species including light intensity, phytoplankton concentration, nutrient concerntration, growth, respiration, and death of phytoplankton, and grazing rates and respiration of zooplankton. Some processes, such as light intensity, can be modelled with some confidence since these mathematical formulations have been well established for many environments. However, other more complex processes, such as grazing rates of zooplankton, were based on general relationships observed in laboratory conditions. These relationships were then incorporated into the model by use of a kinetic expression and associated coefficient whose exact relationship to actual conditions has not been extensively tested.

Some totally empirical coefficients were used as well. Consider the respiration rate of phytoplankton. It was modelled in the form K_2T by DiToro, et al. (1972) where K_2 was the respiration rate coefficient in the range 0.005 ± 0.001 days^{-1}, and T was temperature in degrees Celsius. The respiration rate's dependence on other environmental factors was precluded because of the lack of more precise data.

The total oxygen consumption in the hypolimnion of a lake due to algae (PK), herbivorous (Z1) and carnivorous zooplankton (Z2), oxidation of dissolved organics (DOM) and oxidation of ammonia (N1) may be described by the following:

$$r = K_2T[PK] + K_3T[Z1] + K_4T[Z2] + K_5T[DOM] + K_6T\ RON[N1] / (N1 + 0.54)$$

This is the basic type of formulation which arises from each of these models. Here r is the overall consumption rate, gm/m^3-day, K_3, K_4 are the respiration rates of zooplankton, K_5 is the first-order decay constant for organics, K_6 is the oxidation constant for ammonia and RON is the ratio of oxygen consumed per unit weight of ammonia oxidized. In this formulation, Monod type kinetics have been substituted for the first-order type used by Thomann et al. (1975). The temperature dependency here is expressed as a linear relationship. Alternative formulations more typically take the form of $R_1R_2^{(T-20)}$ where R_1 is analagous to the K's and the exponential is analogous to T. The detail of this model requires estimations of the different mass fluxes controlling PK, Z1, Z2, DOM and RON. This study will, in some ways, be asking whether certain of these fluxes can be ignored if one desires to construct a model of decay in the hypolimnia of a lake.

One question of interest to this work concerns the validity of using models for investigating ecosystem properties. In general, many properties discussed in modelling studies have received very little study as of yet in the natural environment and thus it is not possible to verify the results rigorously by comparison to actual measurements. However, it has been possible to compare some models' results to actual data for a number of these variables and processes described. Additionally, the results of some models show patterns that are in agreement with, or at least not in conflict with the expectations of ecological theory (Chen and Smith, 1979). Models provide a relatively inexpensive way to synthesize existing information and to develop hypotheses concerning these properties (Chen and Smith, 1979, and Canale, et al. 1976). Many models have, however, been constructed without all parts being adequately tested due to a lack of experimental data.

More information on specific processes is needed to assure complete confidence in many of these models. This information often takes the form of more experiments, identified from an analysis of the weaknesses of a model. Few comprehensive studies have been carried out on basic ecosystem properties due to great amounts of time and money that must be expended to carry out such work. Immediate needs of decision makers often require the construction and use of models even though they may have weaknesses. The future requires that experimental and modelling efforts should complement each other in an effort to complete the description of aquatic ecosystems (DePinto, 1979).

CHAPTER 3

EXPERIMENTAL METHODS AND PROCEDURES

3.1 Experimental Methods Chosen

Various measures of community microbial activity are available. They include doubling time, adenosine triphosphate (ATP) production, substrate uptake, substrate utilization, oxygen uptake rate, CO_2 production, and hydrogenase activity. Most of these measures were eliminated from consideration due to problems of cost, reliability, or difficulty of obtaining good reproducible values. Only two were considered as viable alternatives: rate of oxygen uptake and rate of substrate uptake.

The rate of oxygen uptake was chosen as the measure of community activity for two reasons. The first reason is that the measurement of substrate kinetics is specific to those members of the community which are able to utilize the substrate, while the measurement of oxygen uptake rate includes the total aerobic metabolism in a community (Hargrave and Phillips, 1977). The second reason is that analytical, laboratory and other problems were encountered with the carbon analysis.

The rate of oxygen uptake was calculated by measuring the DO concentration at the start and end of the experimental run. The DO concentration was obtained by using a modified Winkler microtitration technique as described by Strickland and Parsons (1968). Appendix A shows more details of this method. All incubations were carried out in the laboratory in the dark. This is due to the fact that light incubation

can stimulate photosynthesis to cause a negative effect upon oxygen uptake.

The experimental test bottles were turned upside-down and shaken for approximately a minute once per day as Hargrave and Phillips (1977) observed that in some cases there was no measurable oxygen uptake in samples that were not agitated. This shaking treatment was also compared to continuous mixing by a magnetic stirrer.

The length of time of an experimental run varied between five and seven days. This period of time allowed for a measurable amount of oxygen uptake but was not long enough for excessive wall growth of bacteria (Zobell, 1943), or inhibitory refractory buildup (Ogura, 1975). Ten litre bottles were chosen to minimize the surface area to volume ratio, again, to limit wall effects.

The carbon source was selected to be glucose as it is easily utilizable by all microorganisms (Parsons and Strickland, 1962, Hamilton, et al., 1966). To assess the validity of glucose as a model carbon source, the effect on the rate of oxygen uptake of glucose and other organic substrates was compared (Appendix F). Appendix A gives details on the types and amounts of chemicals used as nutrient sources.

3.2 Experimental Design

An experimental design is chosen as the means to separate the most important variables from the less important ones, in as few experimental runs as possible. The variables tested are soluble organic carbon (SOC), temperature, nutrients (nitrogen, phosphorous and metals), oxygen concentration, and algal respiration. The response variable is the rate of oxygen uptake.

The experimental design utilized is a two-level fractional factorial type (Box and Hunter, 1961). That is, for every variable, there are two test levels. Temperature, for example, has a high or plus (+) level of 20°C while the low or minus (-) level varied from 7°C to 10°C, depending on the water body. Fig. 3.1 shows the high and low levels of all variables.

Two related fractional designs were tested. Their patterns are shown in Fig. 3.2 and Fig. 3.3. This procedure enabled the investigator to break the main effects (i.e., temperature) from two-factor effects or interactions (i.e., a carbon-temperature interaction). An effect is defined as the change in response, in this case the rate of oxygen uptake, in moving from the plus condition to the minus condition. If two variables do not act additively, they are said to interact. A more complete discussion of these theories is presented in Appendix B.

3.3 Aquatic Sampling

All water samples were collected from hypolimnetic waters of the various water bodies between mid-July and mid-December 1978. In all cases, the hypolimnion was below the euphotic zone. This was deemed a beneficial situation as few actively photosynthesizing algae should be in the sampled water.

All waters were collected by a six litre Van Doren sampler from depths of ten to twenty meters below the surface. The actual depth varied from lake to lake depending on the maximum depth of the lake. Water was sampled from the locations shown in Fig. 3.4 to 3.7 for the various water bodies. Sampling from Hamilton Harbour and the Guelph Reservoir were done from fixed points (a barge and

NUMBER	VARIABLE	LEVELS
1	Temperature	+ 20°C − 10°C Harbour & Guelph 7°C Chub & Red Chalk
2	Soluble Organic Carbon (SOC)	+ spiked − not spiked
3	Nutrients	+ spiked − not spiked
4	Oxygen	+ field conditions − purged with N_2 gas
5	Algae	+ screened − not screened

FIG. 3.1

Experimental Design Levels

BOTTLE NUMBER	1	2	3	4= 12	5= 23
1	+	+	+	+	+
2	−	+	+	−	+
3	+	−	+	−	−
4	−	−	+	+	−
5	+	+	−	+	−
6	−	+	−	−	−
7	+	−	−	−	+
8	−	−	−	+	+

FIG. 3.2

Experimental Design I

BOTTLE NUMBER	1	2	3	4= 12	5= 23
1	−	−	−	−	−
2	+	−	−	+	−
3	−	+	−	+	+
4	+	+	−	−	+
5	−	−	+	−	+
6	+	−	+	+	+
7	−	+	+	+	−
8	+	+	+	−	−

FIG. 3.3

Experimental Design II

FIG.3.4

Hamilton Harbour

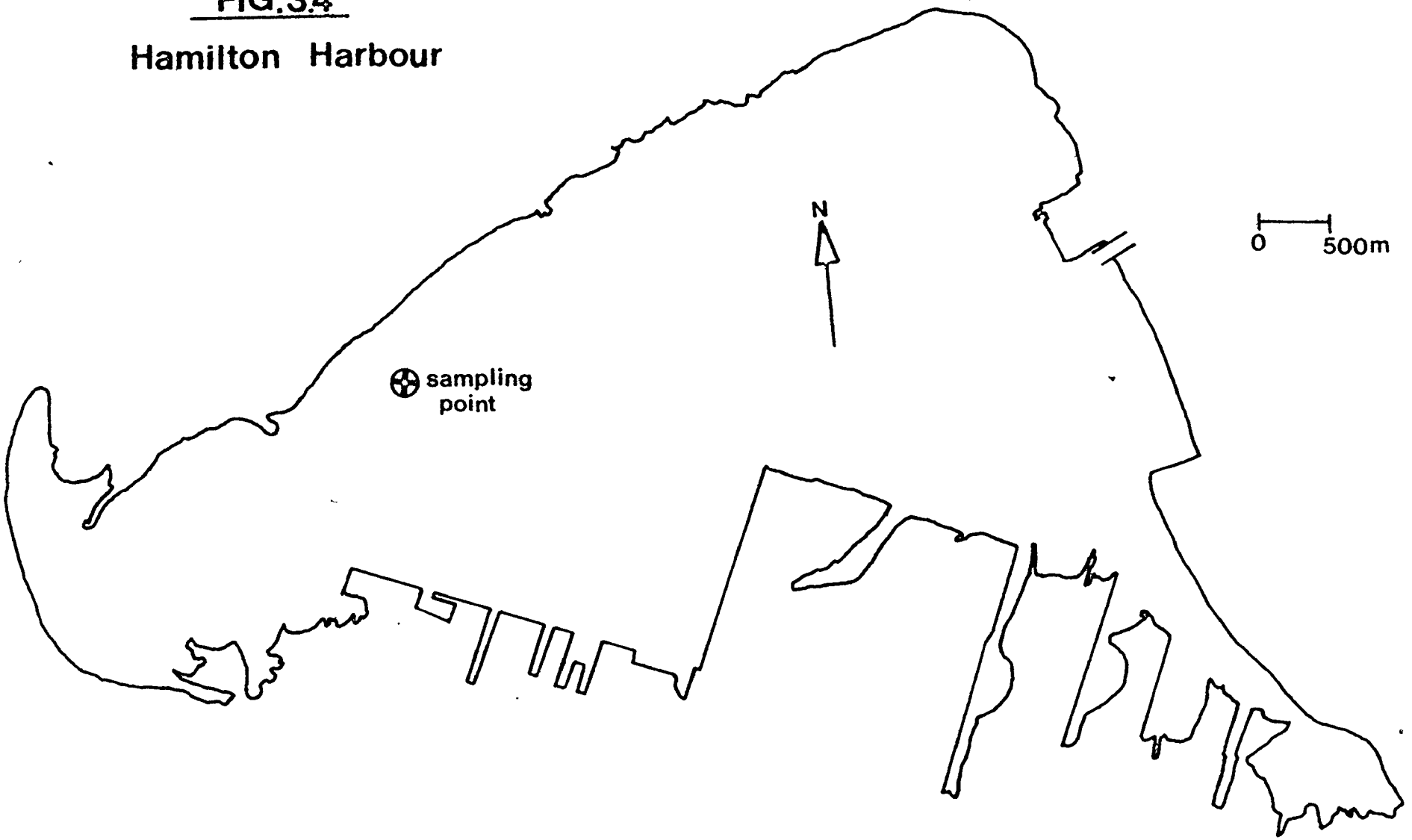


FIG. 3.5

Guelph Reservoir

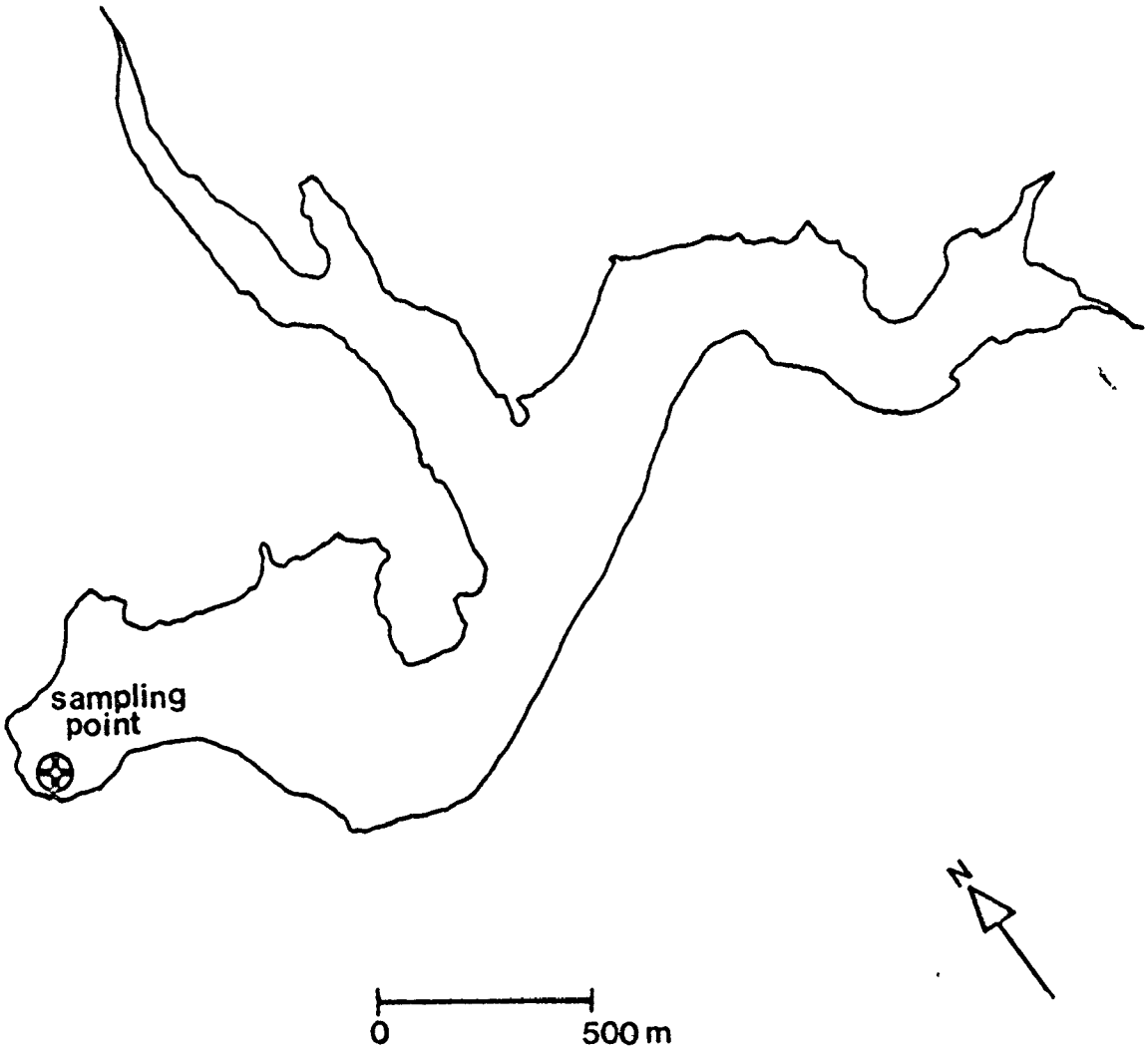
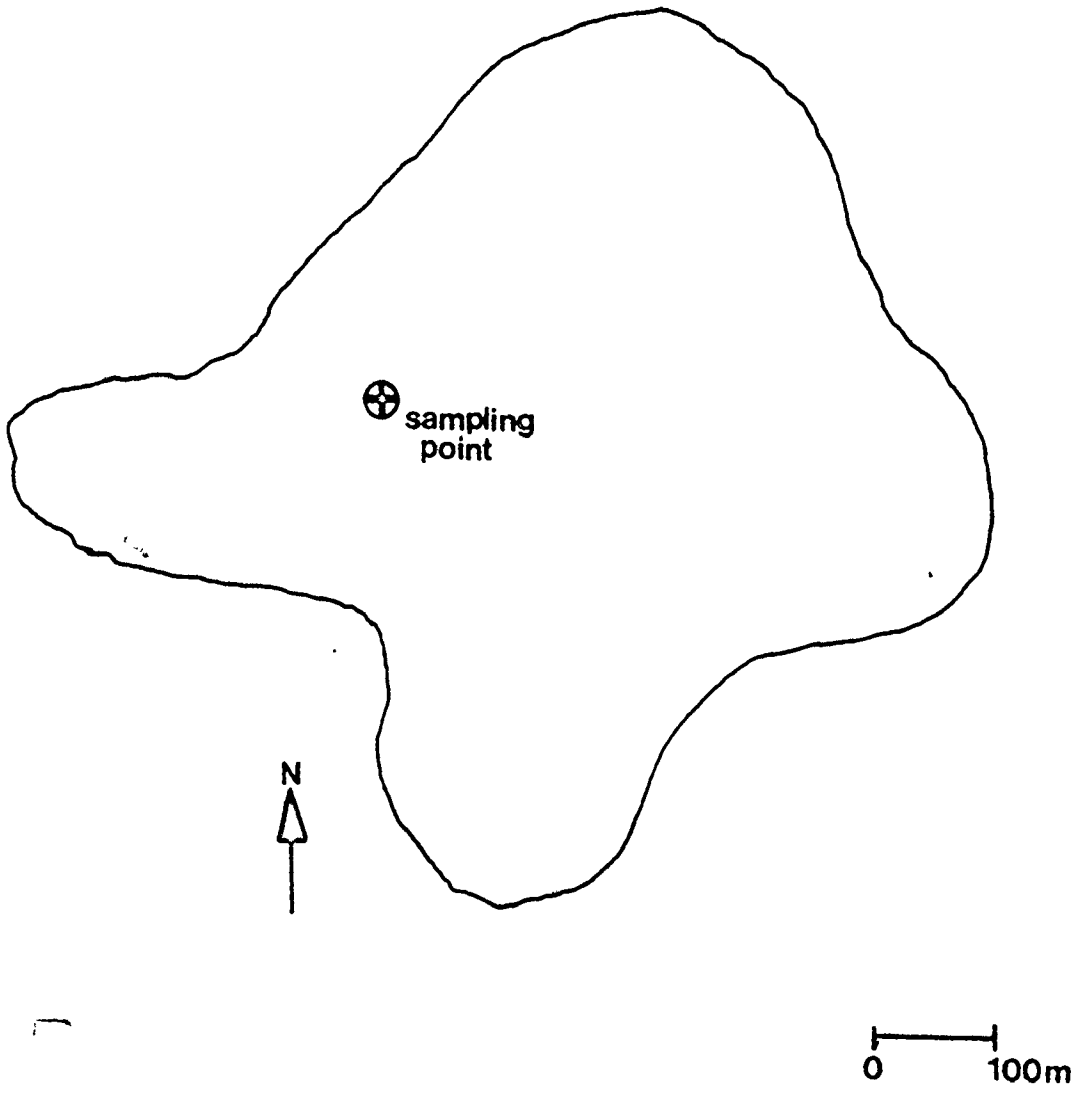


FIG. 3.6

Chub Lake



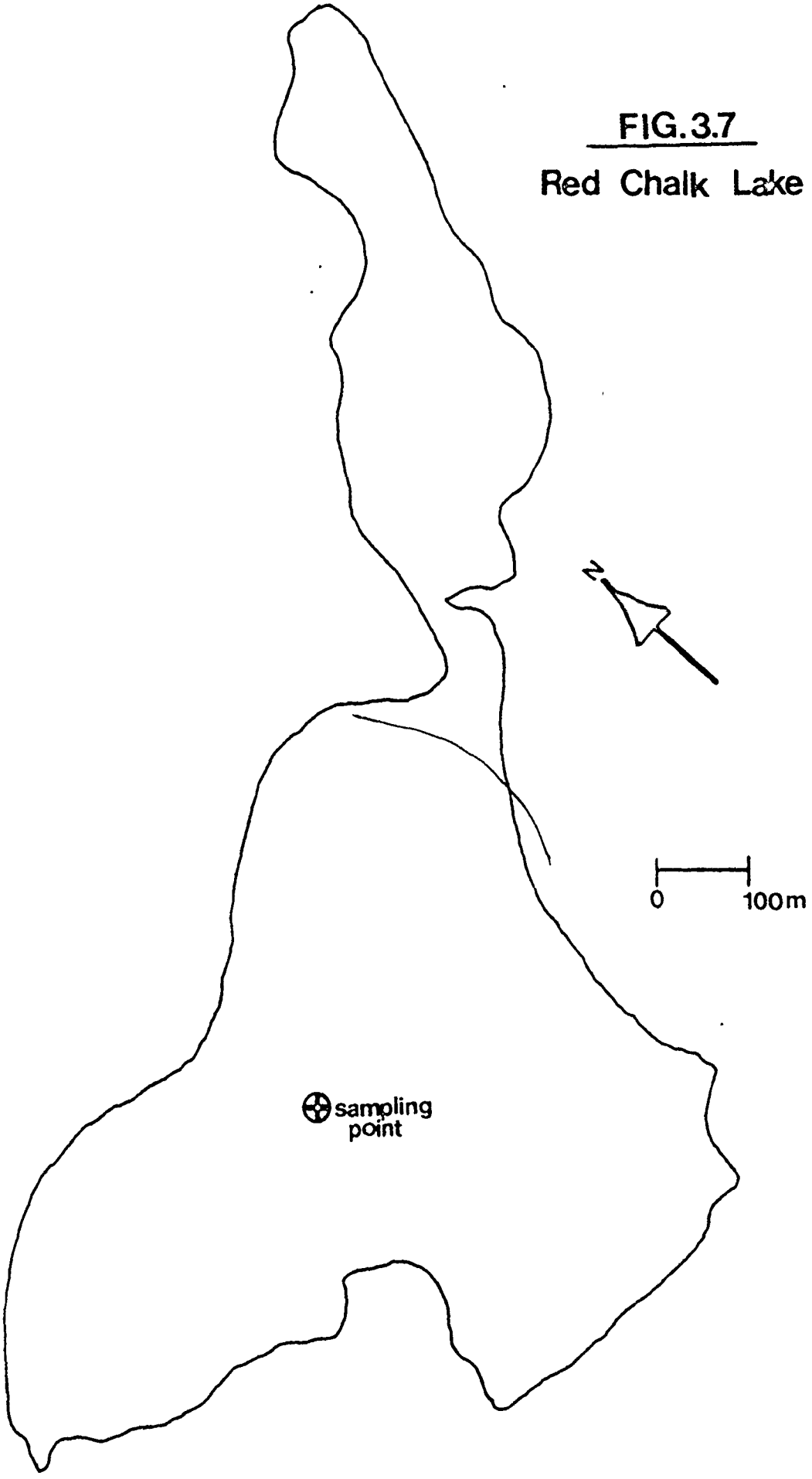


FIG. 3.7

Red Chalk Lake

a dam, respectively) while Red Chalk Lake and Chub Lake samples were collected from a boat.

Approximately 160 litres of water were required for a Main Design Run while 20 litres of water were needed for a Temperature or Oxygen Kinetic Run. Water was transported in 20 litre containers and brought back to the laboratory as soon as possible. If the water was not immediately processed, it was stored at 10°C in the dark.

3.4 Experimental Procedures

Experimental runs were carried out in the following sequence:

1. Test runs for "ballpark" values and investigator experience
2. Main design runs
3. Additional Hamilton Harbour runs
4. Rerun of inconsistent data
5. Oxygen kinetics
6. Temperature kinetics

Appendix D(c) lists the dates and durations of all runs.

3.4.1. Main Design Runs

In all runs, except Run 1, all of the collected water was placed in a 300 litre container to ensure homogeneity. The water was stirred after setting up each experimental bottle. About 15 litres of water were drawn out of this container, screened through a 10 μ mesh, if required, and placed in a 30 litre container. If required, carbon and/or nutrients would be added at this stage. Nitrogen purging took place in the 300 litre container for about one hour before the first amount was transferred to the 30 litre container. A 10 litre experimental bottle was then filled by syphoning such that no air bubbles were introduced into the bottle. A schematic drawing

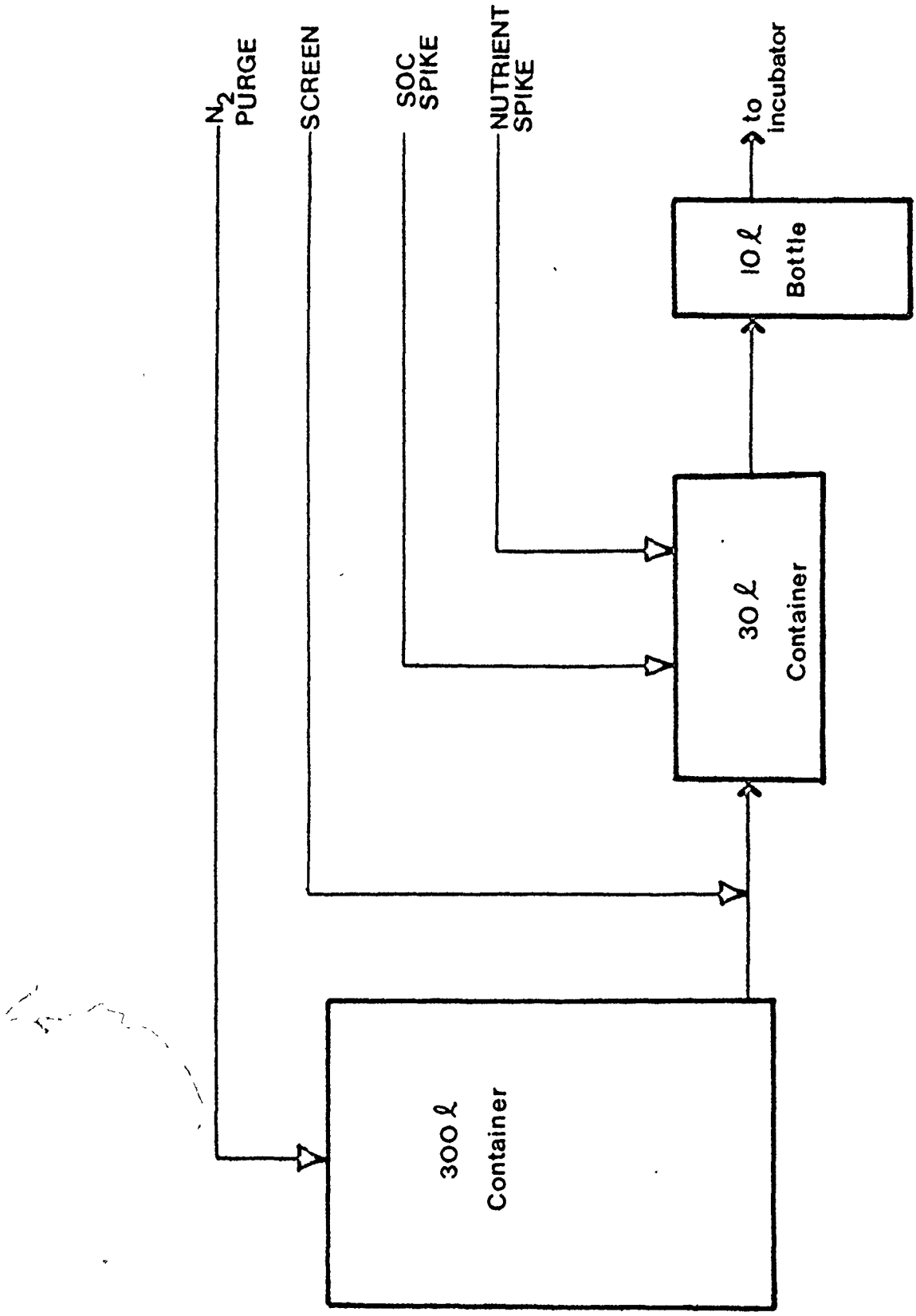


FIG. 3.8

Schematic of Experimental Start-up (NTS)

of the start up procedure is shown in Fig. 3.8.

The following procedure was employed in filling the 10 litre experimental bottles:

1. After the bottle is filled, allow 2 litres of water to overflow from it.
2. Retain the following amounts of water for chemical analyses: 500 mℓ for DO, 500 mℓ for SOC, 100 mℓ for pH and nitrogen.
3. Cap the 10 litre bottle from atmospheric conditions by means of a rubber stopper and place it in the appropriate incubator.
4. Store the SOC, pH, and nitrogen samples at 4°C.
5. Fix (stop all biological activity) and store the DO samples.

The average time for these start up procedures was 4.5 hours, excluding the first three runs because the procedure became more routine for the experimenter.

The termination procedure began by taking the experimental bottles out of the incubators and removing the stoppers. A rubber policeman (plastic spatula taped to an aluminium rod) was used to scrape, best as possible, the sides and the bottom of the experimental bottle and also to uniformly mix the contents. Water was then syphoned into sample bottles and analysed for DO, SOC, pH, and occasionally nitrogen. The average time required for run termination was 1.5 hours. Appendix A gives details of the analyses performed.

In all syphoning activities, either at the start or at the end of the run, about 1 litre of water was allowed to pass through the tubing before the water was sampled. This ensured that the water being sampled was from the container and not left over from the previous sample and eliminated any possible bubbles in the syphon line.

The DO samples were titrated within 48 hours of being fixed. The SOC samples were taken to Toronto and analysed by the Ontario Ministry of the Environment, from between 12 and 48 hours after sampling.

The experimental start up sequence was as follows. The four experimental bottles with the high condition of DO were filled first. Within this group, they were prepared randomly. The water was then purged with nitrogen gas, in the 300 litre container, for about one hour before randomly setting up the remaining four bottles.

When the experimental bottle was sealed with a rubber stopper, extreme care was taken to avoid entraining air in it. Although in some bottles there was a space between the liquid surface and the stopper, this space was thought to be a vacuum produced by the force required to stopper the bottle and the subsequent recoil of the stopper when the force was removed.

All glassware, except the black bottles used for DO analysis, was washed with chromic acid at the end of each run and rinsed three times with distilled water before reuse. The black bottles were only rinsed three times with distilled water and air dried upside-down before reuse.

For Run 1, because the 300 litre container was unavailable, the water was taken directly from the containers used for transportation and transferred to the 30 litre container for perturbation. Nitrogen purging was done in the containers used for transportation. Run 7 was redone due to erroneous initial SOC measurements. The oxygen uptake data was not affected by these measurements. Run 4 was redone because some of the bottles were incorrectly prepared and it was unknown which ones they were. Finally, Run 3 (Conestoga Reservoir)

was not tested on Experimental Design II due to time limitations.

3.4.2 Additional Hamilton Harbour Runs

Towards the end of the experimental period (October, 1978), a nitrogen analysis was done on the four water bodies. The ammonia spike was found to be about three times the ammonia found in the unperturbated water in all water bodies except Hamilton Harbour. The concentration of ammonia added was only about equal to the concentration found in Hamilton Harbour. Thus, at the time Run 7A was done, a double run that included both Experimental Designs, was carried out on Hamilton Harbour (Runs 1E and 1X).

Five litres of water per experimental condition were perturbated as per the Experimental Designs. Five 300 mℓ BOD bottles were used for each experimental condition, as 10 litre bottles were unavailable. SOC, DO, pH, and nitrogen analyses were done at the beginning and end of the experimental run.

Two unperturbated 10 litre bottles were prepared to correspond with ten (two sets of five) 300 mℓ BOD bottles. This procedure allowed for a comparison of effects that container size made on oxygen uptake rates. These results are presented in Appendix E.

3.4.3 Mixing and Non-Mixing Runs

After all eight 10 litre bottles were prepared, about 25 litres of water were placed in the 30 litre container. This water was reaerated, if required, to its approximate initial DO concentration and then syphoned into four 4 litre erlenmeyer flasks. Two of these erlenmeyer flasks contained 3" bar magnets. When all the flasks were filled, they were placed in the 20°C incubator. The length of the Mixing Run was the same as the respective Main Design Run, as they

were tested simultaneously. The two flasks with the bar magnets were mixed by magnetic stirrers adjusted at the same speed setting. The other two flasks were agitated in the same manner and at the same time as the 10 litre bottles.

At the beginning of these runs, measurements of one SOC sample and one triplicate of DO samples were made as the samples in the flasks came from the common 30 litre container. At the runs' termination, one SOC and one triplicate of DO analyses were taken for each erlenmeyer flask.

Due to water shortages, any water left over from the Main Design Run was saved and used in the Mixing and Non-Mixing Run. Although this water was perturbated with respect to field conditions, this change would have been small, as only about one litre remained unused from each experimental bottle prepared in the Main Design. Also, all four flasks, as previously mentioned were filled from the same stock of water. For these reasons, no bias was thought to be introduced.

3.4.4 Temperature and Oxygen Kinetic Runs

These experiments were made towards the end of the experimental program. For the Temperature Kinetic Runs, five different temperatures between 4°C to 20°C were used. The actual temperatures used varied for the different water bodies. Five 120 mℓ black bottles were filled and fixed at the beginning of the run. Two sets of 3 bottles were filled and capped for each different temperature. All black bottles were filled completely at random. The time of incubation varied between two and four days, depending on the water body. The time required to prepare one run was in the order of 20 minutes.

For the Oxygen Kinetic Runs, five different oxygen concentrations were used. Each different oxygen condition had three sets of three 120 mℓ black bottles (initial, intermediate, and final). Although a DO meter gives "ballpark" estimates of DO, titrations were made in order to obtain accurate initial DO values. Nitrogen gas was purged through the water to lower the DO. The black bottles were filled with water in decreasing order of oxygen concentration (highest concentration filled first). These black bottles were then incubated at 12°C for two to four days, depending on the water body. The temperatures in the incubators were set using a U-tube mercury thermometer. The time required to prepare an Oxygen Kinetic Run was in the order of 1 hour. The black bottles (120 mℓ) were not agitated throughout any of these runs unless some accidental mixing occurred such as by the opening and closing of the incubator door.

The water used for the Temperature and Oxygen Kinetic Runs was unperturbated with respect to organic carbon, nutrients, and algae. The only exception to this was one Hamilton Harbour Oxygen Kinetic Run in which a screened and unscreened test was prepared at one oxygen concentration to see if there was any difference between the two conditions. Black bottles were fixed at the start and at four other predetermined times.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Main Design Runs

The analysis of data from Factorial Experimental Designs is not complicated. Recall that an effect is defined as the change in response from the plus (+) condition to the minus (-) condition. If two variables do not act additively, they are said to interact. Third and higher order interactions have been neglected in this work.

First, a set of contrasts for each Design is required (TABLES 4.1 and 4.2). A contrast is defined as the difference between the two averages (plus conditions and minus conditions) of four results. The symbol ℓ_i, the ℓ_i contrast, represents the linear function of the observations which are used to estimate the effect of variable i. If the absolute value of one contrast was noticeably larger than the others, then this contrast could be said to be dominating. From TABLE 4.1, this was not observed consistently, hence a second Experimental Design was developed and tested. The contrasts of this second Design, ℓ_i', are calculated in the same manner as the first Design and shown in TABLE 4.2.

By combining the two Designs, the blocking and average effects can be estimated. The average effect separates the two-factor interactions from the main affects (TABLE 4.3(a)). Conversely, the blocking effect separates the main effects from the two-factor interactions (TABLE 4.3(b)). If the absolute value of the effects is considered,

Water Body	Run #	Contrast ℓ_0	ℓ_1	ℓ_2	ℓ_3	ℓ_4	ℓ_5
Guelph	1	0.603	0.380	0.340	0.135	0.470	0.255
Hamilton Harbour	2	0.660	0.135	0.270	-0.110	0.130	-0.205
Hamilton Harbour (b)	1E	0.554	0.648	0.343	0.108	0.133	0.193
Chub	4A	0.568	0.205	0.865	0.465	0.455	0.035
Red Chalk	5	0.363	0.575	0.330	0.150	0.075	0.340

(b) Big Nutrient Spike

TABLE 4.1 CONTRASTS OF DESIGN I

Contrast

Water Body	Run #	ℓ'_0	ℓ'_1	ℓ'_2	ℓ'_3	ℓ'_4	ℓ'_5
Guelph	6	0.401	0.488	-0.083	0.153	0.103	-0.233
Hamilton Harbour	7	0.465	0.565	0.135	0.015	-0.080	0.110
Hamilton Harbour	7A	0.483	0.325	0.085	0.135	-0.055	0.205
Hamilton Harbour	Ave 7 & 7A	0.474	0.445	0.110	0.075	-0.068	0.158
Hamilton Harbour (b)	1X	0.530	0.400	0.210	-0.185	0.155	0.080
Chub	8	0.285	0.400	0.315	-0.035	-0.080	-0.145
Red Chalk	9	0.290	0.105	0.165	0.220	-0.205	-0.065

(b) Big Nutrient Spike

TABLE 4.2 CONTRASTS OF DESIGN II

the effect with the largest magnitude is taken as most important. A negatively signed effect contributes negatively to the response; in this case, it implies oxygen production.

In TABLE 4.3(a), the 0 (zero) effect is shown. The zero effect is the grand average of all experiments from both experimental runs (sixteen points). Within the set of blocking effects (TABLE 4.3(b)), there is the block effect. This block effect estimates the difference in averages between Design I and Design II. A more complex discussion of these concepts can be found in Appendix B.

4.1.1 Guelph Reservoir

Of the main factors (TABLE 4.3(a)), temperature (1) is observed to be the most dominant main effect. The relative order of importance of the remaining factors is oxygen (4), nutrients (3), carbon (2), and screening (5). The values obtained for the nutrient effect (3) and carbon effect (2) are very close and may not be distinguishable. Only the grand average effect (0) and the temperature effect (1) are statistically significant (95% CI).

Of the two-factor interactions (TABLE 4.3(b)), the carbon-nutrient interaction (23) is the largest, followed by the temperature-oxygen plus nutrient-screening interaction (14 + 35). The two-factor interactions of carbon-oxygen (24) and carbon-screening (25) are very small. None are statistically significant (95% CI). Because the screening main effect (5) is small, any two-factor interaction including it would also be expected to be small. It is interesting to note that the carbon-nutrient interaction (23) is very large when compared to the carbon-oxygen interaction (14). This does not appear to follow the pattern because the main effects of carbon (2) and oxygen (4) are relatively important but their combination is not. No explanation can be given for this occurence.

Water Body	Run #	Effect 0**	1	2	3	4	5
Guelph	1 & 6	0.502*	0.434*	0.129	0.144	0.287	0.011
Hamilton Harbour	2 & 7	0.563*	0.350*	0.203	-0.048	0.025	-0.048
Hamilton Harbour	2 & 7A	0.572*	0.230	0.178	0.013	0.038	0.000
Hamilton Harbour	2 & Ave 7 & 7A	0.567*	0.290*	0.190	-0.018	0.031	-0.024
Hamilton Harbour (b)	1E & 1X	0.542*	0.524*	0.277*	-0.039	0.144	0.137
Chub	4A & 8	0.427*	0.303	0.590*	0.215	0.188	-0.055
Red Chalk	5 & 9	0.327*	0.340*	0.248	0.185	-0.065	0.140

(b) Big Nutrient Spike

* Statistical Significance (95%)

**0 → Grand Average

TABLE 4.3(a) MAIN EFFECTS

Effect

Water Body	Run #	Block	24	14 + 35	25	12	23
Guelph	1 & 6	0.101	-0.054	0.212	-0.009	0.184	0.244
Hamilton Harbour	2 & 7	0.098	-0.215	0.068	-0.063	0.105	-0.158
Hamilton Harbour	2 & 7A	0.089	-0.095	0.093	-0.123	0.093	-0.205
Hamilton Harbour	2 & Ave 7 & 7A	0.093	-0.155	0.080	-0.093	0.099	-0.182
Hamilton Harbour (b)	1E & 1X	0.012	0.124	0.067	0.147	-0.011	0.057
CHUB	4A & 8	0.142	-0.098	0.275	0.250	0.268	0.090
Red Chalk	5 & 9	0.037	0.235	0.083	-0.035	0.140	0.200

(b) Big Nutrient Spike

TABLE 4.3(b) TWO-FACTOR INTERACTIONS

4.1.2 Chub Lake

Of the main factors (TABLE 4.3(a)), the order of importance of the main effects is as follows: carbon (2), temperature (1), nutrients (3), oxygen (4), and screening (5). The screening effect (5) is very small in relation to the others. Only the grand average effect (0) and the carbon effect (2) are statistically significant (95% CI).

Of the two-factor interactions, (TABLE 4.3(b)), the temperature-oxygen plus nutrient-algae interaction (14 + 35) was the most important. The remaining two-factor interactions in decreasing order are temperature-carbon (12), carbon-screening (25), carbon-nutrient (23), and carbon-oxygen (24). None of the two-factor interactions are statistically significant (95% CI). The first three have approximately the same numerical value indicating that further work is essential to distinguish between them. The carbon-nutrient interaction (23) and the carbon-oxygen interaction (24) were very small compared to the first three. The carbon-oxygen interaction (24) is negative. This suggests that this interaction has a negative effect, on the rate of oxygen uptake of the magnitude shown. This occurence may be explained by the presence of the oxygen effect (3) in the interaction.

The carbon main effect (2) is large, but the carbon-nutrient interaction (23) and the carbon-oxygen interaction (24) are small, and negative in the case of the carbon-oxygen interaction (24). Conversely, the main effect of screening (5) is small but the two-factor interaction of carbon-screening (25) is large; this indicates that the carbon effect (2) dominates the carbon-screening interaction (25), but on the other hand, carbon (2) does not directly appear to dominate the carbon-nutrient (23) or carbon-oxygen (24) interactions.

4.1.3 Red Chalk Lake

Of the main factors (TABLE 4.3(a)), the main effects are listed in decreasing order of importance: temperature (1), carbon (2), nutrients (3), screening (5) and oxygen (4). Only the grand average effect (0) and the temperature effect (1) are statistically significant (95% CI). The oxygen effect (4) is small compared to the others, and negative.

Of the two-factor interactions (TABLE 4.3(b)), the carbon-oxygen interaction (24) is the most important followed by the carbon-nutrient interaction (23) and the temperature-carbon interaction (12), respectively. The remaining interactions (temperature-oxygen plus nutrient-screening, 14 + 35, and carbon-screening, 25) are relatively small. None are statistically significant (95% CI).

The main effect of oxygen (4) is small but the two-factor interaction of carbon-oxygen (24) is large. This indicates that the carbon effect (2) is dominant in this situation. Similarily, the carbon effect (2) may also dominate the carbon-screening interaction (25).

A possible explanation for the relative size of the algal effect (5) follows. As algae are exposed to light, photosynthesis occurs which has a negative effect on oxygen uptake. Although incubation took place in the dark, transportation of the water, start-up and termination procedures of the experiments were carried out under light conditions. This hypothesis of photosynthesis is not substantiated by the main effect of screening (5).

4.1.4 Hamilton Harbour

Of the main factors (TABLE 4.3(a)), there are three sets of data for Hamilton Harbour taken from 10 litre bottles. The data from Run 2 and the average of Runs 7 and 7A are shown for demonstration

purposes. Only data from Runs 7 and 7A individually will be discussed.

The most important main effect was temperature (1), followed by the carbon effect (2). The remaining three main effects (nutrients, 3, oxygen, 4, and screening, 5) are small compared to the first two. In Run 7, the grand average effect (0) and the temperature effect (1) are statistically significant (95% CI). For Run 7A, only the grand average effect (0) was found to have statistical significance.

For the two-factor interactions of Run 7 (TABLE 4.3(a)), the carbon-oxygen (24) and carbon-nutrient (23) interactions appear to be more important than the remaining three, and are both negative. The two-factor interactions of carbon-nutrient (23) and, to a lesser extent, carbon-screening (25) seem to be more important than the others from Run 7A data, and again, have negative values. The carbon-screening interaction (25) in Run 7A is numerically similar to the temperature-carbon (12), temperature-oxygen plus nutrient-screening (14 + 35), and carbon-oxygen (24) interactions, thus giving two groups of two-factor interactions: the carbon-nutrient (23) interaction and the others. In both groups, the carbon-nutrient interaction (23) is important but negative. Little can be said about the dominating effect in this case. None of the two-factor interactions are statistically significant (95% CI).

Of the main factors (TABLE 4.3(a)), in the large nutrient spike experiments on Hamilton Harbour water (Runs 1E and 1X), temperature (1) was found to be the dominant main effect. It was followed by carbon (2), oxygen (4), screening (5), and nutrients (3), in decreasing order of importance. The oxygen (4) and screening (5) effects are similar numerically while the nutrient effect (3) is small and negative. The grand average (0), temperature (1), and carbon (2) effects were found to be statistically significant (95% CI).

The most important two-factor interaction (TABLE 4.3(b)) is the carbon-screening interaction (25), closely followed by the carbon-oxygen interaction (24). The temperature-oxygen plus nutrient-screening interaction (14 + 35) is numerically close to the carbon-nutrient interaction (23), but both are relatively small. The temperature-carbon interaction (12) is nearly zero and negative, which is interesting as both the carbon (2) and temperature (1) main effects are quite large. The carbon-screening interaction (25) is the largest two-factor interaction. None of the two-factor interactions are statistically significant (95% CI).

Runs 1E and 1X were incubated in 300 mℓ BOD bottles as opposed to 10 litre bottles. Appendix E shows that there is no statistically significant difference (95% CI) between the two sizes of experimental bottles, meaning that a comparison of results between the two different bottle sizes is valid.

Relating the results from both sets of runs (10 litre and 300 mℓ BOD bottles), the main effects of temperature (1) and carbon (2) are most important. In the 10 litre bottle experimental runs, the remaining three main effects are similar numerically and close to zero. In the final experimental runs (1E and 1X), the only main effect close to zero is the nutrient effect (3), which is also negative. In these runs, the screening (5) and oxygen (4) main effects are similar and large compared to the nutrient effect (3). No trends are observed between the two-factor interactions between the two sets of experimental runs (10 litre size versus 300 mℓ size containers).

4.1.5 Summary of Information from Main Design Runs

Experimental Designs allow one to distinguish which variables are more important than others on a qualitative basis. However, values

close to zero are interpreted as having little effect due to the nature of errors involved in these experiments and due to the need for more degrees of freedom. Values numerically similar to one another ($\pm$ 25%) may not be distinguishable and should be tested further.

The temperature main effect (1) is the most important main effect in the Guelph Reservoir, Red Chalk Lake, and Hamilton Harbour. Carbon (2) is most important in Chub Lake. The second most important main effect, although not statistically significant (95% CI), is carbon (2) in Red Chalk Lake and Hamilton Harbour, temperature (1) in Chub Lake, and oxygen (4) in the Guelph Reservoir.

Novak, et al. (1975) observed that coloured waters contain large amounts of humic material as is probably the situation in Chub Lake. If this is the case, then most of the organic carbon in the water would be unavailable to microbial growth, as it would be refractory material, hence a spike of available organic carbon (such as glucose) could spur microorganisms to increase their metabolic activity; this means that such a level of glucose is not an adequate model compound for simulating degradation processes in Chub Lake.

It was not surprising to see temperature (1) as the dominant main effect, as many others have observed the importance of temperature (Jones, 1971; Jewell and McCarty, 1971; Varma and Nepal, 1972; Cherry, et al. 1974). However, their work was not in the same context as this work. They typically observed the importance of temperature by either comparing the effects of different temperatures or comparing the effects of temperature and one other variable, such as carbon.

Organic carbon (2) is generally the second most important main effect. Had a less easily utilizable form been used (such as oxalic acid), the results may have been somewhat different. An experiment was

made to test the relationship of various forms of organic carbon to oxygen uptake rates. Unfortunately, due to equipment failure, the results obtained from this experiment are unsatisfactory. Appendix F gives a more complete discussion.

The oxygen main effect (4) showed varying degrees of importance in the four water bodies. It was the second most important effect in the Guelph Reservoir but was near zero and negative in Red Chalk Lake. No trend is observed between the oxygen effect in eutrophic-type conditions (Guelph Reservoir, Hamilton Harbour) as opposed to oligotrophic-type conditions (Chub and Red Chalk Lakes).

The nutrient (3) and screening (5) main effects are, generally speaking, smaller numerically than the temperature (1) and carbon (2) main effects. If nutrients are in limiting quantities, such as available carbon in Chub Lake, perhaps one would expect their addition to be important. Since small numerical significance is observed, some reasons are postulated. First, the nutrient spike may have been inhibitory to the microorganisms. This does not seem reasonable because standard BOD chemicals for river analyses were used (Appendix A). Another reason may be because the nutrient concentrations in the water bodies were not limiting to either the degradation process or the nitrifier population.

Because the sample water was taken from the hypolimnion, which in all cases was below the euphotic zone, one would expect algae to be dead and/or decomposing (Kuznetsov, 1968; Verhoff and DePinto, 1977). This would have the effect of increasing oxygen uptake rates. Algae are capable of heterotrophic activity (Parsons and Strickland, 1962)

which would tend to increase the rate of oxygen uptake as well. Also, bacteria may attach themselves on to algae and hence be screened out. Because the results of the Experimental Design show the screening effect (5) to be small, the previous arguments, with respect to algae, are concluded to not significantly contribute to oxygen uptake.

Some errors in simulating hypolimnetic conditions during stratification would occur to sampling during the overturn period. In this period, the lake begins to destratify due to mixing of the upper layers with the lower layers of water. It is possible, therefore, that a sample contained live algae from the hypolimnion. This could also occur during the stratified period but is probably more important during overturn. When exposed to light, these algae could begin to photosynthesize again and hence produce a negative effect on oxygen uptake rates. This problem would have been more acute at the beginning of the run as opposed to the end of the run. This should affect all bottles equally, except the screened ones, making the screening effect (5) important. Because screening is not important, this effect does not contribute significantly to the overall results.

No trends are found with respect to the two-factor interactions between the different bodies of water. These interactions are very complex within each water body and appear to be different under different environmental conditions.

4.2 Nitrogen Effects

Nitrogen analyses were carried out on Runs 7A, 1E, 1X, and partially on Run 9. Results are shown in Appendix J. Runs 1E and 1X were initiated because a nitrogen analysis on Hamilton Harbour showed that the ammonia (NH_3) concentration in the nutrient spike was

not large enough to also include nitrification requirements known to be important because of the large number of nitrifiers present. Hamilton Harbour water contained 0.25 mg/ℓ NH_3 while the concentration of the NH_3 spike in Runs 2, 7, and 7A was of the order of 0.25 mg/ℓ. For Runs 1E and 1X, the water contained about 0.2 mg/ℓ NH_3 while the spike added was 2.5 mg/ℓ NH_3. For the Guelph Reservoir, the field concentration of NH_3 was 0.2 mg/ℓ while the spike added 0.4 mg/ℓ. The field concentration of NH_3 in Red Chalk Lake was 0.15 mg/ℓ while the spike added 0.3 mg/ℓ. Similarily, in Chub Lake, the initial NH_3 concentration was 0.2 mg/ℓ while the spike added 0.4 mg/ℓ. Hence, the NH_3 spike was two to three times the field concentration in all cases except Hamilton Harbour.

TABLE 4.4 shows nitrogen data for two water bodies throughout the year. Hamilton Harbour is an interesting body of water as the NH_3 concentration decreases from 3.5 mg/ℓ in spring, throughout the summer, approaching a minimum in the autumn of < 0.1 mg/ℓ. If microbial activity slows down considerably in the winter due to lower temperature, then the concentration of NH_3 would increase during the winter. The concentration decreases as temperatures increase in spring and summer. Hydraulics can also play an important role with respect to NH_3 concentration in Hamilton Harbour due to exchange flows between Hamilton Harbour and Lake Ontario.

Water Body	Sample Depth (m)	Date (1978)	Nitrogen Concentrations (mg/ℓ)			
			NH_3	NO_2	NO_3	TKN
Hamilton Harbour (Stn. 270)	12.0	May 10	3.05	0.130	1.55	4.0
	12.0	June 7	1.97	0.050	2.60	3.45
	12.0	July 5	1.38	0.155	1.76	1.87
	12.0	Aug. 16	0.374	0.044	--	0.77
	12.0	Sept. 27	0.046	0.067	2.53	0.63
Guelph Reservoir	B*	May 15	0.038	0.048	1.58	0.53
	B*	June 5	0.118	0.029	1.35	0.60
	B*	July 4	0.242	0.036	0.945	0.78
	B*	Aug. 21	0.202	0.032	0.168	0.69
	B*	Sept. 18	0.260	0.013	0.070	1.15
	B*	Oct. 30	0.068	0.012	0.225	0.96

B* -- Bottom

TABLE 4.4

HYPOLIMNETIC NITROGEN CONCENTRATIONS
IN THE GUELPH RESERVOIR AND HAMILTON
HARBOUR THROUGHOUT 1978

Problems were encountered with nitrogen balances; that is, the nitrate (NO_3) plus the total Kjeldahl nitrogen (TKN) at the beginning of the run did not always equal that at the end of the run. Various investigators have observed similar problems (eg. Dawson, 1971) but have not been able to prove the cause irrevocably. It is concluded that the problems observed in obtaining good nitrogen balances are due to sampling and/or analytical techniques used by the researcher. Further discussion is outlined in Appendix J.

4.3 Mixing and Non-Mixing Runs

The results obtained from these experimental runs are shown in TABLE 4.5. In all cases but one (Run 7), the mixed reactors achieved a higher rate of oxygen uptake than the non-mixed reactors. In their work, Hargrave and Phillips (1977) placed sand pebbles, and pieces of detritus taken from the sediments into glass stoppered erlenmeyer flasks, which contained magnets for constant stirring. This procedure was followed because they found that unstirred samples sometimes had no measureable oxygen uptake.

Neither system (mixed versus non-mixed) simulates field conditions exactly. In lakes, wind and currents create complex mixing patterns. Continuous flow of water over sediments is not the same as a batch reactor with continuous mixing; in the field, and a fixed area of sediment as the reference frame (inertial or Newtonian frame of

Run #	Duration (Days)	Rate of O_2 Uptake (mg/ℓ-day)						Significant Difference (yes, no)
		Mix			Non-mix			
		Flask B	Flask D	ci	Flask A	Flask C	ci	
2	6	1.40	1.40	±0.00	1.05	0.91	±0.89	no
3	5	1.37	1.36	±0.06	1.10	1.02	±0.51	no
4	10.5	0.54	--	--	0.35	--	--	--
5	7	0.74	0.79	±0.32	0.63	0.58	±0.32	no
6	5	1.64	1.66	±0.13	1.52	1.49	±0.19	no
7	6	1.22	1.23	±0.06	1.21	1.24	±0.19	no
8	6	1.26	1.20	±0.38	1.05	1.05	±0.00	no
9	7	0.76	0.84	±0.51	0.53	0.52	±0.06	no

TABLE 4.5 CONTINUOUS MIXING VERSUS NON-MIXING RESULTS

reference), one would expect the water flowing over that area of sediment to be replaced with different water through time.

The same applies to the water column. Consider a control parcel or volume of water moving through the lake as the reference frame (Eulerian frame of reference). In this case, the sediments, with respect to the control volume of water, will move. The experiments reported herein are better explained by this system.

The actual field situation would probably lie somewhere in between the continuously mixed and non-mixed reactors. For the purposes of this report, no correction for mixing and non-mixing was made; this conclusion has been made because from TABLE 4.5, although differences in uptake rates can be seen, they are not statistically significant (95% CI).

4.4 Temperature and Oxygen Kinetic Runs

4.4.1 Temperature Kinetic Runs

The results obtained from these experimental runs are given in TABLES 4.6(a) to 4.6(d). These data were not plotted as many of the points lie very close to each other. The data obtained from the Guelph Reservoir was somewhat scattered, but due to time limitations, the run was not retested.

The rate of oxygen uptake was calculated using linear least squares (linear regression). The relationship between DO and time was assumed to be linear for the period under consideration (Gibson, 1975). The length of these runs was between 48 and 72 hours, depending on the water body.

As temperature decreases, the rate of oxygen uptake also decreases. Some exceptions are found. For the majority of experiments however, the

Temperature (°C)	DO_i (mg/ℓ)	DO @ t = 69.0 hrs. (mg/ℓ)	DO_f @ t = 93 hrs. (mg/ℓ)	Rate of DO uptake (mg/ℓ-day)
20	12.15**	11.60	10.97	0.279
12	12.15 **	11.51	11.58	0.164
8	12.15 **	11.55	11.49*	0.179
6	12.15**	11.44*	11.44	0.197
4	12.15**	11.43	11.45	0.196

* 2 replicates

** 5 replicates

TABLE 4.6(a) TEMPERATURE KINETIC
(Run #T-3 Chub Lake)

Temperature (°C)	DO_i (mg/ℓ)	DO @ t = 69.0 hrs. (mg/ℓ)	DO_f @ t = 93 hrs. (mg/ℓ)	Rate of DO uptake (mg/ℓ-day)
20	12.21**	11.79	11.40	0.195
12	12.21**	11.83	11.79	0.114
8	12.21**	11.78	11.80	0.115
6	12.21**	11.86	11.81*	0.107
4	12.21**	11.81	11.98	0.077

* 2 replicates

** 4 replicates

TABLE 4.6(b) TEMPERATURE KINETIC
(Run #T-4, Red Chalk Lake)

Temperature (°C)	DO_i (mg/ℓ)	DO @ t = 43.0 hrs. (mg/ℓ)	DO_f @ t = 93 hrs. (mg/ℓ)	Rate of DO uptake (mg/ℓ-day)
20	13.60**	13.56	12.56	0.326
12	13.60 **	13.82	13.42	0.045
10	13.60 **	13.92	13.08*	0.146
6	13.60 **	13.89	14.45	-0.279
4	13.60 **	13.93	13.90*	-0.110

* 2 replicates

** 5 replicates

TABLE 4.6(c) TEMPERATURE KINETIC
(Run #T-5, Guelph Reservoir)

Temperature (°C)	DO_i (mg/ℓ)	DO @ t = 51.5 hrs. (mg/ℓ)	DO_f @ t = 93 hrs. (mg/ℓ)	Rate of DO uptake (mg/ℓ-day)
20	12.05**	11.33*	10.85	0.385
12	12.05 **	11.62	11.42*	0.207
10	12.05 **	11.60	--	0.209
6	12.05 **	12.05	11.59	0.123
4	12.05 **	11.79*	11.77	0.098

* 2 replicates

** 5 replicates

TABLE 4.6(d) TEMPERATURE KINETIC
(Run #T-6, Hamilton Harbour)

results obtained agree favourably with the literature (Cherry, et al., 1974; Jewell and McCarty, 1971; Varma, et al., 1975; Varma and Nepal, 1972).

4.4.2 Oxygen Kinetic Runs

The results obtained from these experimental runs are plotted in Fig. 4.1 to Fig. 4.4. The length of these runs was between 50 and 70 hours. The tabulated data is given in Appendix D(b). One experiment, using Hamilton Harbour water, was tested to determine if there was a difference between screening and not screening the samples before incubation. As there is no discernable difference (Fig. 4.1(a) and 4.1(b)), non-screened water was used for all subsequent runs.

The rate of oxygen uptake is calculated using linear least squares. This analysis also assumes a linear relationship between DO and time for the period under consideration (Gibson, 1975).

There is a trend in the Hamilton Harbour data such that the higher initial oxygen concentrations resulted in higher oxygen uptake rates. A statistical difference (95% CI) between the higher rates (highest initial DO) and the lower rates (lowest initial DO) was noted. Appendix C shows the calcualtions used to determine statistical difference. No trends were observed in the other water bodies.

4.5 Experimental Errors

The rate data reported (as mgO_2/ℓ-day) has about the same percentage error, with respect to time in both the Main Design Runs and the Kinetic Runs. In both sets of runs, the approximate time was recorded when the last bottle was placed in the incubator. The same procedure

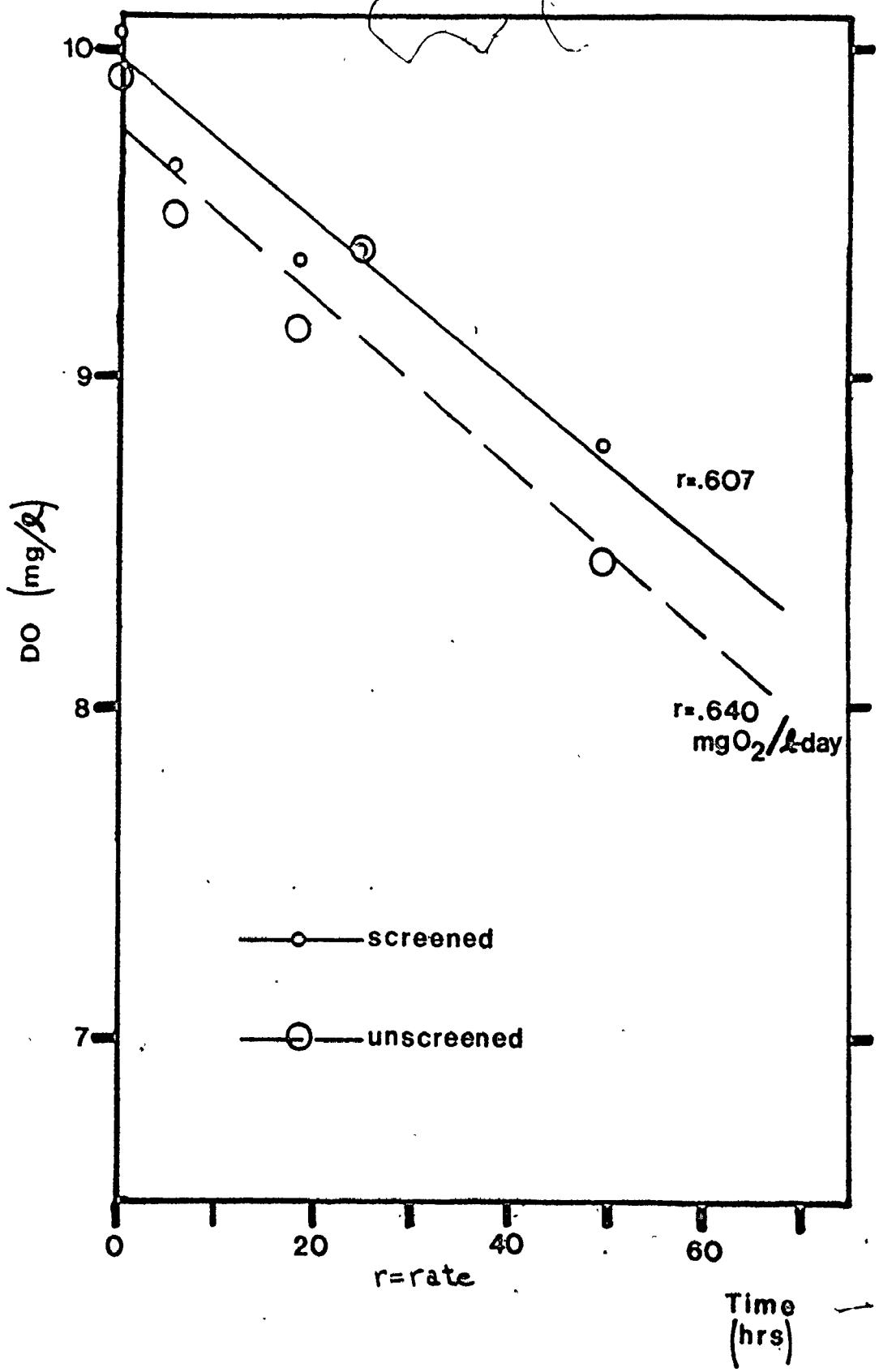


FIG. 4.1(a)

Hamilton Harbour

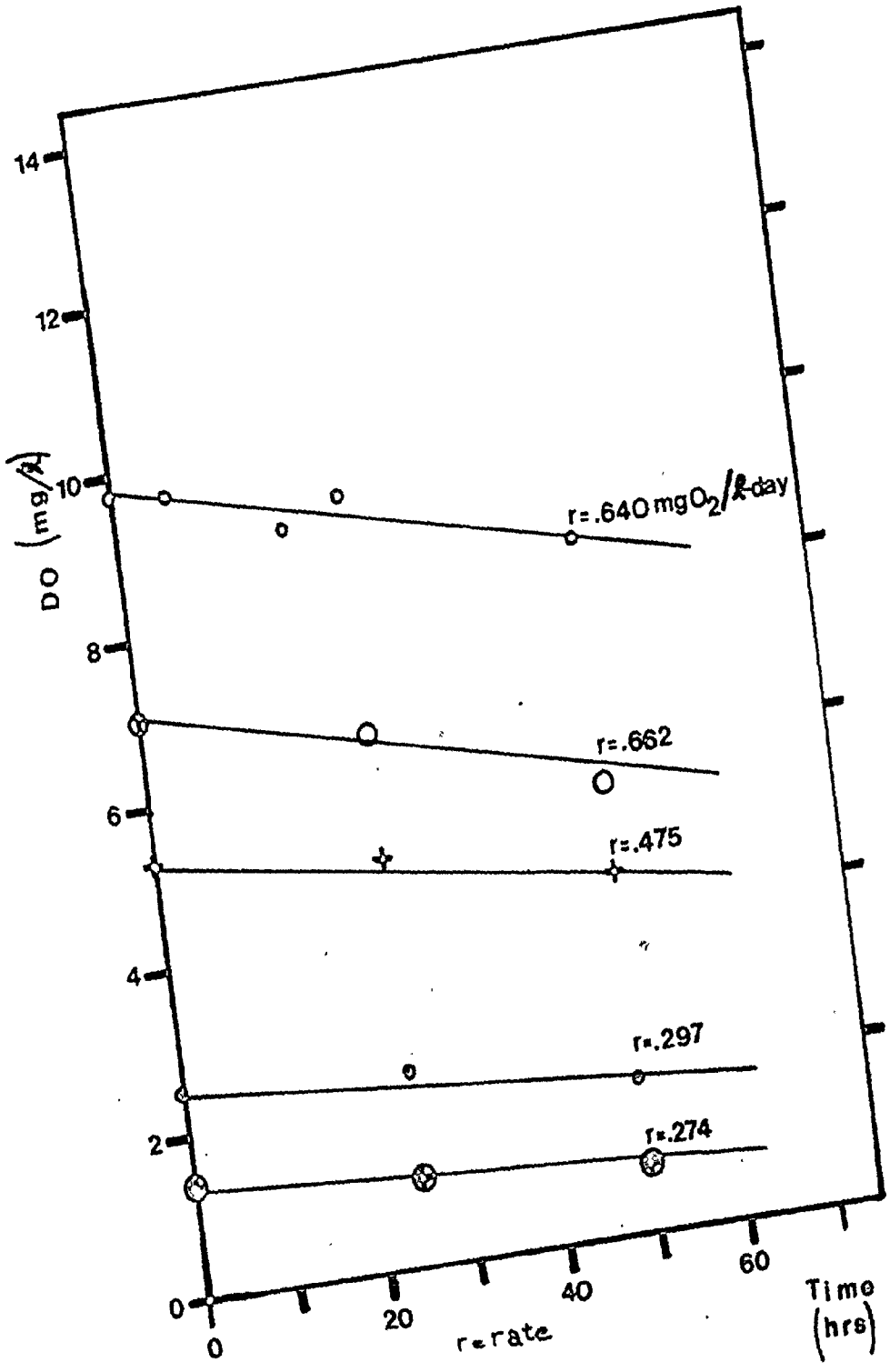


FIG.41(b)
Hamilton Harbour
Oxy K' cs

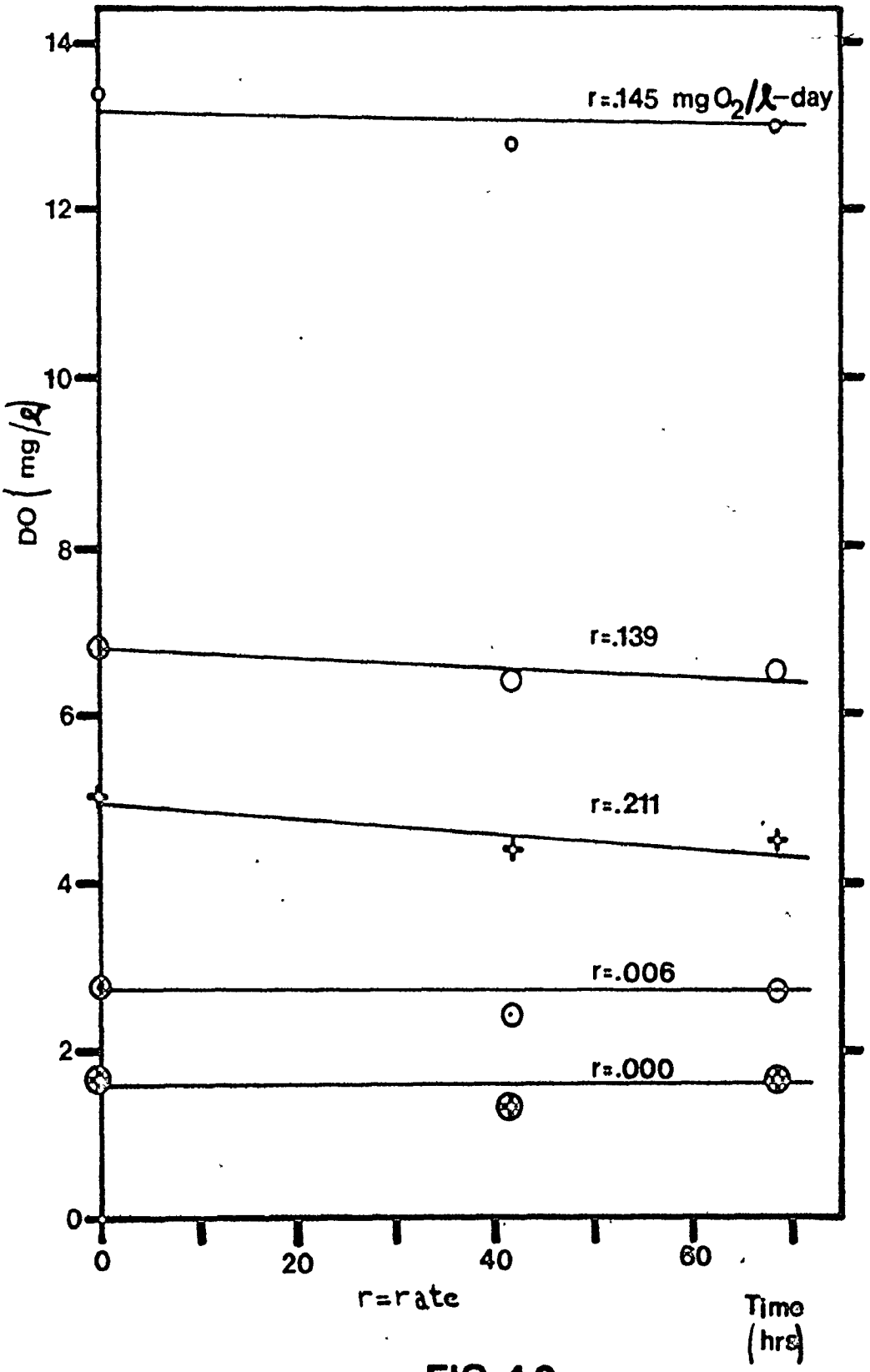


FIG.4.2

Guelph Reservoir
Oxygen Kinetics

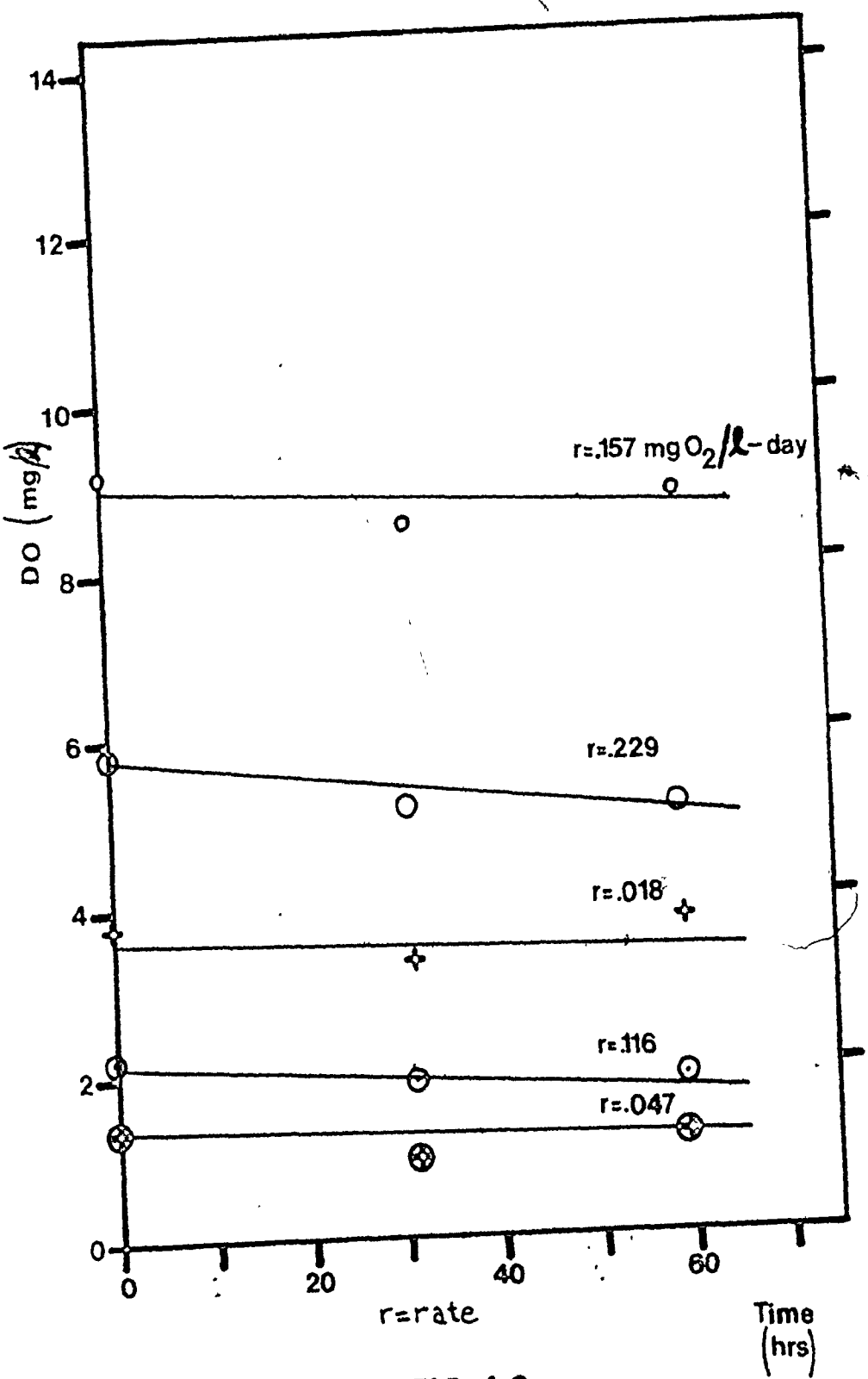


FIG.4.3

Chub Lake
Oxygen Kinetics

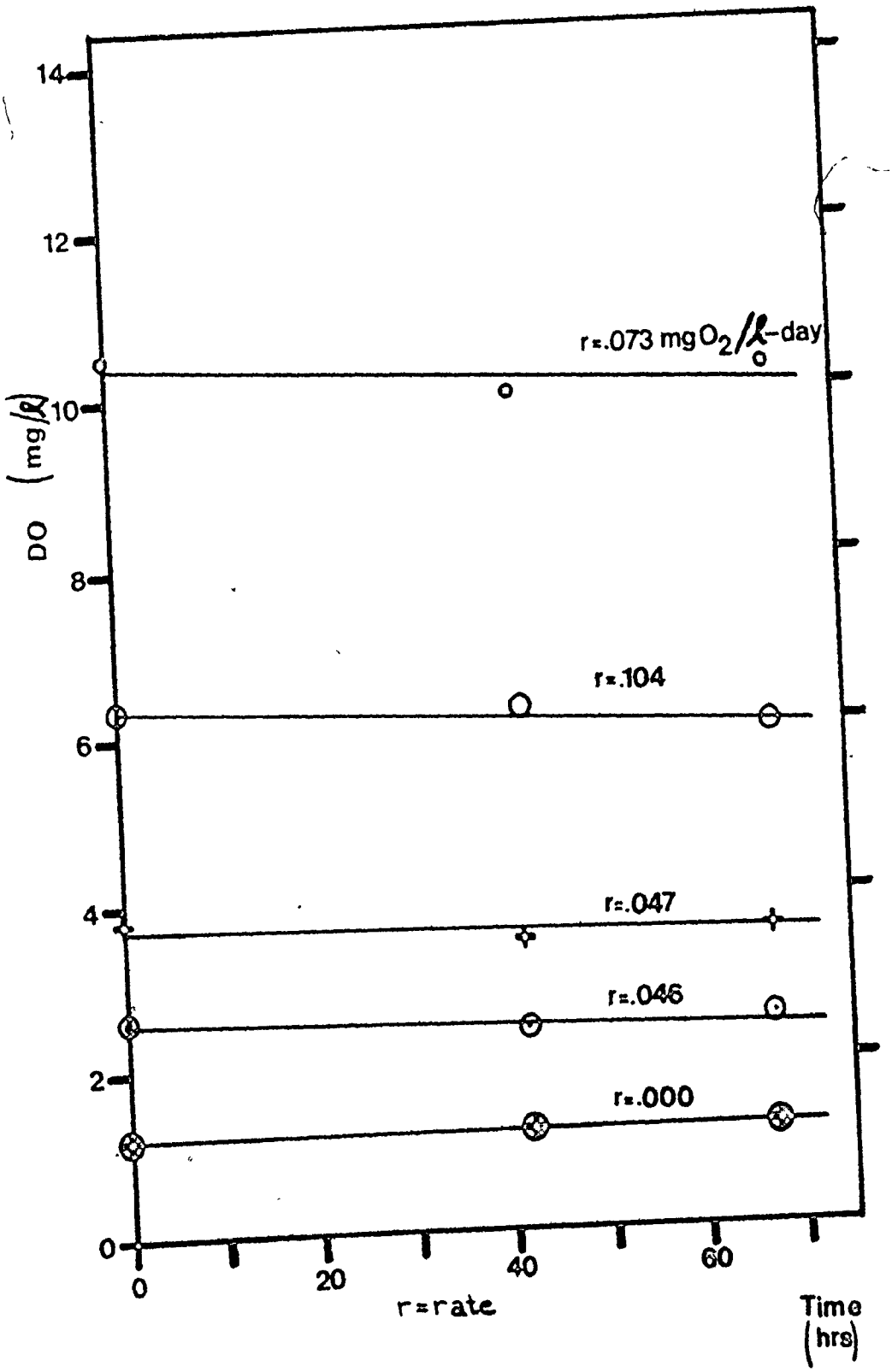


FIG. 4.4

Red Chalk Lake
Oxygen Kinetics

was followed at the end of the run. In the Main Design, the maximum time error would be no more than 5 hours for any experimental bottle. This is relatively insignificant with respect to the minimum length of run (about 200 hours or a 2.5% error). In the kinetic runs, there was, at most, a 1 hour lag time in a total minimum run length of 50 hours (or 2% error); thus, with respect to time frames, the errors are about the same.

Experimental Design theory assumes that all conditions are identical from one run to the next. This is a reasonable assumption for the collection and processing of the sample water. A problem may occur in the sample water itself. In all runs, except Runs 1E and 1X, there was about a two month lag time between performing Design I and Design II on the water bodies. A problem arises with respect to the physics of the water body. For Design I, the water bodies were stratified but by the time Design II was tested, they may have overturned; in other words, they may have been unstratified. A second problem could be caused by the seasonal succession of the microbial communities in the water bodies. Based on the results obtained from Runs 1E and 1X, which were Designs I and II run concurrently, the effects of the physics and biology of the water bodies appear to be minimal in biasing the Experimental Designs. These errors could be minimized by testing both Design I and Design II at the same time.

Glucose was used as a carbon source because it is highly utilizable by all microorganisms. Recall that the definition for an effect, in terms of Experimental Design, is the deviation of the response from the low condition to the high condition of a given variable. By using glucose, this deviation would be maximized. The use of glucose as the carbon spike implies that most of the SOC in the water column is made up of carbohydrates.

In reality, the SOC in the water column would be comprized of many forms including sugars, starches, and refractory materials. This SOC comes from lysed microbial cells and other particulates attacked directly by bacteria from the particulate organic carbon (POC) pool. Although in the real-life situation the organic carbon forms encountered would be more difficult to breakdown than glucose, glucose is considered to be quite acceptable for this work. The next step is to refine the organic carbon model form. Different sources of organic carbon could be tested to see how the response (rate of oxygen uptake) would change. A small experiment was attempted in this context (Appendix F), but due to equipment failure, no meaningful results were obtained.

A 10 μ mesh was used to screen out algae and zooplankton. The actual effect that this mesh had on the removal of algae from the water was unknown. Based on algal dimensions and colony sizes, it was estimated that the mesh removed at least 70% of all algae (Appendix I).

In Chub and Red Chalk Lakes, sampling was not always done from the same location because of boat drift and lack of triangulation using a sextant. This should not contribute significantly to error as vertical stratification has more pronounced effects on the biochemistry of lakes than horizontal variation. Sampling was done from a fixed barge in Hamilton Harbour and from a dam at the Guelph Reservoir.

The water was transported in translucent containers. Some photosynthesis may have, therefore, occurred. However, as the initial DO in all experiments was measured and all experiments were conducted in the dark, such prior photosynthesis should have no significant effect.

In the case of Hamilton Harbour, the water sampled, especially in summer, could contain less than 2 mg/ℓ DO. Before processing, this water was reaerated to about 10 mg/ℓ DO. This was done by emptying the

water from the transportation containers into the 300 ℓ container. This effect does not appear to contribute greatly to error as sufficient time for bacterial adjustment to this new DO was provided.

When purging with nitrogen in the 300 ℓ container, an oxygen probe was not used, except for Runs 1 and 2. The remaining experimental runs were purged for about one hour before preparing the experimental bottles. On occasion, when transferring sample from the 300 ℓ container to the 30 ℓ container, some reaeration occurred; this does not bias the results however, as DO analyses were carried out on every 10 ℓ bottle at the beginning of the run.

Nitrogen purging lowers the concentration of inorganic carbon. A change of 1 mg/ℓ inorganic carbon drop was observed in Hamilton Harbour water which had a field concentration of 30 mg/ℓ (3% drop). The same percentage drop was observed in the other bodies of water as well. This drop in inorganic carbon only changes the field condition slightly and does not contribute significantly to error. The purging done for these experiments was neither long nor intense enough to change the values appreciably.

The analytical methods used (Appendix A) do not appear to contribute significantly to error as all measurement techniques were based on tests recommended by Standard Methods for the Examination of Water and Wastewater (1975). Appendix G shows that an accuracy of ± 0.063 mgO_2/ℓ can be expected from the microtitration technique employed.

CHAPTER 5

ANALYSIS OF RESULTS FROM EXPERIMENTAL DESIGN

5.1 Mathematical Modelling

The model resulting from the Experimental Design is in the form $y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3$. The results of the Experimental Design give parameter estimates (β's) of the above statistical model. An attempt was made to extrapolate the experimental data into a mechanistic model. Combinations of zero-order, first-order, Monod, and linear kinetics were used. The combinations were selected to include the spectrum of probable kinetics. These modelling analyses were made in part to give this researcher a feel for the limits of developing mathematical models from data containing only two levels of information on each variable. Parameter estimation was carried out by a non-linear regression technique. Details of this process, including a program listing can be found in Appendix H.

Firstly, three variables were modelled: temperature, SOC, and oxygen. Data from a few preliminary analyses showed the oxygen variable to be insignificant, hence it was eliminated from further consideration. Temperature was modelled as first-order and Arrhenius kinetics. SOC was modelled as first-order and Monod kinetics. Results of the various model forms (two, three and four parameter) are shown in TABLE 5.1 to TABLE 5.3.

From these tables, it can be seen that all of the model forms are rejected at the 95% confidence interval. The criteria for acceptance

Model	Number of Iterations*	Water Body	θ_1	θ_2	Sum of Squares	Accept/ Reject
$\theta_1 e^{(\frac{1}{T} - \frac{1}{\theta_2})} \times [TOC]$	5	Guelph	0.4199	0.4991	0.9682	R
	5	Red Chalk	0.4347	0.5090	1.037	R
	5	Chub	0.4235	0.5015	1.905	R
	5	Hamilton Harbour	0.5370	0.5815	0.3571	R
	5	Hamilton Harbour (b)	0.4287	0.5050	1.174	R
$(\theta_1 T + \theta_2 TOC)$	9	Guelph	0.9049	1.35×10^{-8}	1.61×10^{5}	R
	9	Red Chalk	0.8395	1.48×10^{-8}	3.93×10^{4}	R
	12	Chub	0.8257	1.17×10^{-9}	1.07×10^{5}	R
	12	Hamilton Harbour	0.9146	3.74×10^{-8}	7.26×10^{4}	R
	14	Hamilton Harbour (b)	0.9093	3.30×10^{-8}	1.28×10^{5}	R
$(\theta_1 T + \theta_2) \times TOC \times O_2$	10	Guelph	0.9035	3.67×10^{-8}	1.18×10^{7}	R
	12	Red Chalk	0.8510	9.28×10^{-9}	3.29×10^{6}	R
	15	CHUB	0.8343	3.21×10^{-8}	7.34×10^{6}	R
	13	Hamilton Harbour	0.9031	6.64×10^{-9}	3.06×10^{6}	R
	16	Hamilton Harbour (b)	0.9181	6.93×10^{-8}	6.81×10^{6}	R

*100 maximum (b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design I

TABLE 5.1(a) TWO PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	Sum of Squares	Accept/ Reject
$\theta_1 e^{(\frac{1}{T} - \frac{1}{\theta_2})} \times [TOC]$	6	Guelph	0.2899	0.4162	0.9356	R
	6	Red Chalk	0.3246	0.4377	0.3199	R
	6	Chub	0.2955	0.4196	0.5386	R
	6	Hamilton Harbour	0.3610	0.4607	0.7837	R
	6	Hamilton Harbour (b)	0.3972	0.4841	0.6584	R
$(\theta_1 T + \theta_2 TOC)$	16	Guelph	0.9059	2.67×10^{-8}	2.54×10^{5}	R
	15	Red Chalk	0.8220	5.38×10^{-8}	6.36×10^{4}	R
	13	Chub	0.8158	2.03×10^{-8}	9.81×10^{4}	R
	14	Hamilton Harbour	0.9038	1.06×10^{-8}	1.50×10^{5}	R
	10	Hamilton Harbour (b)	0.9117	5.08×10^{-9}	1.57×10^{6}	R
$(\theta_1 T + \theta_2) \times TOC \times O_2$	14	Guelph	0.9051	8.60×10^{-8}	1.09×10^{7}	R
	14	Red Chalk	0.8233	1.56×10^{-9}	5.14×10^{6}	R
	14	Chub	0.8110	3.80×10^{-8}	8.07×10^{6}	R
	13	Hamilton Harbour	0.9128	1.01×10^{-7}	7.09×10^{6}	R
	14	Hamilton Harbour (b)	0.8985	2.90×10^{-8}	6.27×10^{6}	R

*100 maximum

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design II

TABLE 5.1(b) TWO PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	Sum of Squares	Accept/ Reject
$\theta_1 e^{(\frac{1}{T} - \frac{1}{\theta_2})} \times [TOC]$	6	Guelph	0.3479	0.4524	2.441	R
	6	Red Chalk	0.3686	0.4656	1.519	R
	6	Chub	0.3667	0.4643	2.882	R
	5	Hamilton Harbour	0.4228	0.5101	1.943	R
	5	Hamilton Harbour (b)	0.4120	0.4939	1.863	R
$(\theta_1 T + \theta_2 TOC)$	13	Guelph	0.9055	4.47×10^{-8}	4.15×10^{5}	R
	16	Red Chalk	0.8285	2.97×10^{-9}	1.03×10^{5}	R
	14	Chub	0.8208	5.60×10^{-9}	2.05×10^{5}	R
	12	Hamilton Harbour	0.9071	7.95×10^{-8}	2.22×10^{5}	R
	14	Hamilton Harbour (b)	0.9106	4.16×10^{-8}	2.85×10^{6}	R
$(\theta_1 T + \theta_2) \times TOC \times O_2$	12	Guelph	0.9043	1.43×10^{-8}	2.27×10^{7}	R
	12	Red Chalk	0.8335	2.91×10^{-8}	8.43×10^{6}	R
	12	Chub	0.8212	4.39×10^{-8}	1.54×10^{7}	R
	11	Hamilton Harbour	0.9096	1.16×10^{-8}	1.01×10^{7}	R
	13	Hamilton Harbour (b)	0.9078	3.86×10^{-8}	1.31×10^{7}	R

*100 maximum

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 16
Design I & II

TABLE 5.1(c) TWO PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	Sum of Squares	Accept/ Reject
$\theta_1 e^{(T\times\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	NC100	Guelph	16.73	0.0735	847.8	0.5138	R
	NC100	Red Chalk	37.31	0.1532	6283.0	0.0567	R
	NC100	Chub	39.42	0.0248	811.8	1.687	R
	39	Hamilton Harbour	0.9779	0.0118	4.243	0.2240	R
	39	Hamilton Harbour (b)	0.1237	0.1326	6.418	0.0914	R
$\theta_1 e^{(T-\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	NC17	Guelph	--	--	--	--	R
	NC100	Red Chalk	9.68×10^{-2}	7.975	1263.0	7.659	R
	NC100	Chub	5.39×10^{-2}	7.188	2493.0	2.759	R
	24	Hamilton Harbour	5.47×10^{-3}	10.40	0.5730	1.722	R
	23	Hamilton Harbour (b)	1.29×10^{-2}	10.48	6.425	0.3241	R
$(\theta_1 T+\theta_2) \times [\frac{TOC}{\theta_3+TOC}]$	NC100	Guelph	4.44×10^{5}	-3.45×10^{5}	1.01×10^{8}	0.5118	R
	NC100	Red Chalk	5.35×10^{3}	-1.77×10^{4}	7.02×10^{5}	0.0566	R
	77	Chub	1.34×10^{4}	3.76×10^{5}	8.17×10^{6}	1.686	R
	9	Hamilton Harbour	0.0138	0.9634	4.253	0.2240	R
	8	Hamilton Harbour (b)	0.1289	-0.8232	6.418	0.0914	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design I

TABLE 5.2(a) THREE PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	Sum of Squares	Accept/ Reject
$\theta_1 e^{(T\times\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	42	Guelph	0.0404	0.1470	1.920	0.1878	R
	38	Red Chalk	0.3638	0.0272	4.645	0.2170	R
	48	Chub	1.214	0.1117	187.4	0.0683	R
	41	Hamilton Harbour	0.1702	0.0686	0.2521	0.2303	R
	39	Hamilton Harbour (b)	0.2268	0.0731	2.138	0.1718	R
$\theta_1 e^{(T-\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	21	Guelph	7.22×10^{-3}	10.51	5.168	0.2726	R
	NC17	Red Chalk	--	--	--	--	R
	NC100	Chub	4.46×10^{-2}	10.61	82.36	0.1111	R
	25	Hamilton Harbour	3.46×10^{-3}	10.20	3.05×10^{-5}	0.6424	R
	23	Hamilton Harbour (b)	6.52×10^{-3}	10.50	1.149	0.6532	R
$(\theta_1 \times T + \theta_2) \times [\frac{TOC}{\theta_3+TOC}]$	12	Guelph	0.0588	-0.4120	1.922	0.1878	R
	10	Red Chalk	0.0141	0.3477	4.675	0.2170	R
	15	Chub	0.6135	-0.9467	187.3	0.0683	R
	10	Hamilton Harbour	0.0334	4.58×10^{-3}	0.2522	0.2302	R
	10	Hamilton Harbour (b)	0.0507	-0.0360	2.138	0.1718	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design II

TABLE 5.2(b) THREE PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	Sum of Squares	Accept/ Reject
$\theta_1 e^{(T\times\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	39	Guelph	0.1583	0.0996	6.055	1.134	R
	39	Red Chalk	0.2523	0.0794	7.903	0.7832	R
	NC100	Chub	14.78	0.0502	580.3	2.193	R
	39	Hamilton Harbour	0.3757	0.0377	0.9752	0.6805	R
	39	Hamilton Harbour (b)	0.1691	0.1003	3.544	0.3995	R
$\theta_1 e^{(T-\theta_2)} \times [\frac{TOC}{\theta_3+TOC}]$	18	Guelph	9.51×10^{-5}	10.31	11.58	1.724	R
	22	Red Chalk	3.76×10^{-5}	9.613	7.719	0.9900	R
	NC100	Chub	5.09×10^{-4}	9.707	209.0	2.963	R
	27	Hamilton Harbour	3.64×10^{-5}	10.16	8.44×10^{-7}	2.380	R
	20	Hamilton Harbour (b)	7.57×10^{-5}	10.33	3.016	1.108	R
$(\theta_1 T+\theta_2)\times[\frac{TOC}{\theta_3+TOC}]$	11	Guelph	0.0731	-0.3026	6.061	1.134	R
	7	Red Chalk	0.0574	0.0889	7.909	0.7832	R
	78	Chub	1212.0	1.01×10^{4}	5.04×10^{5}	2.191	R
	9	Hamilton Harbour	0.0251	0.2969	0.9774	0.6805	R
	8	Hamilton Harbour (b)	0.0797	-0.3354	3.544	0.3995	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 16
Design I & II

TABLE 5.2(c) THREE PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	θ_4	Sum of Squares	Accept/ Reject
$(\theta_1 T+\theta_2)\theta_3 \times [\frac{TOC}{\theta_4+TOC}]$	NC100	Guelph	7.770	-6.021	9.952	1.75×10^4	0.5119	R
	NC100	Red Chalk	5.859	-19.45	17.61	1.36×10^4	0.0566	R
	NC100	Chub	7.592	213.9	3.362	1.56×10^4	1.686	R
	9	Hamilton Harbour	0.1168	8.130	0.1184	4.244	0.2240	R
	8	Hamilton Harbour (b)	0.4656	-2.973	0.2769	6.418	0.0914	R
$\theta_1 e^{\theta_4(\frac{1}{T} - \frac{1}{\theta_2})} \times [\frac{TOC}{\theta_3+TOC}]$	NC100	Guelph	1.014	2.547	1830.0	-14.71	0.5127	R
	NC100	Red Chalk	0.0938	0.2005	4428.0	-1.679	0.6300	R
	NC100	Chub	5.777	0.8306	1288.0	-2.492	1.687	R
	21	Hamilton Harbour	1.179	14.02	4.257	-2.361	0.2240	R
	76	Hamilton Harbour (b)	0.1755	7.306	6.420	-26.51	0.0914	R
$(\theta_1 T+\theta_2) \times [\frac{TOC}{\theta_3+TOC}] \times [\frac{O_2}{\theta_4+TOC}]$	NC100	Guelph	109.2	5.072	1051.0	189.6	0.1463	R
	NC100	Red Chalk	5119.0	-1.66×10^4	4.68×10^5	3.636	0.0375	R
	NC100	Chub	301.2	1.63×10^4	2946.0	736.6	1.003	R
	12	Hamilton Harbour	0.4257	14.31	23.34	37.21	0.1208	R
	11	Hamilton Harbour (b)	0.3206	-1.916	7.180	10.23	0.0267	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design I

TABLE 5.3(a) FOUR PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	θ_4	Sum of Squares	Accept/ Reject
$(\theta_1 T+\theta_2)\theta_3 \times [\frac{TOC}{\theta_4+TOC}]$	9	Guelph	0.3893	-2.730	0.1510	1.920	0.1878	R
	8	Red Chalk	0.1231	3.045	0.1141	4.667	0.2170	R
	12	Chub	0.8369	-1.291	0.7331	187.4	0.0683	R
	10	Hamilton Harbour	0.3698	0.0504	0.0902	0.2521	0.2303	R
	8	Hamilton Harbour (b)	0.3813	-0.2720	0.1330	2.137	0.1718	R
$\theta_1 e^{\theta_4(\frac{1}{T}-\frac{1}{\theta_2})} \times [\frac{TOC}{\theta_3+TOC}]$	38	Guelph	0.2552	1.35×10^{-3}	-3.716	5.84×10^{-6}	0.6358	R
	40	Red Chalk	0.5131	3.54×10^{-3}	4.414	1.92×10^{-5}	0.2459	R
	17	Chub	0.5655	3.141	187.3	-11.17	0.0683	R
	46	Hamilton Harbour	0.5758	5.03×10^{-3}	1.103	2.01×10^{-5}	0.4244	R
	39	Hamilton Harbour (b)	0.8226	2.54×10^{-3}	3.416	4.90×10^{-6}	0.4505	R
$(\theta_1 T+\theta_2) \times [\frac{TOC}{\theta_3+TOC}]$	14	Guelph	0.0708	-0.4946	2.799	-0.8157	0.1865	R
	14	Red Chalk	2.75×10^{-3}	0.1848	4.169	-3.774	0.0776	R
	NC100	Chub	9.681	-11.97	4384.0	-1.909	0.0356	R
	11	Hamilton Harbour	0.0383	-5.28×10^{-4}	3.773	0.6909	0.2274	R
$\times [\frac{O_2}{\theta_4+O_2}]$	11	Hamilton Harbour (b)	0.3605	-0.8823	6.091	22.98	0.0438	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 8
Design II

TABLE 5.3(b) FOUR PARAMETER MODEL RESULTS

Model	Number of Iterations*	Water Body	θ_1	θ_2	θ_3	θ_4	Sum of Squares	Accept/ Reject
$(\theta_1 T+\theta_2)\theta_3$	8	Guelph	0.4010	-1.660	0.1829	6.109	1.134	R
	10	Red Chalk	0.3008	0.4653	0.1919	7.998	0.7832	R
	NC100	Chub	8.884	74.23	2.639	9729.0	2.192	R
$\times [\frac{TOC}{\theta_4+TOC}]$	9	Hamilton Harbour	0.3006	3.558	0.0835	0.9771	0.6805	R
	7	Hamilton Harbour (b)	0.4209	-1.772	0.1893	3.544	0.3995	R
$\theta_1 e^{\theta_4(\frac{1}{T} - \frac{1}{\theta_2})}$	39	Guelph	0.5485	4.76×10^{-3}	0.8723	2.54×10^{-5}	1.958	R
	NC15	Red Chalk	--	--	--	--	--	R
	NC100	Chub	2.353	1.375	1022.0	-5.023	2.192	R
$\times [\frac{TOC}{\theta_3+TOC}]$	34	Hamilton Harbour	0.7325	0.0335	1.457	5.16×10^{-4}	0.8580	R
	28	Hamilton Harbour (b)	0.9603	2.79×10^{-3}	4.849	6.23×10^{-6}	1.462	R
$(\theta_1 T+\theta_2)$	NC100	Guelph	67.46	-208.7	27.40	3231.0	0.6631	R
	9	Red Chalk	0.0466	0.0808	8.278	-1.456	0.7648	R
$\times [\frac{TOC}{\theta_3+TOC}]$	NC100	Chub	146.3	1383.0	3.88×10^{4}	5.189	2.107	R
	11	Hamilton Harbour	0.0390	0.4324	1.491	2.672	0.6409	R
$\times [\frac{O_2}{\theta_4+O_2}]$	9	Hamilton Harbour (b)	0.4451	-1.971	6.865	22.04	0.1134	R

*100 maximum
NC - Non convergent after given # of iterations

(b) Hamilton Harbour Big Nutrient Spike
Number of Data Points 16
Design I & II

TABLE 5.3(c) FOUR PARAMETER MODEL RESULTS

or rejection was based on the actual prediction, confidence interval of the prediction, residual sum of squares, and correlation of the parameters. For most cases, the actual prediction and the confidence interval of the prediction were the main causes of rejection. In many instances, there was a high correlation between various parameters. In a small number of cases, rejection was based on high values of the residual sum of squares.

The model suggested by the experimental results would be a linear and/or product combination of temperature and carbon. For each quarter fraction, the model forms are not significant, despite the fact that these factors have been identified as being statistically significant (95% CI). The lack of model form significance is probably caused by the few observations available for each quarter fraction. Since the block effects are not statistically significant (95% CI), one could combine the two quarter fractions and use data from both fractions for one regression. The relatively large value of the block effect for Chub Lake (and even the Guelph Reservoir) suggests that some care would be needed when interpreting the combined results.

As temperature is the only consistently significant variable, these data suggest the following simple model form as appropriate:

$$R = K_1 T + K_2 \qquad \text{(equation 5.1)}$$

where R is the rate of oxygen uptake (mg/ℓ-day)

T is the temperature of the hypolimnion (°C) and,

K_1 and K_2 are constants for a particular water body (mg/ℓ-day °C and mg/ℓ-day, respectively).

A similar model, but incorporating oxygen concentration dependencies, was used by Polak and Haffner (1978) for Hamilton Harbour.

Consider the hypolimnion as a box (Fig. 5.1). During stratification, there is little transfer of oxygen either in or out of the hypolimnion, therefore, changes in the oxygen concentration are due to uptake reactions. Oxygen consumption in the hypolimnion is due to two functions: water column respiration and sediment oxygen demand (SOD). Water column respiration is made up of bacterial attack of particles settling out of the metalimnion as well as respiration of higher forms of life (zooplankton and fish).

Walker (1980) modelled SOD as follows:

$$TSOD = \hat{\mu} \frac{O_2}{K_{O_2}+O_2} + K_c O_2 \qquad \text{(equation 5.2)}$$

where $\hat{\mu} = 4.85\ (1.085)^{T-25}$,

$K_c = 0.04(0.907)^{20-T}$,

O_2 is the oxygen concentration at the beginning of the period (mg/ℓ),

T is the temperature (°C)

The above model form gives results in units of gO_2/m^2-day. To incorporate the SOD model with the water column model, the SOD model must be divided by Z_m, the mean depth of the hypolimnion, to obtain similar units (g/m^3-day or mg/ℓ-day). Thus, the proposed model for hypolimnetic oxygen depletion is

$$(K_1T+K_2) + \left[4.85(1.085)^{T-25} \frac{O_2}{K_{O_2}+O_2} + 0.04(0.907)^{20-T}O_2 \right] \frac{1}{Z_m} \qquad \text{(equation 5.3)}$$

TABLE 5.4 shows data extracted from the Hamilton Harbour Report, 1976. From TABLE 4.6(d), Temperature Kinetic data for Hamilton Harbour,

$K_1T + K_2^0 = 0.207$ mg/ℓ-day at 12°C

Combining this data with the SOD model, predictions of the oxygen uptake

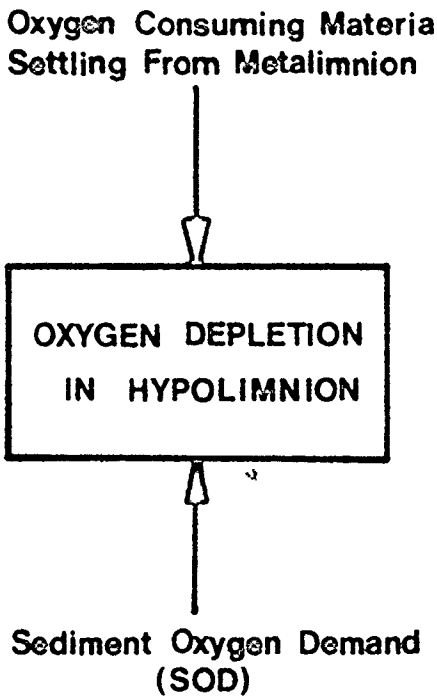


FIG. 5.1
A One—Box Model Of The Hypolimnion

rate at 12°C are made and shown in TABLE 5.4. In all cases, the predicted rates are higher than the observed rates. This may be explained by either exchange with Lake Ontario or transport across the thermocline. Such exchange and transport are highly significant (Polak and Haffner, 1978).

5.2 Procedure for Developing Refined Information

For further work, a new 2^2 Experimental Design in temperature and SOC should be used as follows:

Bottle	Temperature	SOC
1	+	+
2	-	+
3	+	-
4	-	-

This Design would have the same high and low levels as shown in Fig. 3.1 with the following constraints:

$$0°C \leq \text{temperature} \leq 20°C,$$

natural organic carbon concentration

$\leq$ SOC spike

$\leq$ four times natural organic carbon concentration.

After this experiment is run, further experiments would be required to discriminate between the various models proposed.

Let the above four experimental runs be represented by (n-1), and a fifth, or n^{th}, run required. The sequence requires the maximization of the discrimination function at each stage. Mathematically, this is expressed as

$$D = \sum_{i=1}^{m} \sum_{j>1}^{m} \Pi_i \Pi_j D_{ij}$$

Week (1976)	D.O. (mg/ℓ)	T (°C)	Rate of Oxygen Uptake (mg/ℓ-day)	
			Observed	Predicted
May 27	9	12.0	0.29	0.36
June 4	7	12.5	0.29	0.36
June 11	5	12.5	0.29	0.35
June 18	3	12.5	0.29	0.33
June 27	1	12.5	0.29	0.32

Z_m = 10 m

TABLE 5.4

OBSERVED AND PREDICTED OXYGEN UPTAKE

DATA FOR HAMILTON HARBOUR IN 1976.

where

$$D_{ij} = \frac{1}{2}\left[\frac{(\sigma_j^2 - \sigma_i^2)^2}{(\sigma^2+\sigma_i^2)(\sigma^2+\sigma_j^2)} + (\bar{r}_i - \bar{r}_j)^2\left(\frac{1}{\sigma^2+\sigma_i^2} + \frac{1}{\sigma^2+\sigma_j^2}\right)\right]$$

Π_x is the prior probability associated with model x before the n^{th} observation.

σ^2 is the variance of the (n-1) observations $r_1, r_2, \ldots r_{n-1}$,

σ_x^2 is the variance for the predicted value of the n^{th} observation under model x

The experimental point chosen for the next run is where the models differ the greatest, or have the greatest discrepancy, in some sense.

At the start, the prior probabilities are given equal values. With subsequent runs, the posterior probability of one model will increase in relation to the other models'. Posterior probabilities are based on data available up to the subsequent run.

For example, take the case of having (n-1) runs equal to 4 and two different model forms to be discriminated. Assume a fifth run is required. A prior probability of 0.5 is given to each of the two models. D is maximized and the fifth test point found. After the fifth run is tested at that point, a posterior probability is calculated for each of the two models. This procedure is continued until the posterior probability of one model becomes close to 1.0.

The sequential Experimental Design procedure guides one to points of maximum discrimination between two or more models. It is possible, however, that although one model is superior to its rivals, it may be inadequate. In other words, this procedure allows one to determine which model is the better one but says nothing of the correctness of this model.

In general, the adequacy of the best model would have to be checked. This can be done by plotting the residual sum of squares or by doing a standard lack of fit test on the model and data. The lack of fit test compares either the residual sum of squares, or the mean square divided by the sample variance to an F-statistic. Appendix C gives the details of the lack of fit test.

5.3 Utility of Experimental Design in Biodegradation Assays

One premise of this study is that experimentation in the laboratory can provide evidence for determining priorities in modelling research. This is opposite to using the techniques of sensitivity analysis of model coefficients and error analyses in which the model structure is normally presumed to be relatively error free. At first glance, the results described in earlier sections (temperature is the dominant factor affecting oxygen depletion rates) suggest that this research has not discovered any new information. The objective of this section is to evaluate the ability of Experimental Design theory in detecting new information about the factors controlling oxygen depletion when multi-variations and interactions among several factors are possible. Further, this section will present, in summary fashion, the capabilities and limits of Experimental Design theory as evidenced by this research from the point of view that this research is not definitive, but rather a part of the evolution of research into appropriate methods for combining experimental and modelling techniques for predicting the rate of oxygen depletion in the hypolimnia of lakes.

The Experimental Design, itself, may be viewed as a standardized procedure for evaluating relative ranking of degradation of a series of lakes. The average oxygen uptake rates (or O effect in TABLE 4.3(a))

will vary from being a degradation capacity of the lake to being a measure of the actual rate of degradation. It will represent the actual rate of degradation if all perturbations cause an insignificant change in the resultant rate of degradation. It will represent a rate of degradation capacity if all factors cause a significant change in the basal rate of degradation in all lakes. Alternatively, it will represent a degradation capacity if the same factors are significant in all lakes; in this latter case, the other factors not contributing significantly would be disregarded as factors relevant to the development of a concept of degradation capacity. Hence, the concept of degradation capacity may be stated as the rate at which bacteria and other biota respond to a set of perturbations, assuming that their population numbers and physiology are at steady-state with available mass and energy fluxes in the hypolimnia. The relative ranking of lakes gives an indication of the relative ability of the microbial populations to respond and perhaps of the relative degradability of the energy sources available in each lake.

The relative ranking of the average rate of degradation under the standard treatment of the Experimental Design (TABLE 4.3(a)) in decreasing order is Hamilton Harbour, the Guelph Reservoir, Chub Lake and Red Chalk Lake. The differences between the average rates of Hamilton Harbour and Red Chalk Lake are statistically significant at the 95% confidence interval. Differences between all other combinations of the water bodies were not statistically significant at the 95% confidence interval. Appendix C4 gives a more complete discussion as to the methods of evaluation employed. The lack of significance of screening and the overall significance of organic carbon suggests that bacteria dominate oxygen consumption in these experiments.

The relatively small differences in the average degradation of Hamilton Harbour and the Guelph Reservoir suggest that the bacterial populations of the water bodies are approximately similar, hence, have similar energy sources. Hamilton Harbour would appear to have a slightly more degradable energy source. Both water bodies have large standing crops of algae during summer stratification, a common energy source for organics. Hamilton Harbour has significant inputs of BOD and ammonia. Since the recent work of Ng (1981), suggests that total nitrification consumes much more oxygen than organic carbon decomposition, the main effect is probably due to nitrification. The extra energy sources explain the apparently higher average rate in Hamilton Harbour.

Chub and Red Chalk Lakes have substantially lower levels of biomass than do Hamilton Harbour and the Guelph Reservoir, which is reflected in their lower rates. Chub and Red Chalk Lakes both have input of woodland organics. Whether woodlot organics or algal-derived organics dominate as an energy source cannot be concluded with certainty. Glucose is expected to be a good model compound for labile carbon compounds derived from algae but a poor model for woodland organics. Since the organic carbon factor is the most important or second most important variable for both lakes in a fashion similar to Hamilton Harbour, it is expected that algal-derived products are the main energy sources.

Organic carbon is statistically significant (95% CI) in Chub Lake; temperature is not statistically significant (95% CI) althought it is the second most important variable. The reverse order suggests that the bacteria of Chub Lake may be carbon starved. Chub Lake is moderately coloured (15 Hazen Units) whereas Red Chalk Lake is relatively clear. The existance of colour may provide for a larger bacterial population in

Chub Lake than in Red Chalk Lake adapted to oxidizing the colour-causing organics as well as any algal-derived organics which settle through the hypolimnion. Upon receiving the glucose spike, their larger numbers permit a more rapid response.

The lack of statistical significance of temperature in Chub Lake is inconsistant with other observations. The relatively high block effect (TABLE 4.3(b)) for Chub Lake shows that there is a larger difference in experimental conditions between the two quarter fractions than for any other water body. The importance of the organic carbon effect is produced by its dominance in Design I; in Design II, the temperature effect is more important than the organic carbon effect. Since the ℓ_0 and ℓ_0' contrasts for Red Chalk Lake and the ℓ_0' contrast for Chub Lake are quite similar, it is quite probable that Design I for Chub Lake had larger experimental errors associated with it or conditions are significantly different than normal waters. It is concluded that the average rate for Chub Lake is too high, and that it is much closer to that of Red Chalk Lake.

As a model compound, glucose may be overly degradable as there are suggestions that bacteria can metabolize glucose without the use of any extracellular enzymes. It is considered by this research to be a reasonable model compound for the examination of water bodies where a major flux of carbon consists of pools of labile carbohydrates released from algae or obtained from algal derivatives (Jewell and McCarty, 1971; Ogura, 1975). It is a poor model for woodlot or watershed organics. If the carbon flux from algal derivatives is greater than inflows of watershed organics, it would be a good model compound for the Muskoka Lakes. For the objective of an Experimental Design to observe maximum effects and as a standard procedure, it is a good compound.

Its use as a compound for evaluating mathematical models of lakes requires more research.

Oxygen effects can arise from large flocs (diffusion limitations) or low oxygen concentrations (<0.5 mg/ℓ). The Guelph Reservoir is the only water body showing significant oxygen effects. It is probably an artifact of the experiment. Two of eight bottles in Experiment 1 (bottles 1 and 5) show final oxygen concentrations of less than 0.6 mg/ℓ; one is 0.1 mg/ℓ. Since both bottles were given an organic carbon spike and treated at the high temperature, it is probable that consumption rates were limited by oxygen concentration. This would cause the temperature and organic carbon effects to be underestimated in the statistics.

The nutrient factors generally show no significant effects. This implies that bacterial processes in all of the water bodies tested are not nutrient limited, even when the bacteria are given high levels of glucose. This result suggests that the Experimental Design aids in giving a bioassay of bacteria from a nutrient limitation point of view. In the experiment with the large nutrient spike, Hamilton Harbour water does not respond despite the large ammonia input. This suggests that the nitrifier population was not able to respond within the time frame of the experiments. To assess the relative importance of the nitrifier and heterotroph populations, the Experimental Design would have to be changed such that a nitrification inhibitor were used.

Algal and zooplankton effects are not apparently important. A 10μ screen was used to separate algae and zooplankton from the waters. Data from Appendix I shows that approximately 70% (60% to 90%) of algae would have been removed by the screen. If lysed, the algae would be a food source for bacteria. As noted by DePinto (1979), it is difficult

to experimentally separate these two effects. Use of a light-dark procedure in which half of the samples are exposed to light and half are not would be one method that could be substituted. Unfortunately, this probably causes other difficulties including requirements for shorter experiments and questions about the length of time required for algae to cease photosynthesizing after re-entering the dark.

One final question in the use of an Experimental Design for bioassay purposes is with respect to the proper scaling of the parameters. Selection of the parameter scale can be based on a standard deviation about its mean, the observed parameter range, or the maximum possible parameter variation. The temperature range used (10°C to 20°C) matches the range observed in Hamilton Harbour and the Guelph Reservoir (4°C to 15°C) but exceeds that of Chub and Red Chalk Lakes (4°C to 8°C), for their respective hypolimnia. The use of a 10μ mesh for screening algae and zooplankton appears to be effective. Due to the time frames involved, a nutrient spike (ammonia) of three times the concentration naturally found would probably be ineffective for the nitrifier population. Although glucose may be excessively available to bacteria, it would be a useful compound to model labile organic material that could originate from cell lysis. Whether the organic carbon spike were too large cannot be determined until estimates of the flux of organic carbon through the various water columns are available. Although oxygen tensions would not be expected to occur until concentrations were less than or equal to 0.5 mg/ℓ, due to experimental limitations, the low level of initial oxygen concentration (4 to 5 mg/ℓ DO) was taken to be half of the value observed in the field (8 to 10 mg/ℓ DO). The scaling of the parameters, therefore, appears to be adequate for the Experimental Design with respect to experimental lim

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Temperature was found to be the most important effect in all cases except Chub Lake where soluble organic carbon (SOC) was found to be most important.

2. The second most important effect was found to be SOC in Hamilton Harbour and Red Chalk Lake, and temperature in Chub Lake. That of the Guelph Reservoir, initial oxygen concentration being second most important, is an artifact of experimental conditions.

3. The two-factor interactions between the main effects do not appear to show any trends between the different water bodies. The lack of statistically significant differences in the block effects of experiments carried out on samples up to a month apart suggest that biodegradation is approximately constant.

4. The Experimental Design may be thought of as representing a bioassay on biodegradation in various lakes by standardizing variations. The mean rates in decreasing order are Hamilton Harbour, Guelph Reservoir, Chub Lake and Red Chalk Lake. This follows empirical ideas of the relative magnitude of biodegradation. The average rates from this bioassay represent a potential quantification of biodegradation.

5. Nutrient concentrations are not limiting biodegradation processes in any of the lakes tested. A bioassay technique, such as that used, is required to evaluate nutrient effects.

6. Initial oxygen concentration and the presence of algae do not appear to affect biodegradation. This suggests that these factors are not significant in hypolimnetic oxygen models. More work is required regarding the effects of algae as their experimental separation may not have been as complete as desired.

7. Oxygen uptake rates in mixed and non-mixed samples were not significantly different (95% CI).

8. The use of 300 mℓ BOD bottles showed no statistical difference (95% CI) in the rate of oxygen uptake compared to 10 ℓ bottles.

9. A simple model derived from these experiments over-estimates the observed oxygen deficit. Incorporation of transport is required. More experimentation is required on different organic carbon compounds before a slightly more complex form of this model could be used for predictive purposes.

6.2 Recommendations

The work carried out in this research has shown relative importance and unimportance of various factors and established the technique as a potential bioassay and quantification of biodegradation. Further work should refine what has already been developed.

1. Rerun all four water bodies in 300 mℓ BOD bottles. Both Designs I and II should be run concurrently to eliminate the variation between the two if they were run at different times. This can also serve as a check on the data obtained in this work. These experiments should be repeated on a seasonal basis to establish the generality of results obtained herein.

2. Since temperature and SOC were found to be the most important effects, use of a new Experimental Design for these two variables is

in order. Because of its small size, different forms of SOC could also be tested. This experiment, including modelling analyses and field work on forms of carbon flux, could determine if glucose is a good model form for SOC.

3. The failure of the nitrogen budget on many experimental bottles to close adequately needs to be explored. Purging with nitrogen gas to lower initial DO concentrations may aid in releasing some ammonia. Denitrification in an oxic environment is possible, but not probable.

4. Various oxygen concentrations for Hamilton Harbour should be further explored to determine, more conclusively, whether the initial oxygen concentration does have any effect on oxygen uptake rates. The oxygen kinetics suggest a relationship for Hamilton Harbour while little importance was found for oxygen in the five factor experiments.

5. The models shown in Chapter 5 should be tested with data obtained from a two variable Experimental Design. Model discrimination and lack of fit could then be carried out on these models and data.

6. Upon obtaining a model for oxygen demand in the water column, one could incorporate it with a model developed for sediment oxygen demand (SOD). This would then enable modelling of oxygen depletion in both the water column and sediments.

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APPENDIX A

ANALYTICAL TECHNIQUES AND NUTRIENT SOLUTIONS

APPENDIX A

ANALYTICAL TECHNIQUES AND NUTRIENT SOLUTIONS

A.1 Analytical Techniques

A.1.1 Determination of Dissolved Oxygen (DO)

A modified Winkler procedure, as described by Strickland and Parsons (1968), was employed. One hundred-twenty (120) mℓ black bottles were used in lieu of 300 mℓ BOD bottles.

One (1) mℓ of an $MnSO_4$ solution was added to each bottle. This had the effect of stopping, or fixing, microbial activity and oxygen content within the bottle. One (1) mℓ of an alkaline-iodide solution was then added to each bottle. The addition of this solution exchanges dissolved oxygen to dissolved iodide in a 1:1 ratio, and forms a precipitate. To ensure complete mixing of chemicals in the bottles, they were restoppered and shaken twice. The precipitate was then allowed to settle (15 minutes) before adding one (1) mℓ of concentrated sulfuric acid (H_2SO_4). The acid converts the iodide in the precipitate to iodine which is soluble. The bottle was again restoppered and shaken.

Fifty (50) mℓ of this solution, a clear yellow colour, was then titrated with 0.05N sodium thiosulfate ($Na_2S_2O_3$) until it became clear and colourless. Soluble starch was used as an indicator.

The major drawback of this technique is ensuring that no air bubbles get trapped in the bottle. As there is an excess of all added

chemicals, $MnSO_4$, alkaline-iodide and H_2SO_4, oxygen from air bubbles going into solution will be recorded, hence producing erroneous results. To ensure this was kept to a minimum, triplicates were done on all samples.

A.1.1.1 Sample Calculation

$$\text{mg } O_2/\ell = \frac{Y}{Y-2} \times \frac{5.00}{X} \times fxV \times 16.00 \times \frac{N}{0.01}$$

where Y is the sample bottle size (mℓ)

X is the amount of sample titrated (mℓ)

f is a correction factor related to the change in normality of the $Na_2S_2O_3$ solution over time (dimensionless)

V is the volume of titrant ($Na_2S_2O_3$) (mℓ)

N is the normality of the titrant at the start of the titration period.

(ie.) $N = \frac{0.1 \times 2}{V_{STD}}$

where V_{STD} is the volume of tritrant ($Na_2S_2O_3$) required to standardize 2 mℓ of 0.1 N potassium iodate (KIO_3)

The following data is from Run 6

V_{STD1} = 3.9322 mℓ

V_{STD2} = 3.9613 mℓ

V_{STD3} = 3.9724 mℓ

average V_{STD} = 3.9553 mℓ

Therefore N = 0.0506

Y = 120.4 mℓ, f = 1.0

Therefore $\text{mg } O_2/\ell = \frac{120.4}{120.4 - 2} \cdot \frac{5.00}{50} \cdot 16.00 \cdot \frac{0.0506}{0.01} \times V$

for bottle 1, sample 1, V = 0.5572 mℓ $Na_2S_2O_3$

Therefore mg $O_2/\ell = 4.58$

The factor f was taken to vary with time. That is, a set of standards was titrated at the beginning and end of the sample titrations for Runs 1 and 2. Because CO_2 neutralizes thiosulfate, as time proceeds, more thiosulfate is required to titrate the same amount of dissolved oxygen. For the number of bottles titrated at any one time (63 sample bottles in approximately 4 hours), f was found to be 0.9975 at the worst case. It was hence assumed that for further analysis, f would be equal to one (1).

A.1.2 Determination of Soluble Organic and Inorganic Carbon

Many methods were considered to determine soluble organic carbon (SOC). A Beckman TOC unit was first used but it lacked the sensitivity required for this work. Other methods considered were those proposed by Menzel and Vaccaro (1964), Baldwin and McAtee (1974), Goulden and Brooksbank (1975), and Goulden and Anthony (1978). Of these, the most promising was Goulden and Anthony until it was discovered that the equipment in the laboratory was again too crude to use. The method finally implemented was that proposed by Crowther and Evans (1978).

This is a Technicon method based on Ehrhardt's (1969) ultraviolet (UV) digestion technique employing Goulden and Brooksbank (1975) automated system. The sample is sparged in acid medium with nitrogen gas to remove the inorganic carbon. It is then digested in-line with an acid-persulfate solution and irradiated with UV light to convert the dissolved organic carbon (DOC) to CO_2. After dialysis, the CO_2 concentration is determined by measuring the loss of colour in a weakly buffered alkaline phenolphthalein solution. Both DOC

and DIC (dissolved inorganic carbon) are done on the same aliquot of sample. This method can measure between 0.2 mg/ℓ to 20 mg/ℓ carbon and the calibration was linear between 0.5 mg/ℓ to 20 mg/ℓ carbon.

This equipment was located in the Ontario Ministry of the Environment Laboratory on Resources Road in Rexdale. Samples were transported within 36 hours of the termination of a run and were kept cold at 4°C until transported. The samples were processed at the laboratory the same day they arrived except in the case of Run 7.

A.1.3 Nitrogen Analysis

Nitrogen analyses were carried out by methods adapted to the Technicon Auto Analyser as shown in the Manual of Methods for Chemical Analyses of Water and Wastes (1966). For total Kjeldahl nitrogen (TKN) analyses, 50 mℓ of sample were used, except in one case when only 20 mℓ were used due to shortages.

A.1.4 pH

pH was determined by using a Fisher Accumet Model 120 pH meter together with a Broadly-James Combination pH Electrode Model Number 9015.

A.2 Nutrient Solutions

Two nutrient solutions were used. The first, Solution A, contained nitrogen and phosphorous requirements and the second, Solution B, contained heavy metal requirements for microbial growth. Both solutions' formulae were taken from Standard Methods for the Examination of Water and Wastewater, 14th Edition (1975), for the BOD test for rivers.

The following are the chemicals and quantities used in the

two solutions:

Solution A	8.5 g	KH_2PO_4	
	21.75 g	K_2HPO_4	
	20.00 g	Na_2HPO_4*	
	1.7 g	NH_4Cl	to one (1) litre distilled water
Solution B**	11.75 g	$MgSO_4 \cdot 7H_2O$	
	0.25 g	$FeCl_3 \cdot 6H_2O$	
	5.0 g	$Ca(NO_3)_2$	to one (1) litre tap water

*From Standard Methods, this chemical was supposed to be 33.4 g of $Na_2HPO_4 \cdot 7H_2O$. As none could be found, a guess was made as to how much an equivalent amount of Na_2HPO_4 should have been used. A check afterwards showed that the amount should have been 16.7 g.

**From Standard Methods, Solution B should have been made up as follows:

22.5 g	$MgSO_4 \cdot 7H_2O$	
0.25 g	$FeCl_3 \cdot 6H_2O$	
27.5 g	$CaCl_2$	

These amounts were reduced as they would not dissolve well in one litre of water. $Ca(NO_3)_2$ was substituted for $CaCl_2$ because the chloride ion (Cl^-) introduces a negative interference on the method of carbon analysis employed (Crowther and Evans, 1978). It was concluded to use as little Cl^- as possible.

For the main design runs, about 10 mℓ of Solution A and 10 mℓ of Solution B were added to about 15 ℓ of water. In Runs 1E and 1X, 20 mℓ of Solution A and 5 mℓ of Solution B were added to about 5ℓ of water.

Glucose, as dextrose (d-glucose), was used for the carbon spike. A stock solution of 1000 mg/ℓ carbon was made by adding 2.5 g of glucose to 1 (one) ℓ of water. In the main design runs, 50 to 100 mℓ of this solution was added to about 15 ℓ of water. In Runs 1E and 1X, 30 mℓ of this stock solution was added to about 5 ℓ of water.

APPENDIX B

EXPERIMENTAL DESIGN:

THEORY AND APPLICATION

APPENDIX B

EXPERIMENTAL DESIGN: THEORY AND APPLICATION

B.1 Experimental Design Theory

The following has been extracted from Box, Hunter, and Hunter (1978). For a general factorial design, one selects a fixed number of "levels" (conditions) for each of a number of variables (factors) and then runs experiments with all possible combinations. The type of Experimental Design used in this work is known as a two level design; that is, all variables were tested at two levels.

These designs are important for a number of reasons. First is that they yield a maximum amount of information in a minimum number of experimental runs. They are limited, however, to studying only small regions in the factor space, but are able to indicate major trends. This allows determination of a promising direction for further experimentation. Experimental Designs are also helpful when looking at a large number of factors superficially rather than a small number thoroughly in preliminary experimental efforts. Another positive feature is that experiments can be done in blocks. That is, a complete factorial design need not be done when only a part, or fraction, will yield the major results. Finally, the arithmetic is easy and interpretation can largely be done by common sense.

As k increases, the number of experimental runs (runs) required by a full 2^k factorial design increases geometrically. When k is not small ($k \geq 5$) the desired information can often be obtained by only performing a fraction of the full factorial design.

Consider a two-level design in five variables. A full 2^5 factorial design gives 32 data points, or statistics which breakdown as follows: 1 average effect, 5 main effects, 10 two factor interactions (fi), 10 three fi, 5 four fi, and 1 five fi. An "effect" is defined as the change in response in moving from one level of treatment to the other level of treatment. For example, consider temperature. The temperature effect would give some measure of the relative importance of moving from one temperature, say 4°C, to another temperature, say 20°C. If two variables do not act additively, they are said to interact and are called two factor interactions (2 fi). The same is true for 3 factors that do not act additively (three fi), and so on. In terms of absolute magnitude, main effects tend to be larger than two fi which are larger than three fi and so on. Often, at some point, higher order interactions become negligible and can be discarded. Also, there tends to be redundancy in a 2^k design when k is large in that an excess number of interactions can be estimated. Fractional factorial designs take advantage of this redundancy.

Fig. 3.2 and Fig. 3.3 show a 2^{5-2} fractional factorial experiment. This is a one-quarter fraction of a 2^5 factorial design, or eight experiments within an experimental run. There are five quantitative variables: temperature, SOC, nutrients, oxygen concentration, and algae. The response is measured by the rate of oxygen uptake (mgO_2/ℓ-day). The plus signs (+) represent the high levels of the variables and the minus signs (-) represent the low levels of the variables. Fig. 3.1 shows the various levels for all of the variables. A display of levels shown in Fig. 3.2 is called a design matrix.

The design matrix of Fig. 3.2 was constructed as follows: first, a full 2^3 design was written for the three variables 1, 2 and 3. Secondly, the column of signs for the 1x2 and 2x3 column were written to define the levels of the variables 4 and 5, respectively. From the eight runs, eight quantities can be estimated: the mean, five main effects and 2 two fi.

The mystery of the remaining quantities can be explained. For example, multiplying column 1 and column 4 of Fig. 3.2 yields an identical column to that of column 2. Thus, the main effect of variable 2 and the two fi of 14 (one-four) are said to be aliases of each other, or confounded with each other. Obtaining a confounding pattern in fractional factorial designs is important.

The method for this is simple. First, one must keep in mind a few points. A product column (such as 14) is obtained by multiplying the individual elements in the columns that make up that product. Multiplying the elements of any column by an identical column yields a column of plus signs (+). This is known as the identity column and will be represented by the symbol I. For example, $14x2 = 2x2 = 2^2 = 1$.

The Experimental Design employed in this research was constructed by setting

$$4 = 12 \quad \text{and} \quad 5 = 23$$

Multiplying both sides of each of these identities by 4 and 5 respectively, provides the two generating relations in the form

$$I = 124 \quad \text{and} \quad I = 235$$

Combinations such as 124 and 235 may be referred to as "words". The defining relation includes all words that are equal to the identity I. These are the generators 124 and 235 as well as the multiplication of all generators in all combinations. In this simple case, the complete

defining relation is

$$I = 124 = 235 = 1345$$

If there were four generators (say 124, 235, 136, and 1237) then the complete defining relation would contain fifteen words. The smallest word in the defining relation for the design under consideration is 124 or 235; thus this design is known as a Resolution III design.

The confounding pattern for the whole design is then found by multiplying each variable by the defining relation. Thus, for variable 1 the confounding pattern is $1 = 24 = 1235 = 345$

Similarily for variable 2, $2 = 14 = 35 = 12345$

Let the symbol ℓ_2 represent the linear function of the observations which are used to estimate the effect of variable 2. Then

$$\ell_2 = \frac{\text{sum of positive responses}}{4} - \frac{\text{sum of negative responses}}{4}$$

This is known as the ℓ_2 contrast since it is the difference between two averages for four results. The ℓ_2 contrast estimates the sum of the mean values of effects 2, 14, and 35. The notation is indicated as

$$\ell_2 \rightarrow 2 + 14 + 35$$

For the purposes of this work, all three fi and higher have been neglected. The contrast ℓ_0 is the average of all experiments within the run. Table B1(a) shows the confounding pattern for the design shown in Fig. 3.2.

This Experimental Design was then run on the four water bodies and contrasts calculated. If the absolute value of one contrast was noticeably larger than the others, then that contrast could be said to be dominating. Although this process allows the determination of the dominant contrast, it does not give any information as to which of the members of the confounding pattern are the most important.

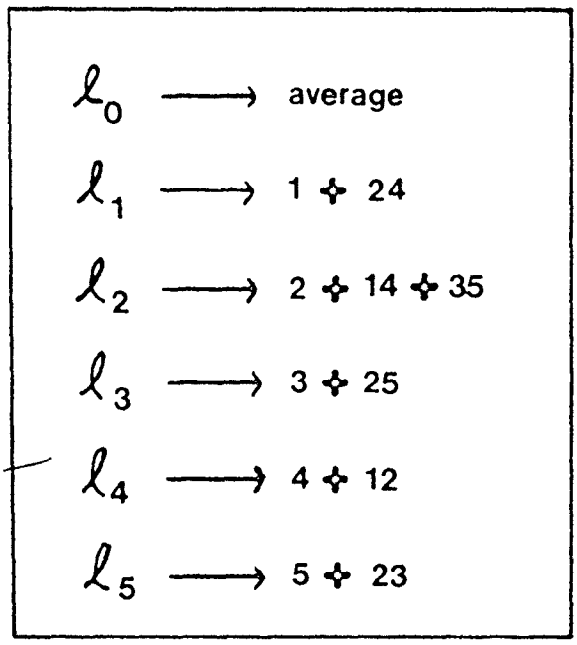

TABLE B1(a)

Confounding of Design I

Say ℓ_1 was much larger than the other contrasts. Recall, from table B1(a), that $\ell_1 \rightarrow 1 + 24$. Nothing further can be said about the relative contribution of the main effect 1 or the two fi 24 to the magnitude of the contrast. It would not be known if the effect of the variable 1 was dominant, or if the two fi 24 was dominant or if both were equally important. Further experimentation would be required to resolve this question. In the work carried out in this program, no contrast was found to be dominant.

In this case, a second quarter-fraction of the Experimental Design was developed. This quarter-fraction is generated by assigning all of the signs in Design I (Fig. 3.2) opposite signs. This is seen in Fig. 3.3 and is called Design II. The defining relation for Design II is

$$I = -124 = -235 = 1345$$

The confounding pattern is found in the identical manner as previously discussed. The confounding pattern for Design II is presented in Table B1(b). To distinguish contrasts between the two Experimental Designs, the symbol ℓ_i' will be used to represent contrasts for Design II.

The second Experimental Design was then run for all four water bodies and the contrasts calculated. By combining this quarter-fraction with the previous quarter-fraction, the block and average effects can be estimated. Table B2 shows the two sets of effects and the method required to obtain their values. From Table B2, the set of average effects shows only the main effects while the set of block effects shows only the two fi.

Within the set of average effects is the 0 (zero) effect. It is the grand average of all experiments from both experimental

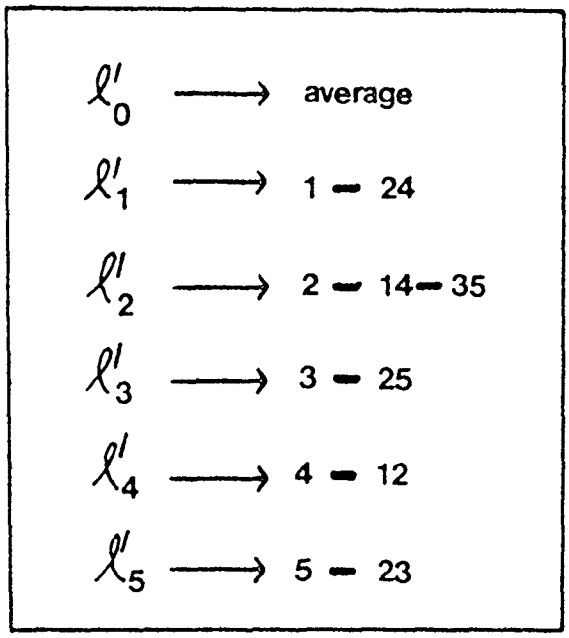

TABLE B1 (b)

Confounding of Design II

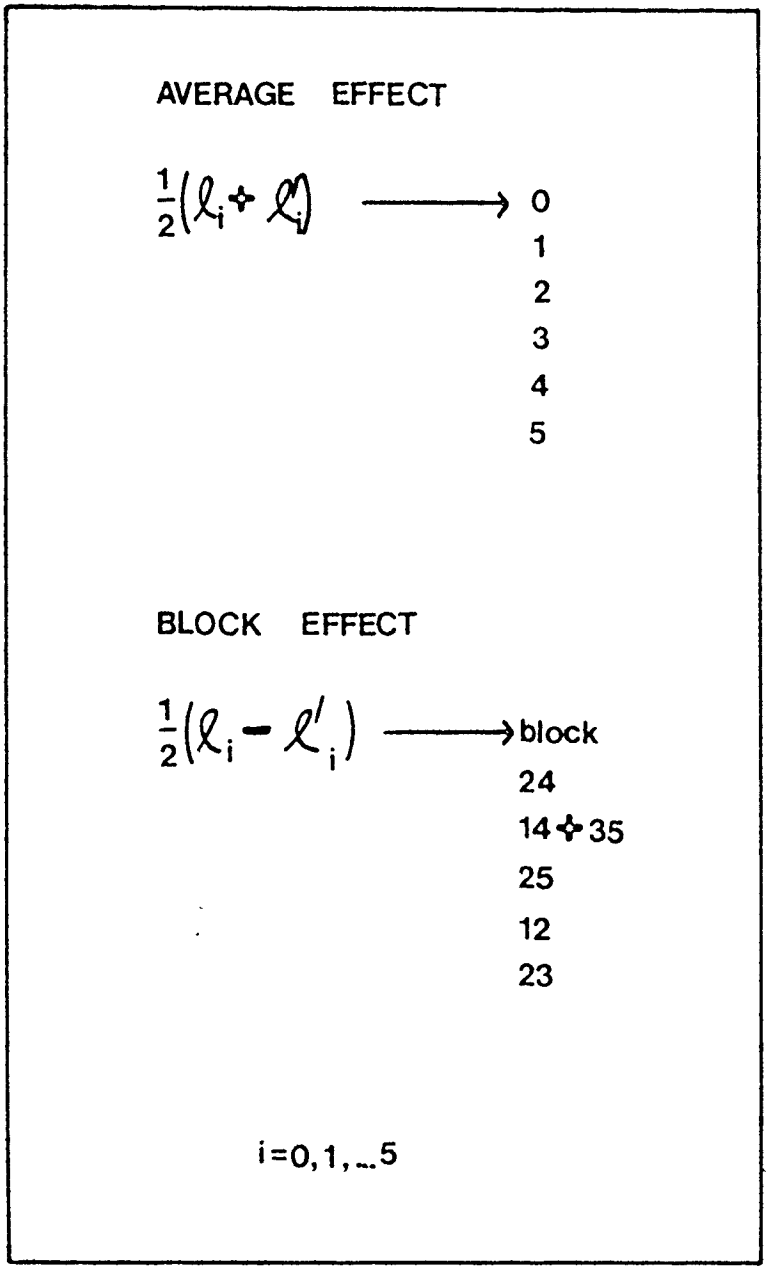

TABLE B2

Calcultion Block and Average Effects

runs. In this case, it is the average of all sixteen experiments. Within the set of block effects is the block effect (when i = 0). This effect is the difference in the averages between Design I and Design II.

Again, the absolute value of the effects are considered. The effect with the largest magnitude is taken as most important. A positive signed effect contributes positively to the response and vice-versa for a negative signed effect. In other words, for this case, a positive number contributes to the rate of oxygen uptake. Conversely, a negative number contributes to a negative rate of oxygen uptake or simply a rate of oxygen production.

B.2 Sample Calculation

From Appendix D, consider the rate of oxygen uptake data for the Guelph Reservoir (Run 1 and Run 6, Table D1 and Table D6, respectively).

	Rate of O_2 Uptake (mgO_2/ℓ-day)	
Bottle Number	Design I	Design II
1	1.41	0.18
2	0.74	0.45
3	0.35	0.00
4	0.18	0.67
5	0.77	0.45
6	0.17	0.69
7	0.64	0.00
8	0.56	0.77

Consider $\ell_1 \rightarrow 1 + 24$

$$\ell_1 = \frac{(1.41 + 0.35 + 0.77 + 0.64)}{4} - \frac{(0.74 + 0.18 + 0.17 + 0.56)}{4}$$

$$\ell_1 = 0.380$$

$$\ell_1' \rightarrow 1 - 24$$

$$\ell_1' = \frac{(0.45 + 0.67 + 0.69 + 0.77)}{4} - \frac{(0.18 + 0.00 + 0.45 + 0.00)}{4}$$

$$\ell_1' = 0.488$$

Average effect $= \frac{1}{2}(\ell_1 + \ell_1')$

$$= \frac{1}{2}(1 + 24 + (1 - 24))$$

$$= 1$$

Substituting the calculated values for ℓ_1 and ℓ_1' yields

$$\frac{1}{2}(0.380 + 0.488)$$

$$= 0.434$$

Block effect $= \frac{1}{2}(\ell_1 - \ell_1')$

$$= \frac{1}{2}(1+24 - (1-24))$$

$$= 24$$

Substituting the calculated values yields

$$\frac{1}{2}(0.380 - 0.488)$$

$$= -0.054$$

Thus, for the Guelph Reservoir, the main effect 1 is estimated as 0.434 and the two fi 24 is estimated as -0.054.

APPENDIX C

STATISTICAL ANALYSIS

APPENDIX C

STATISTICAL ANALYSIS

C.1 General Discussion

The statistical analysis carried out herein is based on the principle of least squares estimates. This analysis minimizes the sum of squares of deviations between observed and expected values. The expected values are provided by a mathematical model.

This was carried out by the method of analysis of variance, or ANOVA for short. This method partitions the total variance of the response, y, into various components. Let $\sum_{u=1}^{n} y_u^2$ represent the total variance of the responses, y. In other words, this term is the total sum of squares. The term can be further broken up as shown below

$$\sum_{u=1}^{n} y_u^2 = \sum_{u=1}^{n} (y_u - \hat{y}_u)^2 + \sum_{u=1}^{n} y_u^2$$

where $\sum_{u=1}^{n} (y_u - \hat{y}_u)^2$ is the residual sum of squares (RSS) and

$\sum_{u=1}^{n} \hat{y}_u^2$ is the sum of squares explained by the model and is also known as the discrepancy sum of squares.

The total and discrepancy sum of squares are calculated from the data and model. The residual sum of squares (RSS) is the difference between the two.

If there are m distinct experimental conditions and there are K_x repeat runs at condition x, then the RSS can be broken up as follows:

$$RSS = \sum_{i=1}^{m} \sum_{j=1}^{k_x} (y_{ij} - \bar{y}_i)^2 + \sum_{i=1}^{m} k_x (\bar{y}_i - \hat{y}_i)^2$$

The first term is known as the sum of squares of pure error (SSPE) and the second term is known as the sum of squares due to lack of fit (SSLOF).

If these are k_u repeat observations at condition x_u, then the contribution of these replicates to the SSPE will be

$SSPE = \sum_{j=1}^{k_u} (y_{ju} - \bar{y}_u)^2$ and will have $(k_u - 1)$ degrees of freedom. A degree of freedom (df) is an independent piece of information on the response, y. Thus, the more pieces of independent information, the more degrees of freedom. The SSPE is calculated from replicate experiments and the SSLOF is the difference between the RSS and the SSPE.

To test for lack of fit, the hypothesis is made that the expected value of the mean square of lack of fit (MSLOF) is equal to the expected value of the mean square of pure error (MSPE). The mean square is calculated by dividing the sum of squares by the degrees of freedom.

Under this hypothesis, the ratio of MSLOF : MSPE is distributed as an F-distribution with degrees of freedom given by LOF and PE. Statistically, if

$$\frac{MSLOF}{MSPE} < F_{\alpha} (df_{LOF}, df_{PE})$$

where α is some confidence factor of being wrong (say 5%), then the hypothesis is accepted. This means that the model is adequate.

C.2 Main Design

From the Experimental Designs, the following statistical model can be obtained:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_2 x_3$$

In the general case of least squares

$$S(\underline{\beta}) = \underline{\varepsilon}' \underline{\varepsilon} = (\underline{Y} - \underline{X\beta})' (\underline{Y} - \underline{X\beta}) \equiv \Sigma (y_u - N_u)^2$$

The symbol $\underline{\beta}$ represents the matrix of all β's. To minimize this, differentiate with respect to $\underline{\beta}$ as in linear least squares, the β's are linear (Appendix H).

Therefore $$\frac{dS(\underline{\beta})}{d\underline{\beta}} = -2\underline{x}' (\underline{Y} - \underline{x\hat{\beta}}) = 0$$

This simplifies to $\underline{\hat{\beta}} = (\underline{x}' \underline{x})^{-1} (\underline{x}' \underline{y})$

where $\underline{\hat{\beta}}$ is the matrix of parameters

$\underline{x}$ is the matrix of experimental conditions

and $\underline{Y}$ is the matrix of responses

$$\underline{x} = \begin{bmatrix}
1 & +1 & +1 & +1 & +1 & +1 \\
1 & -1 & +1 & +1 & -1 & +1 \\
1 & +1 & -1 & +1 & -1 & -1 \\
1 & -1 & -1 & +1 & +1 & -1 \\
1 & +1 & +1 & -1 & +1 & -1 \\
1 & -1 & +1 & -1 & -1 & -1 \\
1 & +1 & -1 & -1 & -1 & +1 \\
1 & -1 & -1 & -1 & +1 & +1 \\
1 & -1 & -1 & -1 & -1 & -1 \\
1 & +1 & -1 & -1 & +1 & -1 \\
1 & -1 & +1 & -1 & +1 & +1 \\
1 & +1 & +1 & -1 & -1 & +1 \\
1 & -1 & -1 & +1 & -1 & +1 \\
1 & +1 & -1 & +1 & +1 & +1 \\
1 & -1 & +1 & +1 & +1 & -1 \\
1 & +1 & +1 & +1 & -1 & -1
\end{bmatrix}$$

The X matrix is composed of the two Experimental Designs. The first eight rows are Design I and the second eight rows are Design II. The $\underline{X}$ transpose ($\underline{X}'$) matrix is the X matrix with the columns and rows interchanged. The first column of the $\underline{X}$ matrix and the first row of the $\underline{X}'$ matrix are all +1. This is the estimate of the grand average, which is the intercept on the response axis.

Sample Calculation

To estimate the parameters (β), the formula used is

$$\hat{\underline{\beta}} = (\underline{X}'\underline{X})^{-1} (\underline{X}'\underline{Y})$$

The $(\underline{X}'\underline{X})$ matrix in this case is a 16 x 16 matrix. In all cases, the matrix is symmetrical about the diagonal. The diagonal, in this case, is made up of the number 16. A portion of this matrix is shown below.

$$(\underline{X}'\underline{X}) = \begin{bmatrix} 16 & 0 & 0 & \cdots & 0 \\ 0 & 16 & 0 & \cdots & 0 \\ 0 & 0 & 16 & \cdots & 0 \\ 0 & 0 & 0 & & 16 \end{bmatrix}$$

The inverse of this matrix, $(\underline{X}'\underline{X})^{-1}$ is then easily calculated and shown below.

$$(\underline{X}'\underline{X})^{-1} = \begin{bmatrix} 1/16 & 0 & 0 & \cdots & 0 \\ 0 & 1/16 & 0 & \cdots & 0 \\ 0 & 0 & 1/16 & & \\ \cdot & \cdot & \cdot & & \\ \cdot & \cdot & \cdot & & \\ \cdot & \cdot & \cdot & & \\ 0 & 0 & 0 & & 1/16 \end{bmatrix}$$

Consider the data for the rate of oxygen uptake for the Guelph Reservoir, Run 1 and Run 6 (Appendix D(a)). The response matrix, $\underline{Y}$, is a column matrix (1 x 16). The first eight rows are data obtained from Design I and the remaining eights rows are data from Design II.

$$\underline{Y} = \begin{bmatrix} 1.41 \\ 0.74 \\ 0.35 \\ 0.18 \\ 0.77 \\ 0.17 \\ 0.64 \\ 0.56 \\ 0.18 \\ 0.45 \\ 0.00 \\ 0.67 \\ 0.45 \\ 0.69 \\ 0.00 \\ 0.77 \end{bmatrix}$$

Now the $(\underline{X}'\underline{Y})$ product can be calculated.

$$(\underline{X}'\underline{Y}) = \begin{bmatrix} 8.03 \\ 3.47 \\ 1.03 \\ 1.15 \\ 0.09 \\ 2.29 \end{bmatrix}$$

Therefore, $(\underline{X}'X)^{-1} = \begin{bmatrix} 0.50 \\ 0.22 \\ 0.06 \\ 0.07 \\ 0.01 \\ 0.14 \end{bmatrix} = \hat{\underline{\beta}}$

These estimates, $\hat{\underline{\beta}}$, should be the same as the estimates obtained from the Experimental Design for the variables (0, 1, ... 5). In fact, a close observation shows that only the 0 variable agrees with the β_0 parameter estimate. The remaining parameters here are one-half the value of the parameters found in the Experimental Design. This can be explained in that the Experimental Design tests the difference of the variables between the + level and the - level. Least squares, however, tests the difference between the 0 level and the + levels or the 0 level and the - level. Finally, the position of β_4 and β_5 parameters are switched with respect to the estimates obtained for the 4 and 5 variables from the Experimental Design. No explanation can be given for this occurrance.

Having these estimates of β, the next thing to do is an analysis of variance or ANOVA. If the model is adequate, $\underline{\beta_0} = 0$, then,

$$(\hat{\underline{\beta}}-\underline{\beta_0})' \; \underline{X}'\underline{X} \; (\hat{\underline{\beta}}-\underline{\beta_0})$$

becomes

$$\hat{\underline{\beta}}' \; \underline{X}'\underline{X} \; \hat{\beta}$$

therefore

$$(\hat{\underline{\beta}}'\underline{X}') = [1.00 \quad 0.54 \quad 0.58 \quad 0.16 \quad 0.58 \quad 0.12 \quad 0.72 \quad 0.30$$
$$0.00 \quad 0.46 \quad 0.42 \quad 0.84 \quad 0.42 \quad 0.88 \quad 0.28 \quad 0.70]$$

This is a row matrix (16 x 1)

$$(\hat{\underline{\beta}}'\underline{X}'\underline{X}) = [8.03 \quad 3.47 \quad 1.03 \quad 1.15 \quad 0.09 \quad 2.29]$$

$$(\hat{\underline{\beta}}'\underline{X}'\underline{X} \; \hat{\underline{\beta}}) = 5.22560$$

This is more simply calculated by

$$(\underline{X}'\underline{Y})' \; (\underline{X}'\underline{X})^{-1} \; (\underline{X}'\underline{Y})$$

or $$(\underline{X}'\underline{Y})' \; \hat{\underline{\beta}}$$

This value is known as the discrepency sum of squares, S_d.

The total sum of squares, $S(\underline{\beta}_0)$, is calculated as

$$S(\underline{\beta}_0) = (\underline{Y}'Y)$$

In this case $S(\underline{\beta}_0) = 5.99090$

Next, an ANOVA table can be set up as follows.

Source	Sum of Squares	Degrees of Freedom
Discrepancy (S_d)	5.22560	6
Residual $S(\hat{\underline{\beta}})$	0.76530	10
Total $S(\underline{\beta}_0)$	5.99090	16

The residual sum of squares, $S(\hat{\underline{\beta}})$, is the difference between $S(\underline{\beta}_0)$ and S_d.

The sample variance, S^2, is the residual sum of squares divided by the degrees of freedom.

$$S^2 = \frac{S(\hat{\underline{\beta}})}{df}$$

For this case $S^2 = \frac{0.76530}{10}$

Therefore $S^2 = 0.076530$

Confidence limits can now be calculated by using the Student's t-distribution. The confidence limit on $\underline{\beta}_i$ is

$$\underline{\beta}_i \pm t_{n-p,\alpha/2} \sqrt{\frac{S^2}{\sum_{u=1}^{n} X_u^2}}$$

where $\sum_{u=1}^{n} X_u^2$ is taken from the diagonal of the $(\underline{X}'\underline{X})$ matrix and equals 16 in this case. For a 95% confidence region with ten degrees of freedom $t_{10,0.025} = 2.2281$

Then

$$t_{n-p,\alpha/2}\sqrt{\frac{S^2}{\sum_{u=1}^{n} X_u^2}} = 2.2281\sqrt{\frac{0.076530}{16}}$$

$$= (2.2281)(0.06916)$$

$$= 0.154$$

Thus, the confidence limits on $\underline{\hat{\beta}}$ are for

β_0 : 0.50 ± 0.154 → (0.654, 0.346)

β_1 : 0.22 ± 0.154 → (1.374, 0.066)

β_2 : 0.06 ± 0.154 → (0.214, -0.094)

β_3 : 0.07 ± 0.154 → (0.224, -0.084)

β_4 : 0.01 ± 0.154 → (0.164, -0.144)

β_5 : 0.14 ± 0.154 → (0.294, -0.014)

A parameter is significant if 0.0 is not included in its confidence interval. From this, the only two parameters that are statistically significant at 95% confidence are β_0 and β_1. It is noted that the more symmetric a parameter is about 0.0, such as β_4, the less significant it is from noise.

One final comment should be made with regards to the magnitude of the confidence interval. Because there was no replication, the confidence interval is large. By repeating runs, the magnitude of the confidence interval will decrease.

If centre-points were run in the Experimental Design (centre-points are analogous to zero (0) conditions which lie between the + and - conditions in the Experimental Design), the residual sum of squares could be broken up into the sum of squares of pure error and the sum of squares of lack of fit. From this, the model form could have been tested for adequacy, as previously explained.

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_o	0.426	0.629, 0.224	Yes
1, β_1	0.151	0.354, -0.051	No
2, β_2	0.295	0.497, 0.093	Yes
3, β_3	0.108	0.310, -0.095	No
4, β_5	0.094	0.296, -0.108	No
5, β_4	-0.028	0.175, -0.230	No

TABLE C1

Run Numbers 4A & 8

Chub Lake

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_0	0.326	0.457, 0.195	Yes
1, β_1	0.170	0.301, 0.039	Yes
2, β_2	0.124	0.255, -0.007	No
3, β_3	0.093	0.224, -0.039	No
4, β_5	-0.033	0.099, -0.164	No
5, β_4	0.070	0.201, -0.061	No

TABLE C2

Run Numbers 5 & 9

Red Chalk Lake

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_0	0.502	0.654, 0.346	Yes
1, β_1	0.217	0.374, 0.066	Yes
2, β_2	0.060	0.214, -0.094	No
3, β_3	0.072	0.224, -0.084	No
4, β_5	0.005	0.164, -0.144	No
5, β_4	0.144	0.294, -0.014	No

TABLE C3

Run Numbers 1 & 6

Guelph Reservoir

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_0	0.563	0.700, 0.425	Yes
1, β_1	0.175	0.312, 0.0379	Yes
2, β_2	0.101	0.238, -0.036	No
3, β_3	-0.024	0.113, -0.161	No
4, β_5	0.013	0.150, -0.125	No
5, β_4	-0.024	0.113, -0.161	No

TABLE C4

Run Numbers 2 & 7

Hamilton Harbour

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_0	0.571	0.703, 0.440	Yes
1, β_1	0.115	0.247, -0.017	No
2, β_2	0.089	0.221, -0.043	No
3, β_3	0.006	0.138, -0.126	No
4, β_5	0.019	0.151, -0.113	No
5, β_4	0.000	0.132, -0.132	No

TABLE C5

Run Numbers 2 & 7A

Hamilton Harbour

Parameter	Estimate	Confidence Interval	95% Significance (Yes/No)
0, β_0	0.542	0.628, 0.456	Yes
1, β_1	0.262	0.348, 0.176	Yes
2, β_2	0.138	0.224, 0.053	Yes
3, β_3	-0.019	0.066, -0.105	No
4, β_5	0.072	0.158, -0.014	No
5, β_4	0.068	0.154, -0.018	No

TABLE C6

Run Numbers 1E & 1X

Hamilton Harbour
(big nutrient spike)

C.3 Mixing Versus Non-Mixing

For these runs, the confidence interval was calculated by using the Student's t-test. First, the sample variance, S_X^2, had to be estimated.

$$S_X^2 = \frac{1}{N-1}\left[\sum_{i=1}^{N} X_i^2 - \frac{\left(\sum_{i=1}^{N} X_i\right)^2}{N}\right]$$

where in this case, N = 2

For Run 2 non-mix $S_X^2 = \frac{1}{2-1}\left[(1.05)^2 + (0.91)^2 - \frac{(1.05 + 0.91)^2}{2}\right]$

Therefore, $S_X^2 = 0.00980$

Therefore, $S_X = 0.0990$

There are N-1 degrees of freedom, so the t-statistic required for a 95% confidence interval is

$$t_{1,\ 0.025} = 12.706$$

therefore,

$$t_{1,\ 0.025}\ \frac{S_X}{\sqrt{N}} = \frac{(12.706)(0.0990)}{\sqrt{2}}$$

$$= 0.89$$

Thus, the confidence interval for Run 2 non-mixed samples is

$$0.98 \pm 0.89$$

The confidence interval for Run 2 mixed samples is

$$1.40 \pm 0.00$$

Since the confidence intervals overlap, it can be concluded that there is no statistical difference at the 95% level between the two treatments. This was done for all experiments.

Because there is little replication, the confidence intervals are very large. By looking at the data, it seems that the mixed samples generally have a higher uptake rate of oxygen than do the

non-mixed samples. More replicates would increase the degrees of freedom which in turn would decrease the magnitude of the t-statistic. Although statistically speaking there is no difference between the two treatments, further work is required to prove this more substantially.

C.4 Estimation of Differences Between Mean Respiration Rates of All Water Bodies

This section shows how the mean respiration rates of all water bodies were tested for similarity.

Suppose that from normal populations 1 and 2 with unknown means μ_1 and μ_2, two samples of sizes N and M are drawn, respectively. If $\bar{X}_1$ and $\bar{X}_2$ are their sample means, then a point estimator of $\mu_1 - \mu_2$ is $\bar{X}_1 - \bar{X}_2$. Consider the case where the variances σ_1^2 and σ_2^2 of the two populations are unknown but assumed to be equal and have estimators S_1^2 and S_2^2, respectively. The pooled sample variance of the two populations, S_p^2, (a pooled estimator) is defined as

$$S_p^2 = \frac{(N-1)S_1^2 + (M-1)S_2^2}{N + M - 2} \qquad \text{(equation C.1)}$$

If $\bar{X}_1$ and $\bar{X}_2$ are both normal, then $\bar{X}_1 - \bar{X}_2$ is also normal with mean $\mu_1 - \mu_2$ and variance equal to

$$\frac{\sigma_1^2}{N} + \frac{\sigma_2^2}{M} \text{. Thus,}$$

$$Z = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{\sqrt{\sigma_1^2/N + \sigma_2^2/M}}$$

If $\sigma_1^2 = \sigma_2^2 = \sigma^2$

Then $Z = \dfrac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{\sigma\sqrt{1/N + 1/M}}$

If σ is replaced by Sp, then the result is a Student's t-statistic with (N + M - 2) degrees of freedom. Thus

$$t = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{Sp\sqrt{1/N + 1/M}} \qquad \text{(equation C.2)}$$

Therefore, the null hypothesis (Ho: $\mu_1 = \mu_2$) is rejected against an alternate hypothesis (Ha: $\mu_1 \neq \mu_2$) at the α level of significance if:

$$t > t_{1-\alpha/2} \quad \text{or,}$$

$$t < -t_{1-\alpha/2}$$

Sample Calculation

The sample variances, S_i^2's, of all of the water bodies were calculated and are listed below.

Guelph Reservoir	0.1307
Red Chalk Lake	0.0996
Chub Lake	0.2276
Hamilton Harbour	0.0618
Hamilton Harbour (big nutrient spike)	0.1201

In this case, N = M = 16, thus the pooled variance becomes

$$S_p^2 = \frac{1}{2}(S_1^2 + S_2^2)$$

Table C.7 shows the pooled variances for all combinations of all water bodies.

Consider the Guelph Reservoir-Chub Lake combination. From Table C.7, the pooled variance, Sp, is 0.4233. The mean respiration rates for the Guelph Reservoir and Chub Lake are 0.502 and 0.427 mgO_2/ℓ-day, respectively (Table 4.3(a)). Substituting the appropriate values in Equation C.2 results in t = 0.5011 (Table C.7) at the 95% level of significance, the hypothesis of the means being equal (Ho: $\mu_1 = \mu_2$) is rejected if

$$t > t_{0.975}\ ,\ 30 \text{ d.f., or}$$

$$t < -t_{0.975}\ ,\ 30 \text{ d.f.}$$

From Statistics Tables, with 30 degrees of freedom

$t_{0.975} = 2.0423$.

Thus, the null hypothesis cannot be rejected; that is, the means are not statistically different.

C.5 Hamilton Harbour Oxygen Kinetics

To determine whether there was a statistical difference in oxygen uptake rates between the various initial oxygen concentrations in Hamilton Harbour, a test similar to that outined in Section C.4 was conducted. The pooled variance, s_p, was estimated between all of the uptake rates (slopes). A t-test was then conducted to determine if there were any statistical differences in the estimated rates. From the table below, three differences are noted.

Initial DO Combinations	Pooled Variance (s_p)	t-estimate	Statistical Difference (Yes/No)
7.0 - 5.4	0.1744	1.608	No
7.0 - 2.5	0.1747	3.133	Yes
7.0 - 1.4	0.1430	4.069	Yes
5.4 - 2.5	0.1436	1.859	No
5.4 - 1.4	0.1028	2.934	Yes
2.5 - 1.4	0.1033	0.334	No

$t_{0.975} = 2.7764$ with 4 d.f.

Water Body Combinations	Pooled Variance (Sp)	t-estimate	Statistical Difference Between Sample Means (Yes/No)
G - C	0.4233	0.5011	No
G - RC	0.3393	1.459	No
G - HH	0.3102	-0.6382	No
G - HHB	0.3542	-0.3195	No
C - RC	0.4045	0.6993	No
C - HH	0.3804	-1.0781	No
C - HHB	0.4170	-0.7800	No
RC - HH	0.2841	-2.4396	Yes
RC - HHB	0.3314	-1.8345	No
HH - HHB	0.3016	0.2814	No

G = Guelph Reservoir
C = Chub Lake
RC = Red Chalk Lake
HH = Hamilton Harbour
HHB = Hamilton Harbour-Big Nutrient Spike

$t_{0.975} = 2.0423$, 30 d.f.

TABLE C7

Analysis of Statistical Differences Between
Mean Respiration Rates of All Water Bodies

APPENDIX D

TABULATED DATA

APPENDIX D
TABULATED DATA

Unless otherwise noted, the data contained in this appendix are in the following units.

Rates of oxygen uptake	:	mg/ℓ-day
Concentrations	:	mg/ℓ
Duration of experiments	:	hours

APPENDIX D

(a) MAIN DESIGN DATA

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f
1	10.47	0.62	1.41	11.6	7.0
2	9.79*	4.61*	0.74	12.9	7.6
3	5.60	3.12	0.35	7.2	6.8
4	6.48	5.25*	0.18	8.2	7.8
5	5.48	0.11	0.77	11.8	7.8
6	5.43	4.25	0.17	11.4	11.0
7	10.79*	6.31*	0.64	8.0	7.0
8	12.24	8.30*	0.56	8.2	6.9

TABLE D1

Main Design - Run #1

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f
1	5.25*	1.12	0.69	9.4	3.2
2	9.09*	3.60	0.92	7.2	4.2
3	6.81*	3.11	0.62	3.8	4.6
4	6.68*	5.56*	0.19	3.8	3.6
5	6.11*	1.13	0.83	8.2	4.8
6	5.24*	0.81	0.74	7.7	5.6
7	10.67*	6.07	0.77	3.8	4.1
8	11.20*	8:10	0.52	4.0	4.9

TABLE D2

Main Design Run #2

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	9.81	0.39	1.88	9.8	6.9	8.0	7.3
2	10.44	3.49*	1.39	11.0	10.8	--	7.4
3	6.18	2.70*	0.70	7.0	5.3	8.0	7.8
4	7.31	6.32	0.20	5.6	8.4	8.1	7.9
5	5.56	0.26	1.06	10.0	6.4	8.0	7.6
6	4.97*	3.70	0.25	9.6	7.0	8.1	8.0
7	10.87*	9.50	0.27	5.6	8.7	8.1	7.9
8	11.60	11.52*	0.02	6.6	7.0	8.1	8.0

TABLE D3

Main Design Run #3

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	7.56*	1.97	0.53	6.0	4.9	6.5	6.2
2	8.93	7.69*	0.12	6.4	6.1	6.4	6.1
3	5.43	3.56	0.18	8.2	4.6	6.6	6.6
4	6.33*	6.04*	0.03	5.5	6.3	6.6	6.6
5	6.29*	1.19	0.49	5.7	4.8	6.4	6.4
6	4.90	4.89	0.00	7.1	6.9	6.3	6.3
7	7.80	7.56	0.02	7.4	4.8	6.1	6.4
8	6.72	7.24	0.00	5.3	6.7	6.1	6.5

TABLE D4

Main Design Run #4

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	10.72*	2.63	1.16	8.2	2.8	6.6	6.1
2	10.82	9.98*	0.12	6.4	7.0	6.5	6.6
3	5.85*	3.68*	0.31	3.4	3.9	6.7	6.7
4	7.10*	6.00	0.16	3.8	3.4	6.7	6.8
5	5.89*	0.22	0.81	6.8	2.7	7.0	6.5
6	5.55	5.38	0.02	6.7	5.4	7.0	6.7
7	11.15*	8.94	0.32	3.0	2.5	6.8	6.6
8	9.52*	10.66	0.00	4.2	3.8	6.6	6.7

TABLE D5

Main Design Run #5

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	5.44	4.53	0.18	8.4	8.2	8.2	8.1
2	6.08	3.81	0.45	8.2	7.6	8.2	8.1
3	7.16	7.26	0.00	16.6	11.0	8.0	7.9
4	7.38	4.05	0.67	15.4	9.7	7.9	7.6
5	10.40*	8.16*	0.45	8.4	8.0	7.8	7.7
6	10.62	7.17	0.69	7.6	8.0	7.8	7.7
7	4.50*	4.61	0.00	15.6	9.4	8.1	8.0
8	4.03	0.16	0.77	15.8	11.6	8.1	7.4

TABLE D6

Main Design Run #6

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	5.79	5.10	0.12	3.2	9.2	7.7	7.8
2	6.14	1.68	0.74	4.8	3.4	7.6	7.3
3	6.97	6.12	0.14	3.4	5.4	7.3	6.8
4	7.93	2.94*	0.83	5.2	5.2	7.2	6.8
5	9.14	9.52	0.00	5.3	3.3	7.3	7.3
6	9.87*	5.52	0.73	4.0	3.2	7.4	7.3
7	5.03	2.21	0.47	5.0	4.7	7.0	7.0
8	4.36	0.22	0.69	4.8	4.5	7.0	7.0

TABLE D7

Main Design Run #7

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	5.85*	6.04*	0.00	6.4	5.6	6.5	5.7
2	7.68*	5.49	0.37	5.3	5.4	6.3	5.6
3	10.89*	10.12*	0.13	13.8	11.0	5.9	4.5
4	10.73	6.49	0.71	13.8	10.6	5.9	5.0
5	10.68*	11.52*	0.00	5.6	8.6	6.6	6.4
6	10.85*	10.00	0.14	5.0	5.6	6.7	6.3
7	6.50	5.23	0.21	10.6	12.4	7.1	5.8
8	4.54	0.23	0.72	10.2	8.1	6.6	5.9

TABLE D8

Main Design Run #8

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	6.11*	5.31	0.11	4.0	2.8	6.7	6.6
2	7.25*	5.24	0.29	4.0	3.1	6.5	6.1
3	10.50	9.90	0.09	10.4	9.8	6.5	6.1
4	10.98*	9.37	0.23	10.8	8.6	6.5	5.8
5	12.81*	10.96	0.26	3.6	3.4	6.8	6.6
6	10.30*	9.08*	0.17	3.0	3.0	6.7	6.6
7	6.21	2.75	0.49	9.4	4.6	6.4	5.9
8	4.96	0.18	0.68	9.0	4.0	6.8	6.0

TABLE D9

Main Design Run #9

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	5.61	5.15	0.08	3.4	4.0	7.9	--
2	6.65*	2.32	0.72	3.6	3.1	7.8	--
3	6.72*	5.34	0.23	13.6	7.4	7.3	--
4	9.02*	5.24*	0.63	13.2	4.4	7.4	--
5	9.66	8.02	0.27	3.5	3.6	7.3	--
6	7.65*	3.54*	0.69	3.5	3.0	7.4	--
7	4.96	0.78	0.70	14.2	7.0	7.5	--
8	3.66*	0.41	0.54	12.6	6.0	7.6	--

TABLE D10

Main Design Run #7A

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	8.59	0.48*	1.35	12.2	6.8	7.1	7.1
2	8.63	6.62*	0.34	10.8	6.0	7.1	7.3
3	5.29	1.94*	0.56	3.4	4.0	7.3	7.0
4	6.45	5.39	0.18	3.4	3.9	7.3	7.4
5	5.87	0.21*	0.94	12.0	4.6	7.8	7.2
6	3.16	1.52	0.27	12.8	5.6	8.0	7.9
7	9.38	5.42	0.66	3.4	3.2	7.4	7.1
8	9.45	8.67	0.13	3.3	3.7	7.4	7.5

TABLE D11

Main Design Run #1E

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f	pH_i	pH_f
1	5.22	4.31	0.15	3.3	4.0	8.0	7.9
2	8.03*	3.66	0.73	3.4	4.1	8.0	7.7
3	8.53*	4.97	0.59	13.2	5.6	7.4	7.5
4	6.91*	0.82	1.02	14.8	5.7	7.3	7.2
5	10.53	9.24	0.22	3.4	5.5	7.2	7.3
6	9.79*	6.17*	0.60	3.4	4.3	7.2	7.0
7	6.34*	4.21	0.36	12.0	7.8	7.2	7.3
8	3.77*	0.38*	0.57	12.2	7.8	7.3	7.0

TABLE D12

Main Design Run #1X

*2 replicates

Bottle #	DO_i	DO_f	Rate of O_2 Uptake (mg/ℓ day)	SOC_i	SOC_f
1	10.37	0.20	1.45	11.4	6.4
2	10.72	0.44	1.47	10.4	7.2
3	4.96	3.70	0.18	6.4	5.2
4	6.52	5.81	0.10	5.2	8.0
5	5.78	0.22	0.79	13.0	8.8
6	4.68	2.64	0.29	13.6	13.6
7	11.36	9.53*	0.26	5.8	5.4
8	10.99	11.45	0.00	6.6	7.0

TABLE D13

Main Design Run #4A

*2 replicates

APPENDIX D

(b) OXYGEN KINETICS DATA

DO_i (mg/ℓ)	DO @ t=25.5 hrs. (mg/ℓ)	DO_f @ t=50.0 hrs.	rate of DO uptake
7.04	6.58	5.66	0.662
5.35	5.02	4.36	0.475
2.52	2.38	1.90	0.297
1.40	1.08	0.83	0.274

TABLE D14(i)

Oxygen Kinetic Run #K-1

Hamilton Harbour

*2 replicates
T = 12°C

DO_i	DO@ t=5.5	DO@ t=18.0	DO@ t=25.5	DO@ t=50	rate DO uptake
10.28*	9.67	9.36	9.39	8.81	0.607 screened
9.94*	9.50	9.16	9.38	8.43	0.640 unscreened

TABLE D14(ii)

Oxygen Kinetic Run #K-1 supplemental

Hamilton Harbour

*5 replicates
T= 12°C

DO_i (mg/ℓ)	DO@ t=33.0 hrs. (mg/ℓ)	DO_f @ t=60.0	rate of DO uptake
9.26	8.60	8.90	0.157
5.78	5.28	5.22	0.229
3.87	3.49	3.85	0.018
2.21	1.92	1.93*	0.116
1.26	0.94	1.16	0.047

TABLE D15

Oxygen Kinetic Run #K-2

Chub Lake

*2 replicates
T = 12°C

DO_i (mg/ℓ)	DO@ t=42.0 hrs. (mg/ℓ)	DO_f @ t=69.0	rate of DO uptake
10.51	10.06	10.35	0.073
6.30	6.19	5.99	0.104
3.78	3.54	3.67	0.047
2.59	2.43	2.47	0.046
1.33	1.34	1.36	(-0.010) 0.000

TABLE D16

Oxygen Kinetic Run #K-3

Red Chalk Lake

*2 replicates
T = 12°C

DO_i (mg/ℓ)	DO@ t=42.0 hrs. (mg/ℓ)	DO_f @ t=69.0	rate of DO uptake
13.59	12.76	13.26*	0.145
6.83	6.39	6.46*	0.139
5.07	4.39	4.51*	0.211
2.75	2.56	2.76	0.006
1.63	1.53	1.85	0.000

TABLE D17

Oxygen Kinetic Run #K-4

Guelph Reservoir

*2 replicates
T = 12°C

APPENDIX D

(c) SAMPLING DATES AND RUN DURATION

Run Number	Date Start	Date End	Duration (days)	Water Body
preliminary	July 12	July 18	6	Guelph
0	July 20	July 26	6	Harbour
1	August 1	August 7	7	Guelph
2	August 10	August 16	6	Harbour
3	August 17	August 22	5	Conestoga
4	August 24	Sept. 4	10.5	Chub
5	Sept. 5	Sept. 12	7	Red Chalk
6	Sept. 13	Sept. 18	5	Guelph
7	Sept. 19	Sept. 25	6	Harbour
8	Sept. 26	Oct. 2	6	Chub
9	Oct. 4	Oct. 11	7	Red Chalk
7A	Oct. 12	Oct. 18	6	Harbour
1E	Oct. 12	Oct. 18	6	Harbour
1X	Oct. 12	Oct. 18	6	Harbour
4A	Oct. 31	Nov. 11	7	Chub

TABLE D18

Main Design

Run Number	Date Start	Date End	Duration (hrs.)	Water Body
K1	Oct. 25	Oct. 27	50	Harbour
K2	Oct. 31	Nov. 3	60	Chub
K3	Oct. 31	Nov. 3	69	Red Chalk
K4	Nov. 28	Dec. 1	69	Guelph

TABLE D19

Oxygen Kinetics

Run Number	Date Start	Date End	Duration (hrs.)	Water Body
T1	Nov. 8	Nov. 12	90	Chub
T2	Nov. 8	Nov. 12	90	Red Chalk
T3	Nov. 20	Nov. 24	93	Chub
T4	Nov. 20	Nov. 24	93	Red Chalk
T5	Nov. 28	Dec. 1	70	Guelph
T6	Dec. 1	Dec. 4	72.5	Harbour

TABLE D20

Temperature Kinetics

Run Number	Date Start	Date End	Duration (hrs.)	Water Body
Preliminary	July 12	July 18	6	Guelph
0	July 20	July 26	6	Harbour
Bottle	Oct. 25	Oct. 30	5	Harbour
Carbon	Dec. 1	Dec. 5	4	Harbour
Titrate	Dec. 6	Dec. 6	--	Hamilton Tap

TABLE D21

Miscellaneous Runs

APPENDIX E

BOTTLE TEST

APPENDIX E

BOTTLE TEST

Because Run 1E and Run 1X were done in 300 mℓ BOD bottles, a correlation was required to compare these bottles to the 10 ℓ bottles of all previous Main Design Runs.

After setting up Runs 1E, 1X and 7A about 15 ℓ of the remaining sample water was placed in the 30 ℓ container. The bottles labelled 1 and 1Z were filled with this water. A second batch of 15 ℓ of sample water was used for the bottles labelled 2 and 2Z. The sample water was from Hamilton Harbour.

The 15 ℓ of water was syphoned as follows:

10 ℓ into 10 ℓ bottle

4-300 mℓ BOD bottles

3-120 mℓ black bottles

1-275 mℓ SOC bottle

The order of filling was random except the 10 ℓ bottle was filled first. The 10 ℓ bottle and the four 300 mℓ BOD bottles were incubated at 20°C in the dark. The remaining bottles were used to determine initial values of DO and SOC. After five days, final determinations of DO and SOC were made for each 10 ℓ bottle and each set of BOD bottles. The results are shown in Table E1.

Using the methods of Appendix C, confidence intervals can be calculated based on the rate of oxygen uptake for both size bottles.

$$Sx^2 = \frac{1}{N-1}\left[\sum_{i=1}^{N} x_i^2 - \frac{\left(\sum_{i=1}^{N} x_i\right)^2}{N}\right] \qquad \text{where } N = 2$$

10 ℓ	300 mℓ
$Sx_{10} = 0.07778$	$Sx_{300} = 0.00707$

$t_{1,0.025} = 12.706$ (1 degree of freedom, 95% confidence)

$$\text{interval} = \frac{Sx}{\sqrt{N}} \cdot t_{1,0.025}$$

$$= (12.706)\frac{(0.07778)}{\sqrt{2}} \qquad = (12.706)\frac{(0.00707)}{\sqrt{2}}$$

confidence interval for 10 ℓ = $\pm$ 0.70

confidence interval for 300 mℓ = $\pm$ 0.06

Thus it can be seen that, statistically, at the 95% confidence interval, there is no difference between the 10 ℓ bottle and the 300 mℓ BOD bottle with respect to the rate of oxygen uptake, as their confidence regions overlap.

Because of the small number of replicates, it is suggested to do more runs to get more degrees of freedom. Because conditions change within the water column of a water body rapidly, it is not recommended to include this data with further data. No information is available comparing the 10 ℓ bottles to the 4 ℓ erlenmeyer flasks. This was not considered important as the 4 ℓ erlenmeyer flasks were used solely to distinguish between mixing and non-mixing effects.

Bottle #	DO_i	DO_f	Rate of O_2 uptake (mg/ℓ-day)	SOC_i	SOC_f	Bottle Volume (ℓ)
1	9.25	4.66	0.92	3.5	4.0	10
2	9.93	4.76	1.03	3.6	4.0	10
1Z	9.25	4.63	0.92	3.5	3.6	0.3
2Z	9.93	5.26	0.93	3.6	4.0	0.3

TABLE E1

BOTTLE TEST RUN

APPENDIX F

CARBON RUN

APPENDIX F

CARBON RUN

Dextrose (d-glucose) was used as the carbon spike in all runs. It was desired to see the effects of different forms of organic carbon on microbial activity in lake water. Stock solutions of dextrose (d-glucose), methanol and oxalic acid of 1000 mg/ℓ were made up. About 20 ℓ of Hamilton Harbour water was screened through the 10μ mesh. The algae were then washed off the mesh with about 80 mℓ of distilled water. One (1) mℓ of concentrated H_2SO_4 was added to the solution. It was then boiled for about 5 minutes, then autoclaved for 15 minutes. Finally, this solution was filtered through a glass-fibre filter. Secondary effluent from the Dundas Water Pollution Control Plant was concentrated by a flash evaporator. Hamilton Harbour water was spiked with these various organic carbon sources. The rate of oxygen uptake was calculated at the end of the run (which had a duration of 4 days). This data is shown in Table F1.

The data shown in Table F1 do not follow the expected trend. The reason was not found until sometime later. The SOC analyses were done on a Beckman TOC machine. It was found that, on the Total Carbon side, although the temperature indicated 900°C, in actuality, it was somewhat lower. The thermocouple was found to be defective. Due to the lower temperature, it was thought that incomplete combustion occurred for the various organic carbon forms used. Thus, it was not known how much carbon was spiked into each of the bottles. As a result, the data

in Table F1 is virtually meaningless. It is therefore, recommended to redo this experiment before postulating any conclusions.

Bottle #	DO_i	DO_f	Rate of O_2 uptake (mg/ℓ-day)	SOC_i	SOC_f
1	8.67*	3.69	1.23	25	18
2	12.28	0.24	2.98	26	30
3	12.63	7.19	1.35	18	18
4	11.32*	9.71	0.40	22	21
5	11.64	7.54	1.01	15	14
6	9.68*	3.14	1.62	19	19
Hamilton Harbour Water				12	

1 Dextrose (D-Glucose)
2 Methanol
3 Oxalic Acid
4 Autoclaved Algae
5 Concentrated-Autoclaved Secondary Effluent (Dundas STP)
6 Secondary Effluent (Dundas STP)

*2 replicates

TABLE F1

EFFECTS OF ORGANIC CARBON ON OXYGEN UPTAKE RATES

APPENDIX G

TITRATION ERROR

APPENDIX G

TITRATION ERROR

This exercise was carried out to check the precision of the experimentor. About seven litres of Hamilton tap water was aerated for about 30 minutes. It was then allowed to stand for a further 30 minutes. This was to let it equilibrate with respect to DO. Six 120 mℓ black bottles were filled and immediately fixed with 1 mℓ of $MnSO_4$ solution and 1 mℓ of the alkaline-iodide solution (see Appendix A for further details). Two 50 mℓ samples were taken from each bottle and titrated with 0.05 N thiosulfate. Results are shown in Table G.1.

From Appendix C, Sx was calculated and found to be

$$Sx = 0.09432 \quad \text{for } N = 12$$

therefore, there are 11 degrees of freedom,

therefore, $t_{11,0.025} = 2.2010$

$$\text{confidence interval} = (2.2010)\frac{(0.09432)}{\sqrt{11}}$$

$$\text{confidence interval} = \pm 0.063 \text{ mgO}_2/\ell$$

This exercise was carried out at the end of the experimental program. At this time, the investigator had the benefit of about 1350 titrations (1400 including standards) behind him. At the beginning of the experimental program, it is not thought that the confidence interval was as good as at the end.

Bottle #	D.O. I	D.O. II	AVE D.O.	STD. DEV.
1	8.65	8.55	8.60	0.071
2	8.74	8.50	8.62	0.170
3	8.71	8.52	8.62	0.134
4	8.77	8.51	8.64	0.184
5	8.70	8.59	8.65	0.078
6	8.57	8.59	8.58	0.014

TABLE G1

TITRATION ERRORS FOUND

USING HAMILTON TAP WATER

APPENDIX H

NON-LINEAR LEAST SQUARES PARAMETER ESTIMATION

APPENDIX H

NON-LINEAR LEAST SQUARES

PARAMETER ESTIMATION

Models such as the following

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots \beta_n x_n + \varepsilon$$

are said to be linear in the parameters (β). A model such as

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_2 x_3 + \varepsilon$$

is also linear, as the parameters are linear. It is noted here that the above model is the model obtained from the Experimental Design.

In many real life situations, models estimating certain relationships are non-linear in their parameters. For example, consider the BOD model:

$$Y = \theta_1(1 - \exp(-\theta_2 t)) + \varepsilon$$

In this discussion, it is assumed that the response, Y, is normally distributed with mean η, and variance σ^2. This will be expressed as $Y \sim N(\eta, \sigma^2)$. Similarily, the error term, ε, is assumed to be $\varepsilon \sim N(o, \sigma^2)$. This error term includes:

1) measurement or instrument errors
2) errors in setting the conditions (X) which probogate into the response (Y)
3) the effect of conditions (X_i) not included in the model
4) incorrect model form

5) anything else not included by the expectation function or the model.

The criterion for least squares is to minimize the sum of squares of deviations between observed and expected (predicted) values. Mathematically, this is expressed as the minimization of

$$\sum_{u=1}^{n} (y_u - \eta_u)^2 \text{ and with respect to } \beta,$$

$$\beta = \text{minimum} \sum_{u=1}^{n} \varepsilon_u^2 .$$

For linear models, the least squares solution can be solved directly since

$$S(\beta) = \sum_{u=1}^{n} (y_u - \beta x_u)^2$$

then $\frac{\partial S}{\partial \beta} = 2 \sum_{u=1}^{n} (y_u - \hat{\beta} x_u)(-x_u) = 0$, for minimum

where $$\hat{\beta} = \left[\frac{\sum_{u=1}^{n} x_u y_u}{\sum_{i=1}^{n} x_u^2} \right]$$

For non-linear models, however,

$$\frac{\partial \eta}{\partial \beta} = f(\beta)$$

thus other techniques must be employed.

One method, proposed by Gauss, was to linearize the model by expanding a Taylor Series about some parameter vector, $\underline{\beta}_0^\circ$, where

$$\underline{\beta}_0^{\circ\prime} = (\beta_1^\circ, \beta_2^\circ, \ldots \beta_p^\circ)$$

The Taylor Series is

$$\eta(\underline{X}_u, \underline{\beta}) \simeq \eta(\underline{X}_u, \underline{\beta}^\circ) + \sum_{i=1}^{P} \left[\frac{\partial \eta(\underline{X}_u, \underline{\beta})}{\partial \beta_i} \right]^\circ (\beta_i - \beta_i^\circ) + \text{higher order terms}$$

Let $$X_{iu}^\circ = \left[\frac{\partial \eta(\underline{X}_u, \underline{\beta})}{\partial \beta_i} \right]_{\underline{\beta}^\circ}$$

$$\eta_u^\circ = \eta(\underline{X}_u, \underline{\beta}^\circ)$$

and $$y_u = \eta(\underline{X}_u, \underline{\beta})$$

then $$(y_u - \eta_u^\circ) = \sum_{i=1}^{p} X_{iu}^\circ(\beta_i - \beta_i^\circ) + \varepsilon_u$$

The above is now linear in β_i, thus $(\beta_i - \beta_i^\circ)$ can be estimated from linear least squares by an iterative method.

This method has two problems with respect to convergence. The first is brought about if η is extremely non-linear. The second problem arises from poor initial estimates. These may result in estimates of β where there may be little or no experimental data.

A modification to this process is the method of steepest descent.

If $$\hat{\delta} = (\beta_i - \beta_i^\circ)$$

then, by the steepest descent method

$$\hat{\delta} = \frac{1}{1+\lambda} (\underline{X}^{\circ\prime}\ \underline{X}^\circ)^{-1}\ \underline{X}^{\circ\prime}\ \underline{R}^\circ$$

where $\lambda \geq 0$ and usually starts at $\lambda = 1.0$.
$(\underline{X}^{\circ\prime}\ \underline{X})^{-1}\ \underline{X}^{\circ\prime}\ \underline{R}^\circ$ is essentially linear least squares, hence $\hat{\delta}$ is linear least squares with a correction factor. This method obtains the perpendicular to the confidence contours of the parameter estimates. When $\lambda = 0$, convergence is complete. Although this method always converges, if there is a minimum, the convergence can be very slow, requiring hundreds of iterations.

A method was developed in 1963 known as Marquardt's Compromise, in which he combined the best points of the linearization and steepest descent techniques.

For a linearized model

$$\underline{R}^\circ = \underline{X}^\circ\ \underline{\delta}^\circ + \underline{\varepsilon}$$

linear least squares gave

$$(\underline{X^{o\prime}}\ \underline{X^{o}})\underline{\delta^{o}} = \underline{X^{o\prime}}\ \underline{R^{o}}$$

Marquardt said

$$(\underline{X^{o\prime}}\ \underline{X^{o}} + \lambda I)\underline{\delta}^{0} = \underline{X^{o\prime}}\ \underline{R^{o}}$$

Thus when $\lambda \to 0$, Gauss' linearization comes out

$$(\underline{X^{o\prime}}\ \underline{X^{o}})\underline{\delta^{o}} = \underline{X^{o\prime}}\ \underline{R^{o}}$$

and when λ is large such that $\lambda \underline{I} >> \underline{X^{o\prime}}\ \underline{X^{o}}$

then $$\underline{\delta}^{0} = \frac{1}{\lambda}\ \underline{X^{o\prime}}\ \underline{R^{o}}$$

which gives the vector of steepest descent. Generally, λ is started large and on subsequent iterations is divided by ν and made smaller.

In 1966, Wolfe (adapted from Meeter) wrote a computer program called GAUSHAUS which made use of Marquardt's Compromise for non-linear parameter estimation. Wertz converted this program to Fortran II and called in UWHAUS. MacGregor adapted this program to the CDC6400 computer.

UWHAUS allows one to obtain least squares estimates of non-linear parameters in a mathematical model. An iterative method is used based on Marquardt's Compromise. Since any mathematical model can be used, the user must specify its form in a subroutine. The user must also supply the main program for input data and initialization of certain constants. Output from UWHAUS includes a description of the problem, summary of each iteration, and information relating the precision of the estimates and possibly the adequacy of the model. Provisions are made for multiple problems. A sample of the output is shown at the end of this appendix.

For more complete details of linear and non-linear least squares, the reader is referred to Draper and Smith (1966). Similarily, for more details of GAUSHAUS and its operation, the reader is referred to Wolfe (1966).

```
NON-LINEAR ESTIMATION, PROBLEM NUMBER  1
   8 OBSERVATIONS,    2 PARAMETERS            46 SCRATCH REQUIRED

INITIAL PARAMETER VALUES

  .1000E+01    .1000E+01

PROPORTIONS USED IN CALCULATING DIFFERENCE QUOTIENTS

  .1000E+00    .1000E+00

INITIAL SUM OF SQUARES =    .9367E+02


                                          ITERATION NO.   1
DETERMINANT =    .3000E+01       ANGLE IN SCALED COORD. = 0.00DEGREES
TEST POINT PARAMETER VALUES
  .7180E+00    .7037E+00
TEST POINT SUM OF SQUARES =    .1400E+02

PARAMETER VALUES VIA REGRESSION

  .7180E+00    .7037E+00

LAMBDA =   .100E+01                          SUM OF SQUARES AFTER REGRESSION =
                                                          .1400500E+02
```

ITERATION NO. 2

DETERMINANT = .2100E+00 ANGLE IN SCALED COORD. = 0.00DEGREES

TEST POINT PARAMETER VALUES
.4877E+00 .5401E+00

TEST POINT SUM OF SQUARES = .1349E+01

PARAMETER VALUES VIA REGRESSION

1 2
.4877E+00 .5401E+00

LAMBDA = .100E+00 SUM OF SQUARES AFTER REGRESSION =

.1348873E+01

ITERATION NO. 3

DETERMINANT = .2010E-01 ANGLE IN SCALED COORD. = 0.00DEGREES

TEST POINT PARAMETER VALUES
.4245E+00 .5019E+00

TEST POINT SUM OF SQUARES = .9698E+00

PARAMETER VALUES VIA REGRESSION

1 2
.4245E+00 .5019E+00

LAMBDA = .100E-01 SUM OF SQUARES AFTER REGRESSION =

.9697574E+00

ITERATION NO. 4

DETERMINANT = .2001E-02 ANGLE IN SCALED COORD. = 0.000EGREES

TEST POINT PARAMETER VALUES
.4199E+00 .4991E+00

TEST POINT SUM OF SQUARES = .9682E+00

PARAMETER VALUES VIA REGRESSION

.4199E+00 .4991E+00

LAMBDA = .100E-02 SUM OF SQUARES AFTER REGRESSION =
.9682236E+00

ITERATION NO. 5

DETERMINANT = .2000E-03 ANGLE IN SCALED COORD. = .000EGREES

TEST POINT PARAMETER VALUES
.4199E+00 .4991E+00

TEST POINT SUM OF SQUARES = .9682E+00

PARAMETER VALUES VIA REGRESSION

.4199E+00 .4991E+00

LAMBDA = .100E-03 SUM OF SQUARES AFTER REGRESSION =
.9682230E+00

ITERATION STOPS - RELATIVE CHANGE IN EACH PARAMETER LESS THAN .100CE-03

FINAL FUNCTION VALUES

.6904E+00 .8072E+00 .4286E+00 .5131E+00 .7024E+00 .7133E+00 .4762E+00 .5131E+00

RESIDUALS

.7196E+00 -.6719E-01 -.7855E-01 -.3331E+00 .6705E-01 -.5433E+00 .1638E+00 .4690E-01

CORRELATION MATRIX

	1	2
1	1.0000	
2	-1.0000	1.0000

NORMALIZING ELEMENTS

1	2
.1681E+11	.1000E+11

VARIANCE OF RESIDUALS = .1614E+00, 6 DEGREES OF FREEDOM

INDIVIDUAL CONFIDENCE LIMITS FOR EACH PARAMETER (ON LINEAR HYPOTHESIS)

1	2
.1350E+11	.8034E+10
-.1350E+11	-.8034E+10

APPROXIMATE CONFIDENCE LIMITS FOR EACH FUNCTION VALUE

.5106E+05	.5976E+05	.3174E+05	.3797E+05	.5198E+05	.5276E+05	.3527E+05	.3797E+05
-.5106E+05	-.5976E+05	-.3173E+05	-.3797E+05	-.5198E+05	-.5275E+05	-.3527E+05	-.3797E+05

END OF PROBLEM NO. 1

APPENDIX I

PHYTOPLANKTON COUNTS AND DIMENSIONS

APPENDIX I

PHYTOPLANKTON COUNTS AND DIMENSIONS

This appendix contains counts and dimensions of the major species of phytoplankton that occur in Hamilton Harbour and Red Chalk Lake. Although some species have individual dimensions less than 10 μ, they are normally found in colonies greater in size than 10 μ and are identified on the following tables as such.

Species	Mean Dimensions (μ)	No. cells/mℓ	% Total	Retained by 10μ Mesh (Yes, No)
Chlamydomonas sphagnicola	15.6	515	9.7	Yes
Eudorina	82.5	94	1.8	Yes
Pediastrum	57.9 x 6.9	62	1.2	No
Oocystis 4-celled	32.1	593	11.1	Yes
1-celled	10.9	608	11.4	Yes
Coelastrum*	21.1	769	14.4	Yes
Lagerheimia	11.8	562	10.5	Yes
Scenedesmus quadricauda	17.5 x 22.1 x 5.1	109	2.0	No
S. acuminatus	17.3 x 13.7 x 4.3	94	1.8	No
S. bijuga	5.8 x 5.6 x 2.5	94	1.8	No
S. denticulatus	20.6 x 14.7 x 5.3	125	2.3	No
Chlorella	--	125	2.3	--
Stephanodiscus	28.7 x 13.2	406	7.6	Yes
Cyclotella	10.5 x 7.1	499	9.4	No
Cryptomonas erosa	21.3 x 12.4	62	1.2	Yes
Rhodomonas	9.6 x 6.2	312	5.9	No

Total % Retained = 71.8%
*Colonies > 10μ

TABLE II

PHYTOPLANKTON IN HAMILTON HARBOUR (July 31, 1978)

Species	Mean Dimensions (μ)	No. cells/mℓ	% Total	Retained by 10μ Mesh (Yes, No)
Chlamydomonas sphagnicola	15.6	421	11.5	Yes
Pandorina	38.3	94	2.6	Yes
Pediastrum	57.9 x 6.9	78	2.1	No
Oocystis 4-celled	32.1	562	15.4	Yes
1-celled	10.9	1092	29.9	Yes
Coelastrum*	21.1	577	15.8	Yes
Kirchneriella	8.9 x 2.5	47	1.3	No
Scenedesmus quadricauda	17.5 x 22.1 x 5.1	78	2.1	No
Chlorella	--	172	4.7	--
Stephanodiscus	28.7 x 13.2	140	3.8	Yes
Melosira*	11.3 x 10.5	47	1.3	Yes
Rhodomonas	9.6 x 6.2	125	3.4	No

Total % Retained = 89.9%
*Colonies > 10μ

TABLE I2

PHYTOPLANKTON IN HAMILTON HARBOUR (August 8, 1978)

Species	Mean Dimensions (μ)	No. cells/mℓ	% Total	Retained by 10μ Mesh (Yes, No)
Chlamydomonas sphagnicola	15.6	140	4.7	Yes
Golenkinia	15.2	31	1.1	Yes
Oocystis 4-celled	32.1	78	2.6	Yes
1-celled	10.9	94	3.2	Yes
Coelastrum*	21.1	78	2.6	Yes
Lagerheimia	11.8	125	4.2	Yes
Selenastrum	14.5 x 3.8	31	1.1	No
Scenedesmus quadricauda	17.5 x 22.1 x 5.1	31	1.1	No
S. acuminatus	17.3 x 22.1 x 5.1	31	1.1	No
Dictyosphaerium	66.0	47	1.6	Yes
Tetrastrum	9.8 x 8.6 x 3	125	4.2	No
Micractinium*	12.5	31	1.1	Yes
Euglena	14.9	109	3.7	Yes
Asterionella*	80.2 x 2.5 x 2.5	47	1.6	Yes
Synedra	91 x 1.3 x 1.3	47	1.6	No
Cyclotella	10.5 x 7.1	218	7.4	No
Cryptomonas erosa	21.3 x 12.4	702	23.8	Yes
C. ovata	41.4 x 17.3	104	4.7	Yes
Rhodomonas	9.6 x 6.2	546	18.5	No

Total % Retained = 60.7%
*Colonies > 10μ

TABLE I3

PHYTOPLANKTON IN HAMILTON HARBOUR (October 19, 1978)

Species	Mean Dimensions (μ)	No. cells/mℓ	% Total	Retained by 10μ Mesh (Yes, No)
Aphanothece*	1.5 x 7.5	23	5.4	Yes
Chroococcus*	5 x 5	51	12.0	Yes
Aphanocapsa *	2 x 1.5 x 1.5	27	6.3	Yes
Dinophyceae	8 x 6 x 6	2	0.5	No
Cryptomonas	11 x 4.5 x 4.5	43	10.1	No
Dinobryon*	11.9 x 3 x 3	75	17.6	Yes
Aned. Cryptomonad	5 x 5 x 5	25	5.9	No
Coneigenia	4.5 x 2 x 2	41	9.6	No
Gloeocystis*	6 x 6 x 6	11	2.6	Yes
Staurastrum*	40 x 8	12	2.8	Yes
Cyclotella	21 x 10	60	14.1	Yes
Asterionella	70 x 3.5 x 2.5	10	2.3	Yes
Tabellaria*	40 x 6 x 3	41	9.6	Yes
Synedra	60 x 2 x 2	5	1.2	No

Total % Retained = 72.8%
*Colonies > 10μ

TABLE I4

PHYTOPLANKTON IN RED CHALK LAKE (October 23, 1978)

APPENDIX J

NITROGEN ANALYSES AND BALANCES

APPENDIX J

NITROGEN ANALYSES AND BALANCES

Tables J.1 to J.4 show the data obtained from the nitrogen analyses performed on Runs 9, 7A, 1E, and 1X. In many cases, the NO_3 plus TKN at the beginning of the run does not balance with that at the end of the run. The following is a brief discussion of what others have observed.

One possible explanation for the lack of balance could be through the loss of nitrogen gas from the system through the denitrification process. Denitrification is the production of nitrogen gas (N_2) from NO_3 and only occurs in anaerobic environments. Jannasch (1960), however, reported to have observed denitrification occurring in an oxygenated culture. According to McCarty (1972), a common observation in mixed bacterial systems is that denitrification does not occur in the presence of oxygen. Similarily, sulfate (SO_4) and CO_2 are not used as terminal electron acceptors in the presence of oxygen (O_2) or NO_3. The use of a thermodynamic argument to explain these occurances is incorrect, because thermodynamically speaking, it is possible to have organic carbon uptake and denitrification occur simultaneously in an aerobic environment. A more proper argument would be that oxygen is inhibitory to those organisms which can mediate these reactions.

Koyama and Tomino (1967), however, observed denitrification occurring throughout a water column tested on two separate occasions in July and September. On both occasions, the water column contained sub-

stantial concentrations of oxygen, except at the sediments. They suggested that denitrification follows the equation:

$$4\ CH_2NH_2\ .\ COOH + 9\ O_2 \rightarrow 8\ CO_2 + 2N_2 + 10\ H_2O$$

This reaction is a combination of the following steps:

$$2\ CH_2NH_2\ .\ COOH + 3\ O_2 \rightarrow 4\ CO_2 + 2\ NH_3 + 2\ H_2O$$

$$NH_3 + 2\ O_2 \rightarrow HNO_3 + H_2O$$

$$5\ NH_3 + 3\ HNO_3 \rightarrow 4\ N_2 + 9\ H_2O$$

This system has not been proven microbiologically, although it is valid thermodynamically. At the end of the stagnation period, the amount of denitrified nitrogen gas found was considerably large in comparison to ammonium (NH_4), nitrite (NO_2), NH_2OH, and NO_3; thus, the authors concluded that denitrification was a dominant process in determining nitrogen metabolism in water.

Other researchers such as Brezonik (1975), postulated that within an aerobic environment, there are micro-anoxic zones possibly surrounding a bacterial cell or organic particle in which denitrification can occur.

In denitrification experiments conducted in batch reactors, Dawson (1971) observed that the nitrogen balances to within 2% to 6% with the odd value to 10%. He postulated that the most important error was in the organic nitrogen fraction as the TKN's were 4% to 6% of the solids concentration which is lower than the expected values of 8% to 12%. The dissolved nitrogen accounted for a significant amount of the total nitrogen in the system (up to 25% in some cases).

The above discussion could possibly account for some nitrogen losses from the system. From Tables J.2 and J.3, significant increases in the total nitrogen from the beginning to the end of the runs are noted. In

lieu of these data, it is concluded that the problems observed in obtaining good nitrogen balances are due to sampling and/or analytical techniques used by the researcher.

Bottle Number	NH_{3i}	NH_{3f}	NO_{3i}	NO_{3f}	TKN_i	TKN_f	$(TKN+NO_3)_i$	$(TKN+NO_3)_f$
1	0.15	0.08			0.17	0.70		
2	0.15	0.10				0.70		
3	0.13	0.05				0.80		
4	0.13	0.05			0.30	0.90		
5	0.45	0.48			0.67	1.10		
6	0.53	0.50				1.20		
7	0.45	0.02				1.25		
8	0.48	0.13			0.77	1.35		

all measurements mg/ℓ

TABLE J1. NITROGEN ANALYSIS
(Run #9, Red Chalk Lake)

Bottle Number	NH_{3i}	NH_{3f}	NO_{3i}	NO_{3f}	TKN_i	TKN_f	$(TKN+NO_3)_i$	$(TKN+NO_3)_f$
1	0.05	0.10	0.12	3.00	1.00	1.05	1.12	4.05
2	0.05	0.02	0.15	2.25	1.00	1.00	1.15	3.25
3	0.10	0.05	0.15	3.00	1.40	1.35	1.55	4.35
4	0.18	0.20	0.15	1.90	1.60	1.35	1.75	3.25
5	0.05	0.50	0.65	3.25	1.05	0.90	1.70	4.15
6	0.05	0.03	0.68	1.75	1.30	0.65	1.98	2.40
7	0.38	0.10	0.20	2.50	1.90	1.55	1.98	4.05
8	0.13	0.38	0.00	0.00	1.50	1.25	1.50	1.25

all measurements mg/ℓ

TABLE J2. NITROGEN ANALYSIS
(Run #7A, Hamilton Harbour)

Bottle Number	NH_{3i}	NH_{3f}	NO_{3i}	NO_{3f}	TKN_i	TKN_f	$(TKN+NO_3)_i$	$(TKN+NO_3)_f$
1	2.75	2.05	3.75	1.78	3.25	2.85	7.00	4.63
2	2.50	1.65	3.75	2.50	3.55	3.40	7.30	5.90
3	2.80	2.18	3.50	2.50	3.20	1.60	6.70	4.10
4	3.00	2.48	4.00	1.75	3.55	3.25	7.55	5.00
5	0.20	0.60	2.00	0.28	1.35	0.80	3.35	1.08
6	0.20	0.05	2.00	2.92	1.35	0.95	3.35	3.87
7	0.20	0.05	2.75	3.00	0.80	1.38*	3.55	4.38
8	0.20	0.15	2.75	2.95	0.80	0.70	3.55	3.65

all measurements mg/ℓ

* 20 ml sample

TABLE J3. NITROGEN ANALYSIS
(Run #1E, Hamilton Harbour)

Bottle Number	NH_{3i}	NH_{3f}	NO_{3i}	NO_{3f}	TKN_i	TKN_f	$(TKN+NO_3)_i$	$(TKN+NO_3)_f$
1	0.23	0.12	2.75	0.00	1.00	1.00	3.75	1.00
2	0.23	0.05	3.00	0.00	0.90	0.90	3.90	0.90
3	0.25	0.05	2.00	0.00	1.45	1.30	3.45	1.30
4	0.25	0.23	2.50	0.35	1.40	1.85	3.90	2.20
5	2.43	2.63	3.25	2.50	3.10	3.30	6.35	5.80
6	2.60	1.70	3.25	2.00	3.10	0.85	6.35	2.85
7	2.43	1.85	2.75	1.25	3.10	3.60	5.85	4.85
8	2.60	2.25	3.25	0.20	3.90	3.15	7.15	3.35

all measurements mg/ℓ

TABLE J4. NITROGEN ANALYSIS
(Run #1X, Hamilton Harbour)