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IRON DYNAMICS IN A GAS-WATER-SEDIMENT MICROCOSM

by

Peter A. Cowan
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ABSTRACT

Iron dynamics in eutrophic systems were studied in the laboratory utilizing gas-water-sediment phase sealed microcosms. Sediments from Hyrum Reservoir (2.4 percent iron by weight) were placed in the dark to simulate the hypolimnetic regions of a eutrophic impoundment. Iron both chemically and physically was readily available to microorganisms of the aqueous phase because iron in these systems was soluble. In the light microcosms, which simulated shallow littoral regions of eutrophic impoundments, iron was found in higher aqueous phase concentrations than was predicted chemically and physically; this was rationalized through biological mechanisms.

The experiment was conducted in two phases: Phase I lasted 189 days (0 and 0.300 mg NO_3-N/l inputs) and Phase II lasted 175 days (10 mg NO_3-N/l input). Average light microcosm effluent iron concentrations increased from 0.092 mg Fe/l (Phase I) to 0.246 mg Fe/l (Phase II) given higher inorganic nitrogen inputs. In Phase II, when nitrogen input into the microcosms ceased (nitrogen perturbation, day 115), aqueous phase iron concentrations in the dark microcosms increased dramatically (0.011 to 0.624 mg Fe/l).

ACKNOWLEDGMENTS

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INTRODUCTION

Definition of Problem

Eutrophication of surface waters is a process where an aqueous system ages through the addition of nutrients from the watershed. The eutrophication process has been greatly accelerated through man's usage of water resources and excessive nutrient inputs. The ultimate fate of lakes and reservoirs receiving excessive nutrient inputs is high plant productivity, which manifests itself in algal blooms and eventual dominance by the nuisance blue-green algae. This situation is complicated by density stratification resulting from temperature differences where nuisance levels of algae are located in the photic zone (epilimnion) above a nutrient rich, anaerobic hypolimnion. Control of nutrient inputs to surface waters and watersheds can reduce these unaesthetic eutrophication problems and improve or maintain the resources' beneficial uses.

Legislation has been passed (PL 92-500) with the ultimate and highly idealized goal of eliminating all discharges of wastes (growing stimulating or toxic substances) into the nation's waterways. This particular law is directed principally at the point sources of pollution, and is less specific about nonpoint sources of pollution. These latter sources of nutrients would be either allochthonous (enters the system from outside the lake or reservoir (Mortimer, 1941, 1942)) from diffuse sources and the atmosphere (precipitation, carbon and nitrogen fixation) or autochthonous (is produced chemically or biologically within the lake or reservoir), from the sediments within the system (Foess and Feng, 1971; Fillos and Swanson, 1975). Benthic deposits will release nutrients into the overlying waters long after point and other allochthonous sources of pollution into the waterways are eliminated (Fillos and Molof, 1972).

Studies have been performed on water-sediment systems in order to investigate the role of carbon, nitrogen and phosphorus compounds (Wentz and Lee, 1969; King, 1970; Porcella et al., 1970; Shapiro, 1970, Goldman et al., 1972; Kamp-Nielsen, 1974; Porcella et al., 1975) which control plant productivity in lakes, but little has been done concerning iron (and other trace metals and nonmetals) and its effect upon the eutrophication process.

Given a historical high iron input to a system, chemical considerations favor deposition of iron into lake bottom sediments (Lee, 1962). Elimination of iron inputs from outside the system will usually result in a decrease in the aqueous phase iron concentration. The important question from an environmental management point of view is: Will iron remain permanently in the sedimentary phase, or will there be times during the year when it is released from the sediments and will be utilized by algae and other plants? Resolution of this question takes on even more importance when iron is considered as a possible algal growth rate limiting nutrient (Browne, 1942; Winder and O'Hara, 1962; Goldman and Carter, 1965; Goldman, 1972; Porcella et al., 1973).

Iron, under natural conditions, not only exerts a profound effect upon biological systems (Goldman, 1972; Morton and Lee, 1974) but can also play a major role in the distribution of phosphorus between the sediment and aqueous phases (Ohle, 1937; Mortimer, 1941, 1942; MacKenzie, 1962; Gorham and Swaine, 1965; Stumm and Morgan, 1970; Shulka et al., 1971; Williams et al., 1971; Wildung and Schmidt, 1973; Bortleson and Lee, 1974; Fitzgerald and Uttormark, 1974; Fillos and Swanson, 1975; Hwang et al., 1975). Iron and phosphorus are sometimes found in nature in comparable concentrations, and any extensive complex formation involving iron and phosphorus would have a significant effect upon the distribution of iron, phosphorus, or both.

Objectives

The approach of studying iron in simulated aqueous environments was used to obtain further understanding about iron sorption and release from sediments. The specific objectives necessary to achieve the overall goal were:
1. To determine iron dynamics by utilizing laboratory scale gas-water-sediment microcosms to simulate a eutrophic reservoir as follows:
   a. Phase I: Variation in light conditions, N and Hg concentrations (iron constant input at 33 μg Fe/l) and measurement of iron and phosphorus concentrations and the

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determination of iron and phosphorus relationships.

b. Phase II: Variation in light conditions and iron inputs to microcosms and the measurement of iron and phosphorus concentrations and relationships.

2. To determine mass balances for iron in the microcosms to quantify the amounts of iron entering or leaving the aqueous phase with respect to the sediments.

3. To determine if iron has any effect upon the algal productivity of the system at the concentrations studied.

LITERATURE REVIEW

Biological organisms require a variety of nutrients to grow and maintain themselves. Included among these nutrients are trace elements, those constituents required by organisms in micro-quantities. Iron (Fe) is the most essential trace element for biological systems, but like many trace elements, it is also very insoluble in aqueous environments at physiological pH values (Neilands, 1974).

Iron is an essential portion of various enzymes (organic catalysts) and is required for the synthesis of other essential enzymes (Mahler and Cordes, 1968; Wood and Tchobanoglous, 1975). Iron is important to biological systems because a large number of biological reactions are catalytic (Schutte, 1964). The chemistry of iron is ideally suited to perform catalytic functions in electron transport reactions (Neilands, 1974). The primary function of iron in aerobic microbes lies in respiration: "the reduction of O_2 by means of the cytochrome (Fe) chain with resultant generation of chemical energy" (Neilands, 1974, p. 4). Neilands concluded that iron is "the most versatile of all the biocatalytic elements."

Using the green alga *Selenastrum capricornutum*, PRINTZ in bioassays, Fitzgerald and Uttormark (1974) showed a dependence of algal growth on iron concentrations up to 20 μg Fe/l when other nutrients were in excess. This dependence was shown in two different media (Gorham's and AAM).

Kauppinen (1963) determined a K_s value of 0.40 μg Fe/l and a μ_{max} value of 0.22 hours^{-1} for *Candida gulliermondii* in batch culture using Michaelis-Menten (1913) growth kinetics equations modified for microbial growth rate studies by Monod (1942) and Hinshelwood (1946):

$$\mu = \mu_{max} \left[\frac{S}{K_s + S} \right]$$

in which

μ = specific growth rate, hrs^{-1}

μ_{max} = maximum specific growth rate, hrs^{-1}

S = substrate concentration, μg/l

K_s = saturation constant (numerically equal to the concentration of substrate at $1/2$ μ_{max}), μg/l

Iron is indeed required in micro-quantities, and the levels at which iron will limit microbial growth are < 1 μg Fe/l. Liebig's law of the minimum states that growth is limited by the substance that is present in minimal quantity in respect to the needs of the organism (Browne, 1942). In order for iron to limit microbial growth, other nutrients would have to be in excess of the microbes need, and iron would have to be < 1 μg Fe/l.

In addition to the possibility of iron as a limiting nutrient, iron at high levels (100 to 1000 μg Fe/l) was found to cause a shift in algal dominance from green to blue-green algae (Morton and Lee, 1974). Iron at these higher concentrations resulted in the succession of algae to nuisance species which caused adverse effects upon the aqueous environments.

Voisin's law of the maximum states that the nutrient in relative maximum (in addition to consideration of limiting nutrients) determines the yield (Schutte, 1964).

Vollenweider (1968) hypothesized a reversal of phosphorus (P) induced eutrophication by stopping phosphorus input into a lake system. The question of significance is, can this principle be applied to a eutrophic system when considering the micro-nutrient iron? Considering the chemical, physical, and biological composition of a lake or reservoir, can the concentration of iron be reduced in the aqueous phase to result in the beneficial limitation of microbial populations?

Aqueous Chemistry of Iron

In order to study iron and its effects upon biological systems, a basic understanding of iron chemistry is essential. Iron is the second most abundant metal and the fourth most abundant element in the earth's crust (Cotton and Wilkinson, 1962). The source of iron in the natural aqueous environment results from input of drainage basin water and from the constant interaction between the sediments and the water overlying the sediments.

Iron is present primarily in two oxidation states: +2 ferrous (Fe(II)) and +3 ferric (Fe(III)). In ferrous-ferric aqueous systems, it is the pH, the redox

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potential, and the presence of complexing ligands which dictate the composition and the stability of the iron oxidation states (Cotton and Wilkinson, 1962). In order to understand iron in aqueous systems, these three factors will be discussed separately. It must be emphasized that in nature, all three must be considered simultaneously to describe iron in the aqueous environment.

pH

Most natural aquatic systems have a pH in the range of 6.5 to 8.5. Under these pH conditions, iron would be present as the ions Fe(II) and Fe(III), or any one of a number of hydroxide complexes, the most prevalent being $FeOH^{++}$, $Fe(OH)_2^+$, $Fe(OH)_4^-$, $FeOH^+$, and $Fe(OH)_3^-$ (Figures 1 and 2).
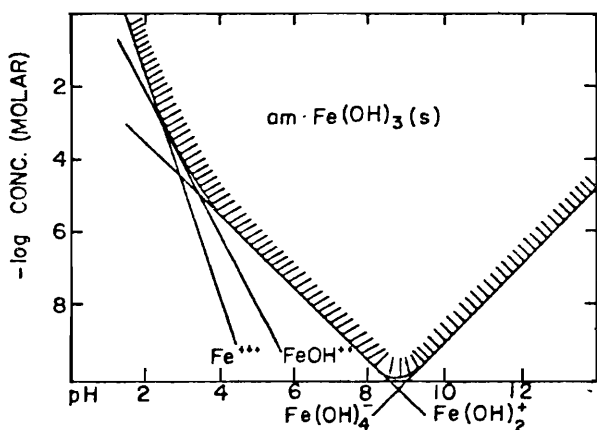
Figure 1. Phase diagram for the solubility of amorphous $Fe(OH)_3$ (pk_{sp} 38.7) in natural aquatic systems. The possible occurrence of polynuclear complexes, i.e. $Fe_2(OH)_4^{++}$ has been ignored; such a complex would not change the solubility characteristics markedly (Stumm and Morgan, 1970).
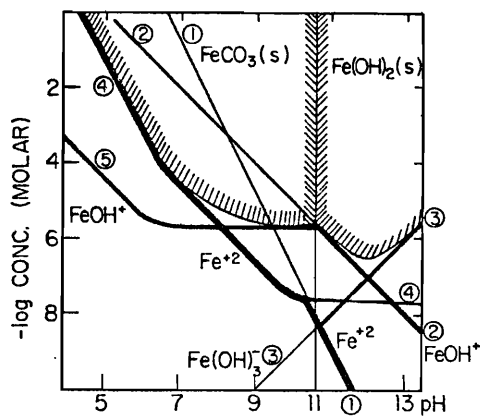
Figure 2. Phase diagram for the solubility of Fe(II) in a 10^{-3} M carbonate system (Stumm and Morgan, 1970).

All metal cations in aqueous solution are hydrated (Stumm and Morgan, 1970). Iron reacts with water molecules to form aquo complexes; i.e., $Fe(H_2O)_6^{+++}$. These complexes can act as Bronsted acids (Stumm and Morgan, 1970) and upon hydrolysis (proton transfer) yield various hydroxo complexes (Cotton and Wilkinson, 1962; Stumm and Morgan, 1970), i.e., $Fe(H_2O)_5OH^{++}$, $Fe(H_2O)_4(OH)_2^+$.

Insoluble $Fe(OH)_3$ or its anhydrous form, Fe_2O_3, totally dominates the solubility of Fe(III) at pH ranges found in nature (Figure 1). Theis and Singer (1973) indicated that the hydrated Fe(III) ion concentration in solution is negligible (Figure 3). This point is further emphasized in Figure 1 where, when using pH as a master variable, the presence of Fe(III) as a free ion is shown to be limited to relatively highly acidic solutions. Even in the pH range of 6.5 - 8.5 the hydroxo complexes, which are the predominate form of soluble iron, exist at concentration levels of $< 10^{-10}$ m/l. Therefore, it is obvious that the soluble Fe(III) in natural systems exist at very low concentrations and may be considered negligible.

Most natural systems are buffered by the carbonate alkalinity system since they are open to the atmosphere; therefore, in discussing Fe(II) equilibria of natural systems, a phase diagram of the solubility of Fe(II) in a carbonate system must be considered (Figure 2).

The solubility of $FeCO_3$ controls the concentration of Fe(II) at pH < 11. The molar solubility of $FeCO_3$ was calculated as 4.5 x 10^{-6} m from the reported K_{sp} value of 2.0 x 10^{-11} (Stumm and Morgan, 1970). This means that Fe(II) ion (hydrated) occurs in an equilibrium solution at a concentration of 250 μg Fe/l. When considering iron as a trace element, this is an extremely high concentration. Hydrated Fe(II) ion is subject to proton transfer
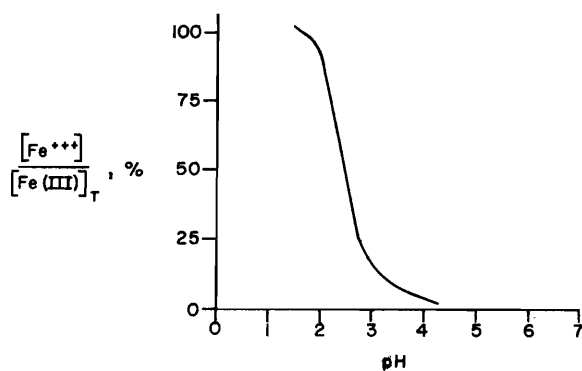
Figure 3. Percent of total Fe which is free Fe(III), as a function of pH (Theis and Singer, 1973).

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(hydrolysis) and yields the hydroxo complexes $FeOH^+$ and $Fe(OH)_3^-$. The $Fe(OH)_3^-$ complex is of very limited solubility, but $FeOH^+$, which can result from the hydrolysis of both $FeCO_3$ and $Fe(OH)_2$, is relatively soluble (Figure 2).

In a system which is open to the atmosphere (partial pressure $CO_2 = 10^{-3.5}$ ATM), $Fe(OH)_2$ is not the stable form and its conversion to $FeCO_3$ (Stumm and Morgan, 1970) emphasizes the control of $FeCO_3$ on Fe(II) solubility.

Redox potential

The Fe(II) - Fe(III) couple has a considerable range of oxidation-reduction potentials (Neilands, 1974). Oxidation-reduction potential is an electro chemical parameter, in volts, which measures the tendency of an element to give up or receive electrons (Brock, 1970). This potential is usually measured with reference to a hydrogen (H_2) electrode and is expressed at a given pH, i.e. pH 7, E_7.

The redox potential, along with the concentration of iron and other dissolved substances in aqueous solution, is controlled by the dissolved oxygen concentration (Mortimer, 1941).

Fe(II) is oxidized to Fe(III) in the presence of dissolved oxygen (D.O.):

$$Fe^{++} \xrightarrow{\ O_2\ } Fe^{+++} \xrightarrow{\ OH^-\ } Fe(OH)_3 \downarrow$$
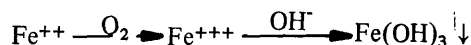
The rate of these reactions is rapid and a function of the partial pressure of O_2 (P_{O_2}) and the concentrations of [Fe(II)] and [OH$^-$] (Stumm and Lee, 1961) as given in the following kinetic expression:

$$- \frac{d[Fe(II)]}{dt} = k\ [Fe(II)]\ (P_{O_2})\ [OH^-]^2$$

Considering only the parameter of D.O. (and therefore redox potential) the hydrated Fe(II) ion can only exist under extreme reducing conditions, that is, D.O. < 0.1 mg/l and $E_7 < 0.25$ v (Pearsall and Mortimer, 1939; Mortimer, 1941).

It must also be noted that under extremely reducing conditions of $E_7 < 0.08$ v, sulfide $(S^=)$ will be produced (given the presence of sulfate, $SO_4^=$) and the hydrated Fe(II) will then precipitate as FeS (Mortimer, 1941). Ferrous sulfide formation would occur at low D.O. concentrations.

Complexing ligands

Due to the limited solubility of iron in natural aerobic systems, complexing ligands must be present to coordinate with iron and keep it in solution. The presence of organic complexes is used to rationalize the higher concentrations of metals found in solution than can be explained by their molar solubilities (Stumm and Morgan, 1970).

Complex formation (coordination) is defined as "any combination of cations with molecules or anions containing free pairs of electrons (bases)" (Stumm and Morgan, 1970, p. 239). Iron is the central metal atom and the anion(s) complexed to it is(are) ligand(s). Chelation is a complex formation of a cation with an anion which contains more than one ligand atom (a multidentate ligand). These complexes are soluble, thus placing iron in solution and available to microorganisms. Iron chelation, therefore, plays an essential role in microbial physiology (Price, 1968).

Figure 4 schematically shows the chemistry of iron in the presence of organic matter and oxygen.
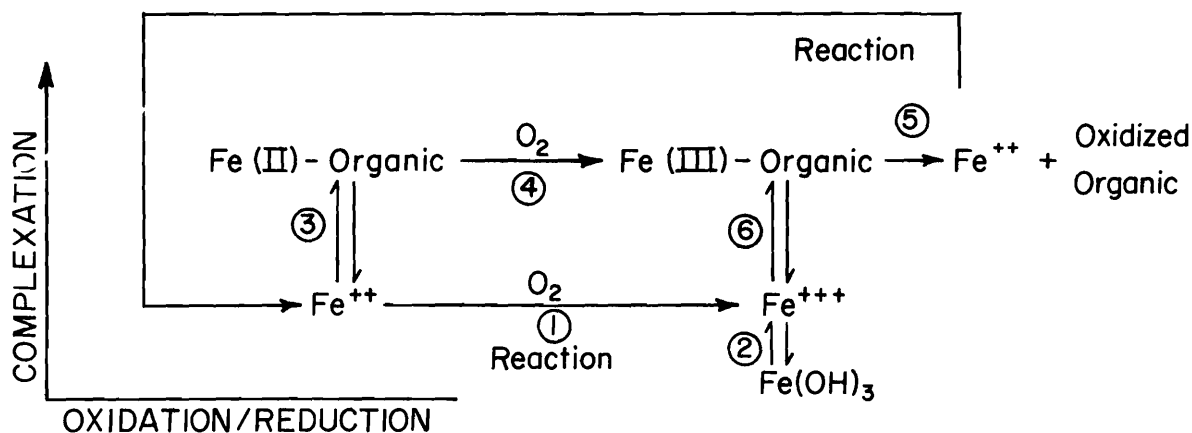
Figure 4. Iron in presence of organic matter and oxygen, a simplified schematic by Theis and Singer (1974) to show Fe(II) and Fe(III) in natural water systems. Complexation reactions are indicated vertically, while redox reactions are indicated horizontally.

Reactions ① and ② have been explained previously, and will proceed rapidly in the absence of organic material. Dissolved organic matter can stabilize Fe(II) (Reaction ③) and Fe(III) (Reaction ⑥) in an aquatic system through complex formation. Although resistant to oxidation, Fe(II)-organic complexes are susceptible to oxidation given highly oxidizing conditions (Reaction ④). Some reduction of organically bound Fe(III) does occur (Reaction ⑤); the extent of this occurrence is dependent upon the organic species complexed to Fe(III) since the holding capacity of organic matter is variable (Plumb and Lee, 1973). Resultant Fe(II) produced is then free to enter the cycle again.

Humic matter (naturally occurring organic compounds) is effective in dissolving metallic compounds and their presence will delay the precipitation of iron by formation of soluble, stable complexes (Rashid and Leonard, 1973; Theis and Singer, 1973).

In strictly anaerobic conditions one would expect Fe(II) to predominate, while under natural aerobic conditions, oxidation would occur and Fe(III) would dominate. However, since organic matter is present in nature, both Fe(II)-organic complexes and Fe(III)-organic complexes exist in aqueous systems (Figure 4). The former are resistant to oxidation under aerobic conditions and the latter are resistant to reduction under anaerobic conditions (Theis and Singer, 1974). Therefore, it is possible to have appreciable amounts of Fe(II) under aerobic conditions and Fe(III) under anaerobic conditions when organic complexing agents are present.

From the previous discussion it is concluded that iron found in the aqueous phase of an aerobic aquatic environment would consist primarily of Fe(III)-organic and Fe(II)-organic (both particulate and soluble), $Fe(OH)_3$ (suspended), and $FeOH^+$ forms. Given anaerobic conditions the hydrated Fe(II) ion would be present in considerable concentrations (Theis and Singer, 1973; Brock, 1970; Cotton and Wilkinson, 1962).

Because of the insolubility of iron in natural systems, a method must be utilized which insures that any iron added to a system under study will remain in solution. Metals are coordinated by ligands which are normally found in natural waters (Pittwell, 1974). Chelation of the Fe(III) ion is one mechanism used to keep iron in solution and thus more available to microorganisms than would otherwise be the case (Theis and Singer, 1973). Microorganisms and plants possess the ability to utilize iron supplied as iron chelates (Chaney et al., 1972). Because of the insolubility of Fe(III), it is extremely difficult for microorganisms in aqueous systems to assimilate iron. Iron must first be soluble in the extracellular environment before it can be transported into the micro-

organism (Emery, 1971). The transport of soluble iron is accomplished by the siderochromes (a series of iron binding enzymes produced by microorganisms) which have specific iron binding constants in excess of 10^{30}. These extremely high binding constants insure that these ligands (siderochromes) can adequately remove iron from other chelates which coordinate with iron in the aqueous environment.

Disodium ethylenediaminetetraacetate, Na_2EDTA, was the chelator selected in these experiments to keep iron in solution because it is a highly effective, very stable complexer (Lockhart and Blakeley, 1975; Schutte, 1964) which forms strong 1:1 complexes with metal ions (Hanck and Dillard, 1973). Na_2EDTA has been used to insure availability of trace elements for algal growth in bioassay procedures (Miller et al., 1975).

Competition for Na_2EDTA from aqueous calcium (Ca^{++}) and magnesium (Mg^{++}) can decrease the efficiency of Fe(III) and Na_2EDTA forming soluble chelates (Stumm and Morgan, 1970); therefore, the presence of high concentrations of calcium and magnesium can decrease the solubilization effects of chelators on iron in natural systems.

Iron in Sediments

Sediments are accumulated almost continuously by sedimentation of particulate matter in lakes and reservoirs (Mortimer, 1950). This particulate matter is both organic and inorganic, and is either allochthonous (enters the system from outside the lake or reservoir) or autochthonous (is produced chemically or biologically within the lake or reservoir).

A significant portion of nutrient material entering a lake or reservoir is deposited, and therefore accumulates in the sediments (Huang et al., 1974). Lee (1962) studied Lake Mendota (Wisconsin) and found that 80 percent of the iron transported into the basin accumulated in the bottom sediments. Wentz and Lee (1969) reported a maximum iron concentration in Lake Mendota sediments of 24 mg Fe/g sediment (2.4 percent iron). These sediments represent a considerable source of iron to the aqueous phase biota. This same study showed a close correlation between iron and phosphorus in the sediment.

Mortimer (1941, 1942) in studying Lake Windermere (English Lake District) sediments found color distinctions relative to redox potentials (and therefore iron oxidation states). Brown mud was Fe(III) containing sediments ($Fe(OH)_3$). Mud immediately below the surface layers was grey; this was reduced (low redox potential) mud, $E_7 < 0.25$ v. When redox potential fell to extremely low levels ($E_7 < 0.08$ v), sulfate, $SO_4^=$, if present was reduced to

sulfide, $S^=$, and FeS was formed. Sediments containing FeS were black. This phenomenon of FeS precipitation was observed in laboratory studies (extremely reducing conditions were induced) but was not observed in field observations (extremely reducing conditions did not exist).

Laboratory studies (Mortimer, 1941, 1942) were made in jars (microcosms). When the system was aerated, the brown $Fe(OH)_3$ containing layer of mud increased in thickness. Precipitation and adsorption of material at the mud-water interface would immobilize dissolved species and remove them from the aqueous phase. If the system was allowed to go anaerobic, the brown layer decreased in thickness and disappeared. After disappearance of this oxidized microzone, nutrients and other chemical components (ammonia, orthophosphate, iron, silica, and alkalinity and conductivity elements) began to solubilize and the aqueous concentrations of these elements increased markedly. These laboratory experiments of Mortimer (1941, 1942) emphasized physico-chemical processes; however, biological aspects must not be neglected.

Algae, and other microorganisms which require light as an energy source, are situated in the upper layers (epilimnion) of lakes and reservoirs. When these microorganisms die, they begin to sink and fall through the water column and settle upon the mud's surface. Any nutrients and trace elements incorporated in these cells are therefore relocated to the bottom of the water column.

A committee report on nutrients in water (Committee on Nutrients in Water, 1970) concluded that not all of the nutrients deposited on lake sediments were readily available to the biota in the overlying waters due to the formation of refractory (resistant to biodegradation) biological materials. Because chemical and physical factors prevent dissolution of solid phase nutrients and due to incomplete cycling of nutrient material via formation of refractory matter, a net loss of nutrients from the aqueous phase results. The unanswered question is: how much of the trace element iron, which has been deposited in or on the sediments, is available to organisms in the aqueous phase?

The answer to the problem of eutrophication and eutrophic aqueous systems lies in a process which would maximize nutrients in the sedimentary phase without creating potentially toxic conditions.

Sediment-Water Interchange of Iron

Exchange mechanisms between the solid and liquid phases of an aquatic system are functions of the entire chemical, physical, and biological nature of the system (McKee et al., 1970).

Considering chemical and physical parameters, iron sorption to or iron release from the sediments almost solely depends upon the D.O. concentrations in the waters overlying the sediments. The release rate of iron from the sediments of rivers and lakes is inversely related to the D.O. concentration of the aqueous phase (Fillos and Swanson, 1975).

Oxygen, if present in the aqueous phase, will penetrate into the mud only to a depth of a few millimeters (Mortimer, 1950; Hayes et al., 1958), since it can only enter the muds by molecular diffusion (Mortimer, 1950).

Anaerobic hypolimnion

Given low D.O. conditions in the waters overlying the sediments, iron will be released from the sediments because, in its reduced ferrous state, iron is soluble. Complete lack of oxygen (< 0.08 v E_7) is very rare, and only under these extreme conditions (and presence of sulfide) does iron precipitate out of solution as FeS.

Aerobic hypolimnion

If aerobic conditions exist in the waters overlying the sediments, conditions will be such that it will be impossible for iron to migrate from the sediments and enter the aqueous phase. Even though conditions (anaerobic) exist just below the surface of the mud which would reduce Fe(III) to Fe(II), iron would still not reach aqueous phase because as soon as the reduced form (Fe(II)) crossed the solid liquid interface into O_2 bearing water, it would be oxidized and reprecipitated as $Fe(OH)_3$. This barrier which exists at the interface has been referred to as oxidized microlayer (Mortimer, 1941; Hayes et al., 1958; Gorham, 1958). This barrier exists only if there is an oxygenated hypolimnion and prevents iron entry into the aqueous phase (Einsele, 1938; Pearsall and Mortimer, 1939; Kuznetsov, 1968).

Chelating agents naturally occurring in the system or added to a system (Na_2 EDTA) will increase the concentration of iron (and other trace elements) in waters overlying lake sediments (Barica et al., 1973; Hanck and Dillard, 1973). Na_2 EDTA was the most effective mobilizing agent tested (Barica et al., 1973). Thus, natural chelators present in an oxygenated hypolimnion might allow iron to migrate from the sediments and be available to algae and other organisms.

There are biological mechanisms which affect iron transport into or out of the sediments. The existence of algal mats on the sediment surface has been observed in nature (Gahler, 1969) and in laboratory microcosms (Porcella et al., 1970); these

mats represent a sink for extracting nutrients from the sediments. Also the mats, in lifting off the sediments, due to gas bubble formation, would physically disrupt and mix the sediments with the overlying water.

Bacterial populations can affect the exchange of iron between phases (Fillos and Swanson, 1975). In studying Russian lakes, Kuznetsov (1968) correlated bacterial counts of a given species with conditions (iron oxidation and deposition on the sediments or iron reduction and release from sediments) expected to be caused by those species. He concluded that specific bacteria were responsible for the oxidation of Fe(II) to Fe(III). The process of reduction resulted from the activities of a nonspecific flora in the iron cycle within the lake basin. Kuznetsov concluded that even though physico-chemical processes play a role in nutrient cycling, biological considerations of bacteria and algae are of greater importance.

In work performed in the laboratory by Huang et al. (1974) it was noted that mechanisms for nutrient release were mostly chemical and physical, with biological or microbial activities having only a minor role. These experiments were performed over short periods (seven days) in buffered dechlorinated tap water with sewage seed spikes (mercuric chloride was utilized to inhibit microbial activities) to assay the effects of microbial populations on pollutant release from the sediments. The assays were performed in the dark in a walk-in incubator. Given these conditions (short time and no light) it is understandable that physico-chemical aspects were more important than biological aspects in considering pollutant release. In order to assess biological effects upon nutrient release from sediments, longer experimental runs and light must be utilized.

Benthic organisms, through burrowing activities, can resuspend or redeposit nutrients (McKee, 1970), and, therefore must also be considered in nutrient and trace element fluxes between the aqueous and solid phases of any aquatic ecosystem.

The aqueous chemistry of iron presents a unique paradox with respect to iron exchange processes between the sediment and the aqueous phases. An oligotrophic (nutrient poor) lake system will generally have an aerobic hypolimnetic region all year around, thus any sedimentary phase iron is immobilized in the solid phase by the oxidized microzone. A eutrophic (nutrient rich) lake system will develop an anaerobic hypolimnetic region during the summer and thus yield chemical and physical conditions favorable to iron transport to the aqueous phase with resultant removal of any (if present) iron

limitation. The iron flux in a eutrophic reservoir is tremendous (Mortimer, 1941). This flux can be attributed to spatial and temporal changes in D.O. within the system. Kamp-Nielsen (1974) measured iron fluxes from 5.0 ± 3.3 mg $Fe/m^2/day$ adsorption onto sediments in an aerobic system to 7.4 ± 4.0 mg $Fe/m^2/day$ liberation to the aqueous phase in an anaerobic system using Lake Esrom (Denmark) sediments in the laboratory.

The iron paradox results in iron deficiencies being more prevalent in oligotrophic rather than eutrophic systems (Goldman, 1972).

Iron and Phosphorus

Phosphorus is a very important nutrient in algal productivity (Sawyer, 1966). The patterns of iron deposition closely follow the patterns of phosphorus deposition in various Wisconsin lakes (Bortleson and Lee, 1974); the percent phosphorus sorbed to the sediments was directly related to the concentration of iron in the sediments. Iron was concluded to be the primary factor which determined the levels of phosphorus in sediments.

Although inorganic phosphorus has been inversely related to $CaCO_3$ in calcareous sediments (Wentz and Lee, 1969; Shulka et al., 1971), it has been found that iron containing components are of equal if not greater importance than $CaCO_3$ in determining inorganic phosphorus accumulations in calcareous sediments from Wisconsin lakes (Williams et al., 1971). The same study revealed that in impoundments with an anaerobic hypolimnion (Fe(II) and inorganic phosphorus present in hypolimnion) the disappearance of aqueous phase inorganic phosphorus and Fe(II) occurred simultaneously at overturn (when the redox potential of the aqueous phase increased).

In studies performed in the English Lake District, under oxidizing conditions, orthophosphate-phosphorus (PO_4-P) was precipitated in the presence of iron as insoluble $FePO_4$ (pk_{sp} = 23, Stumm and Morgan, 1970) on the sediment surface (Mortimer, 1941). When reducing conditions returned, iron and phosphorus were liberated from the sediments and appeared in the aqueous phase. Mortimer concluded that the main factors controlling such deposition were located at the mud's surface (oxidized microzone).

Fillos and Swanson (1975) in studying sediments from Muddy River and Lake Warner (Massachusetts) in microcosms found that the release rates of both iron and PO_4-P were closely related and concluded that iron played a dominant role in phosphorus release mechanisms.

Shulka et al. (1971) in laboratory studies found that the amount of phosphorus sorbed to sediments was closely related to the amount of iron in the sediments. Calcareous sediments generally sorb lower amounts of added phosphorus in the laboratory and contain lower levels of phosphorus than non-calcareous sediments; therefore, $CaCO_3$ was less effective than iron in sorbing added inorganic phosphorus in the laboratory (Shulka et al., 1971).

The study of the fate of iron in an aquatic microcosm could lead to results involving phosphorus, a nutrient which if removed from the aqueous phase could certainly limit algal growth.

MATERIALS AND METHODS

The determination of iron dynamics in a eutrophic reservoir was simulated in the laboratory utilizing gas-liquid-sediment microcosms (Figure 5). These units were sealed systems, containing approximately 3 kilograms of wet sediment (15 cm deep) and 9.05 liters of medium (60 cm deep). The sediment came from Hyrum Reservoir, a eutrophic impoundment in northern Utah. The sampling point coincided with the station used by Drury et al. (1975). The medium, adapted in some cases for the various nutrient variables and in other cases to approximate the aqueous chemical environment at Hyrum Reservoir, was based upon the nutrient algal assay medium (EPA, 1971). The medium was replaced semi-continuously on a 10 day residence time basis.

The experiment was conducted in two phases: Phase I started on November 30, 1972, and lasted a total of 189 days until June 7, 1973, and Phase II started on January 17, 1974, and lasted a total of 175 days until July 10, 1974. The sediment for Phase I was collected on November 28, 1972; the sediment for Phase II was collected on January 14, 1974. On both occasions, the sediment was collected only after the microcosm units were completely ready for operation. Procedures for collection, mixing, and distributing the sediment to the microcosms were according to Porcella et al. (1975).

In order to completely analyze all aspects of the microcosms and in order to perform complete mass balances, the sediments were analyzed prior to and after each experimental run of the microcosms. The methodology for analysis of sediment, aqueous and gas phases is given in Appendix A: Analytical Methods.

Phase I Experiment

The first experimental phase of the microcosms involved 16 units (Figure 6) and included the variables of light, nitrate-nitrogen (NO_3-N) and mercury ($HgCl_2$). The experimental design and lighting scheme (Figure 6), the nutrient and gas analyses methods, and the nutrient medium exchange protocol were described in Porcella et al. (1975).

In these experiments, chelated iron was added daily ($FeCl_3 \cdot 6H_2O$) at a concentration of 33 μg Fe/l

(an excess amount so iron would not be a limiting nutrient (EPA, 1971)); the hexadentate ligand used was Na_2EDTA, at a concentration of 300 μg/l.

Total and total soluble iron analyses were performed spectrophotometrically according to Strickland and Parsons (1968). This bathophenanthroline technique for iron in the aqueous phase was sensitive to $\geq$ 10 μg Fe/l. Samples were acidified, buffered, reduced and allowed to react with bathophenanthroline. Absorbance of the colored complex was determined in a 5 cm cuvette at 533 mμ and 650 mμ on a Bausch and Lomb Spec 70 (band width 8 μ); the latter absorbance was a turbidity background correction.

Total ferrous iron, Fe(II), was determined using the phenanthroline technique (APHA, 1971), and the results are not reported because the data were irregular and lacked the sensitivity to detect Fe(II) in the microgram per liter range in the chemically and biologically complex aqueous phase effluent from the microcosms.

The daily addition of phosphorus as K_2HPO_4 was 93 μg P/l; this concentration approximated the average daily summer input to Hyrum Reservoir (Luce, 1974). Phosphorus was added in excess to prevent it from being a limiting nutrient (EPA, 1971).

The soluble fraction represents compounds which passed through a Whatman GF/C filter. Soluble analyses were not performed on 0.45 μ MF Millipore filter samples (EPA, 1974); this was so that the particulate fraction (iron and phosphorus) could be directly related to that material (suspended solids, SS, in mg/l) which could be quantified on the GF/C filter. This comparison would not be possible if a 0.45 μ MF Millipore filtration step followed the SS determination because the amount of material removed by such a membrane filter is not quantitative. The data on the particulate fraction equaled total unfiltered less total filtered analyses.

Phase II Experiment

In this experimental phase, iron and light were the variables (Figure 7) within the microcosms.

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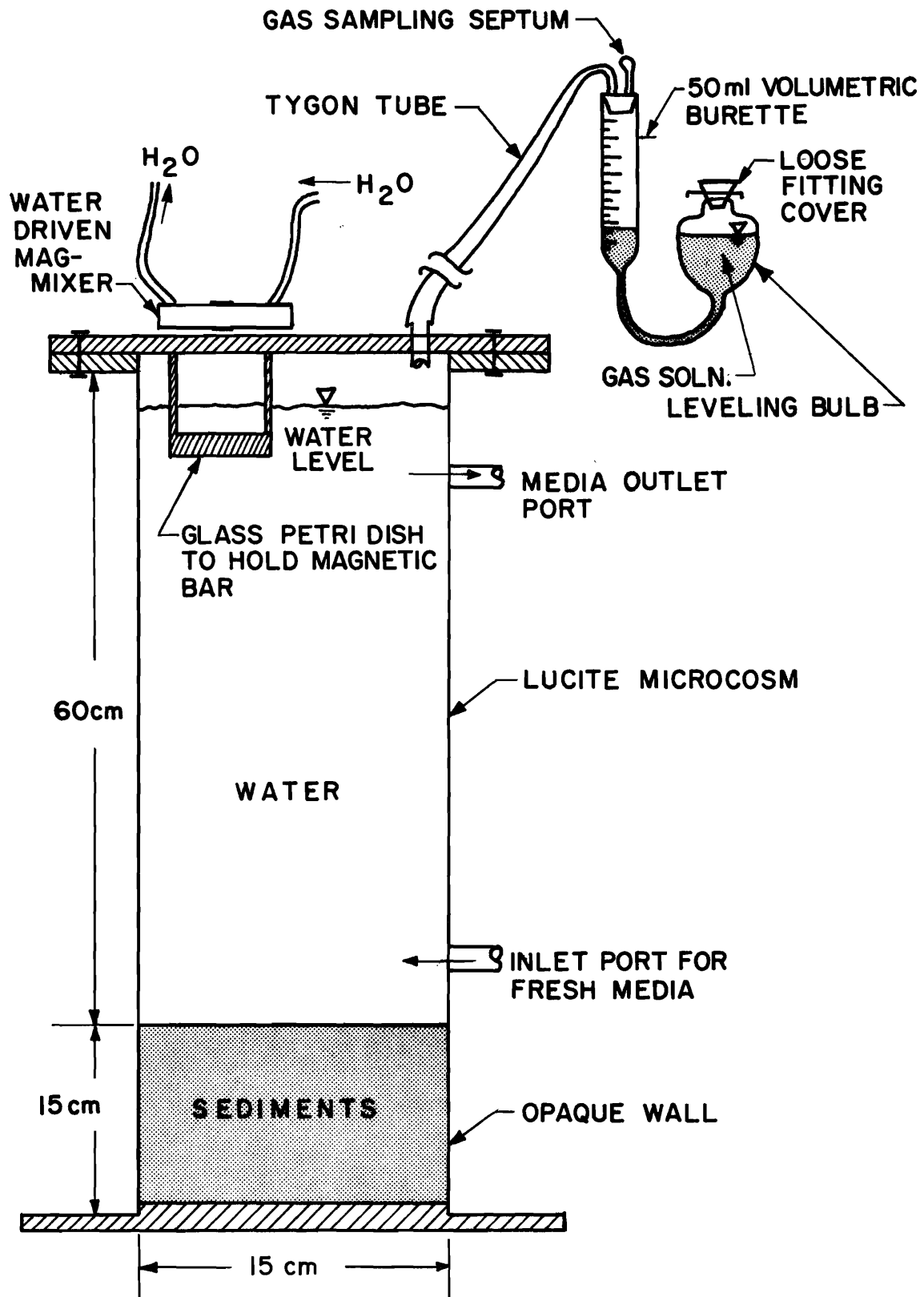
Figure 5. Schematic of a microcosm.
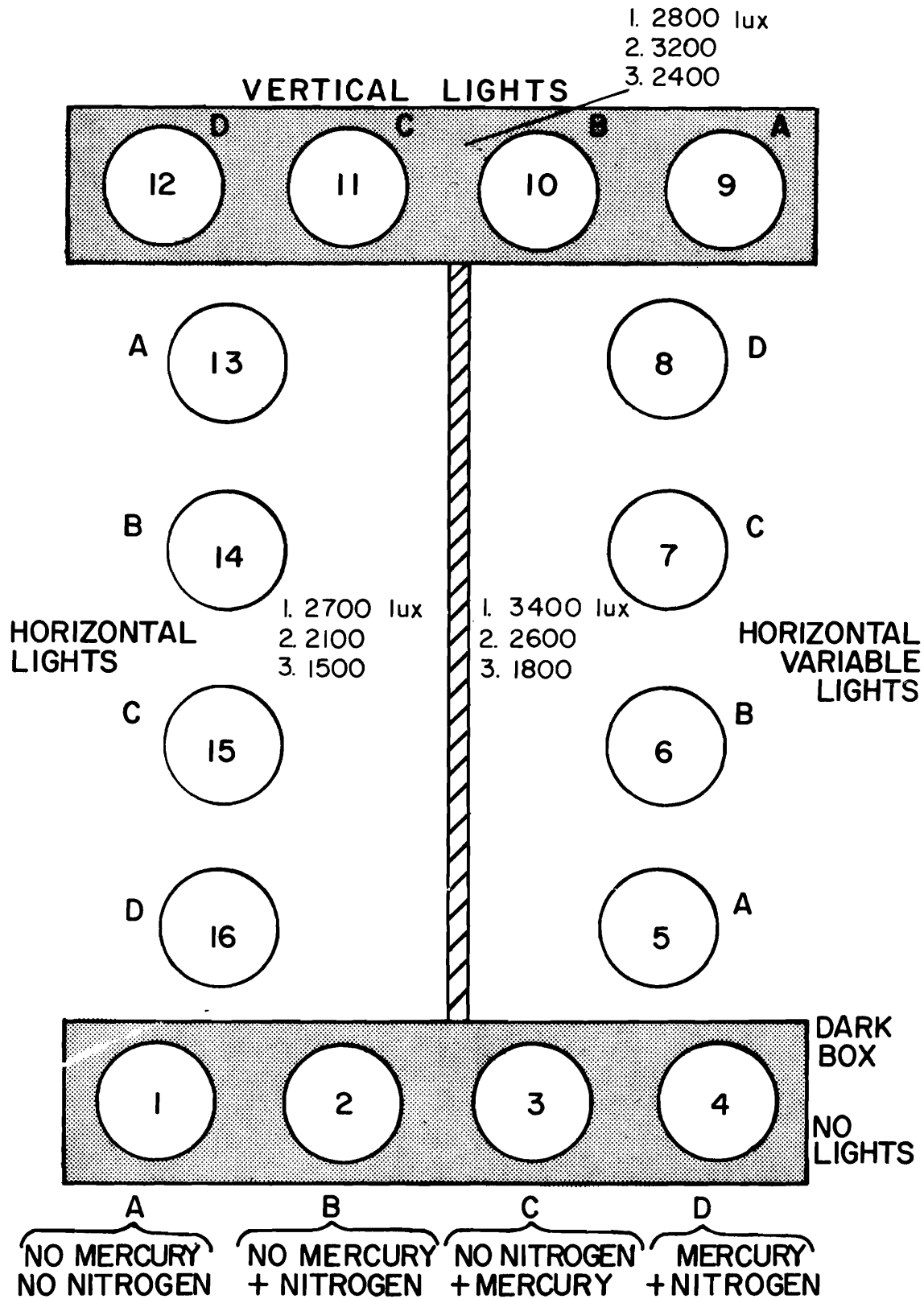
Figure 6. Phase I. Microcosm positions in constant temperature room. Outside light intensities were measured at: (1) water surface, (2) mid water depth, (3) sediment surface.

The same units (Figure 5) and the same nutrient medium exchange protocol (Porcella et al., 1975) used in Phase I were utilized in Phase II. The nutrient medium (Table 1) was altered as follows: the phosphorus concentration was reduced to 30 μg P/l and the nitrogen concentrations in the added medium were raised to an excessive level (10,000 μg N/l). Thus, phosphorus was closer to iron concentrations; nitrate was in excess (therefore nitrogen was not limiting).

The nutrient medium (Table 1) was prepared differently with respect to iron in this second experiment. All the nutrients were added to a large 5 gallon polyethylene bottle except the variable trace element, iron. Thus, the chelator (Na$_2$EDTA) was in solution prior to the addition of the FeCl$_3 \cdot 6H_2O$ solution. The nutrient medium was then split into four 1 gallon polyethylene bottles, one each for treatments A and B and two for treatment C (see Figure 7). The iron was then added to each gallon bottle as indicated to yield the proper concentration of iron in each unit input medium.

Chelator (stock solution D, Table 1) was added to each microcosm. This addition diminished the possibility of iron limitation in the Phase II experiments (variable iron input). The presence of aqueous phase chelators will increase the concentration of iron (and other trace elements) in the water overlying the sediments.

Chelation of iron

Sillen and Martell (1964) list various complexation constants for metal ion complexes with organic ligands. These values were used by Stumm and Morgan (1970) in summarizing Fe(III)-EDTA complex formation with competition by the cations calcium and magnesium for the chelator, EDTA:
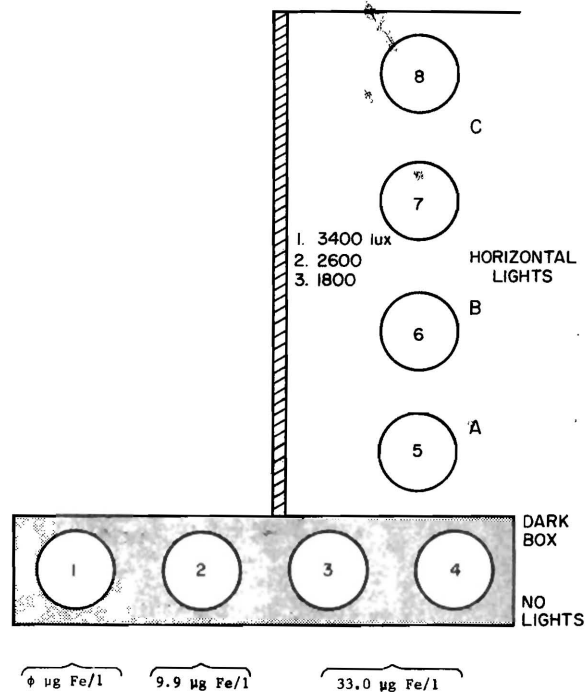
Figure 7. Phase II. Microcosm positions in constant temperature room. Outside light intensities were measured at: (1) water surface, (2) mid water depth, (3) sediment surface.

Table 1. Medium constituents and concentrations, Phase II.

Stock Solution		Compound	Conc. in Stock (mg/l)	Dilution in Feed D.W.	Element	Final Concentration in Microcosm, μg/l			
						I	II	III	IV
A.	A$_1$	NaNO$_3$	6,072.	10→1000	N	10,000.	⟶		
	A$_2$	MgSO$_4 \cdot 7H_2O$	12,167.	10→1000	Mg	12,000.	⟶		
	A$_3$	CaCl$_2 \cdot 2H_2O$	8,070.	10→1000	Ca	22,000.	⟶		
	A$_4$	KCl	7,181.	1→1000	K	4,000.	⟶		
B.		K$_2$HPO$_4$	168.7	1→1000	P	30.	⟶		
C.		H$_3$BO$_3$	187.	1→1000	B	33.	⟶		
	C$_1$	MnCl$_2$; (MnCl$_2 \cdot 4H_2O$)	415.		Mn	115.	⟶		
		ZnCl$_2$	33.		Zn	16.	⟶		
		Na$_2$MoO$_4 \cdot 2H_2O$	7.3		Mo	2.9	⟶		
	C$_2$	CoCl$_2$ (CoCl$_2 \cdot 6H_2O$)	1.5	1→1000	Co	0.37	⟶		
		CuCl$_2$ (CuCl$_2 \cdot 2H_2O$)	0.013		Cu	0.005	⟶		
D.		Na$_2$EDTA$\cdot 2H_2O$	300.	1→1000	(Na$_2$EDTA)	300.	⟶		
E.		NaHCO$_3$	15,000.	1→1000	(NaHCO$_3$)	15,000.	⟶		
F.		FeCl$_3 \cdot 6H_2O$	660.						
	Dilute F	FeCl$_3 \cdot 6H_2O$	3.3	Variable	Fe	ϕ	9.9	33.	33.

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$$Fe(III) + EDTA \rightleftarrows Fe(III) \cdot EDTA$$
$$\text{Log } K_{Fe(III)EDTA} = 25.1$$

$$Ca(II) + EDTA \rightleftarrows Ca(II) \cdot EDTA$$
$$\text{Log } K_{Ca(II)EDTA} = 10.7$$

$$Ca(II) \cdot EDTA + Fe(III) \rightleftarrows Fe(III) \cdot EDTA + Ca(II)$$
$$\text{Log } K_1 = 14.4$$
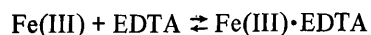
$$Mg(II) + EDTA \rightleftarrows Mg(II) \cdot EDTA$$
$$\text{Log } K_{Mg(II)EDTA} = 8.7$$
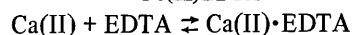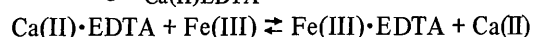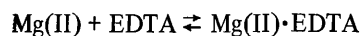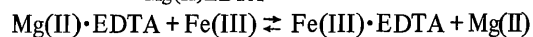
$$Mg(II) \cdot EDTA + Fe(III) \rightleftarrows Fe(III) \cdot EDTA + Mg(II)$$
$$\text{Log } K_2 = 16.4$$

The extremely high equilibrium constants for the reactions involving addition of Fe(III) to complexed Ca(II) and Mg(II) (log K_1 = 14.4, log K_2 = 16.4) indicated that nearly all of the Fe(III) added to the medium would be complexed and therefore soluble and available to microorganisms in the microcosms. The extent of complexation of Fe(III) with EDTA in a solution containing Ca(II) and Mg(II) is pH dependent (Stumm and Morgan, 1970). At the pH of the input media (pH 7.2) and at the concentrations of Fe(III), EDTA, Ca(II), and Mg(II), all of the Fe(III) is complexed (Stumm and Morgan, 1970; Spence, 1975).

Chemical analyses—overall

Analyses were performed every 14 days on the aqueous effluent and the gaseous phase (Figure 8). As was the case in the first experiment, the soluble fraction represents that portion which passed through a Whatman GF/C filter. The gas samples were collected by the syringe and rubber stopper technique and analyzed on a Hewlett Packard 5750 research gas chromatograph (Porcella et al., 1975).

Chemical analyses—iron

The bathophenanthroline technique of Strickland and Parsons (1968), which was used in the first experiment, was found to be inadequate. The aqueous phase, which was nutrient rich (Algal Assay Medium, EP^., 1971) and which was in direct contact with sediments from a eutrophic reservoir, represented a complicated chemical-biological system. The effluent from such a system, when analyzed directly, created suspensions of varying turbidity. Turbidity interfered with colorimetric analyses, and yielded variable data which led to rejection of this direct method (Strickland and Parsons, 1968) in favor of a procedure which would extract metals from the aqueous phase into some organic solvent.

Solvent extraction is used to preconcentrate trace metals prior to determination of a particular metal. Diphenylthiocarbazone was used as the major constituent of an extraction solution (Sachdev and West, 1970); this organic solvent was then aspirated

into a Perkin Elmer 303 atomic absorption spectrophotometer (AAS). However, precipitation occurred in the solvent layer after its separation from microcosm samples, resulting in excessive mixing chamber clogging. Further testing of solutions on another AAS system (Perkin Elmer 303) gave the same results. Precipitation in the organic solvent gave erratic aspiration and produced variable data.

Because solvent extraction (preconcentration) was essential to work in the low microgram iron per liter range of concentrations, a method of n-hexyl alcohol extraction of complexed iron (bathophenanthroline) followed by spectrophotometric determination of iron was used for the second experiment (Lee and Stumm, 1960). Extraction allowed removal of metals from the complicated chemical-biological aqueous phase into a pure organic solvent phase.

The total (unfiltered) and total soluble (GF/C filtered) iron samples were acidified, boiled, buffered, reduced, and then complexed with bathophenanthroline. The total ferrous iron samples, Fe(II), were analyzed identically except the reducing step was omitted. Acidification and boiling of the samples were the two steps utilized to free any iron associated with biological material, complexed to organic material, or from mixed ferrous-ferric oxides, and therefore, allowed it to react with the bathophenanthroline to form the necessary colored complex.

Shapiro (1965) determined that while the boiling step did not affect the total analyses, it did, however, lead to erroneously high results for ferrous iron, Fe(II), in solutions containing organic material. An acceleration of the breakdown of Fe(III) · organic complexes to Fe(II) and oxidized organic material (Reaction ⑤, Figure 4) gave high Fe(II) results. Also, once dissolved oxygen had been expelled from the boiling water, subsequent reduction of Fe(III) to Fe(II) would occur; again, accounting for high Fe(II) values.

Neilands (1974) discussed the chromogenic reagent bathophenanthroline and defined the experimental hazards involved in attempting to define the oxidation states of iron. Neilands (1974) supports Shapiro (1965) and states that Fe(II) will increase during boiling indicating that the Fe(II) data presented in this research could be high.

Emery (1971) modeled iron transport across the cell membrane in the laboratory. Iron in solution, Fe(II), or bound to an organic ligand, Fe(II) ·organic or Fe(III)· organic, in solution (outside compartment) was transferred through an organic phase (simulated cell membrane) into an inside compartment. In order for iron to be transferred, it had to be reduced to

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Fe(II). Because Fe(II) is so important to iron transport, and because it is present in biological organisms, it must be freed in order to react with the bathophenanthroline.

Given the excessively high binding constants of siderochromes for iron (Fe(II) and Fe(III)), in the range of 10^{30} (Emery, 1971), boiling was concluded to be a necessary step to free biologically bound iron.

Experiments were performed in distilled deionized water (DDW) and in Hyrum Reservoir water (HRW) under aerobic (D.O. saturated water) and anaerobic conditions (low to zero D.O. water) to

determine the actual effects boiling had upon the samples when utilizing the iron analyses of Lee and Stumm (1960).

Ferrous Iron Analytical Techniques

Experiments were performed (Table 2) to determine the effects of boiling the sample prior to treatment with bathophenanthroline (Lee and Stumm, 1960). Distilled deionized water (DDW, low nutrient water) and Hyrum Reservoir water (HRW, natural, eutrophic impoundment water) were utilized to simulate nutrient extremes. Samples were aerated for one half hour to attain aerobic water and were
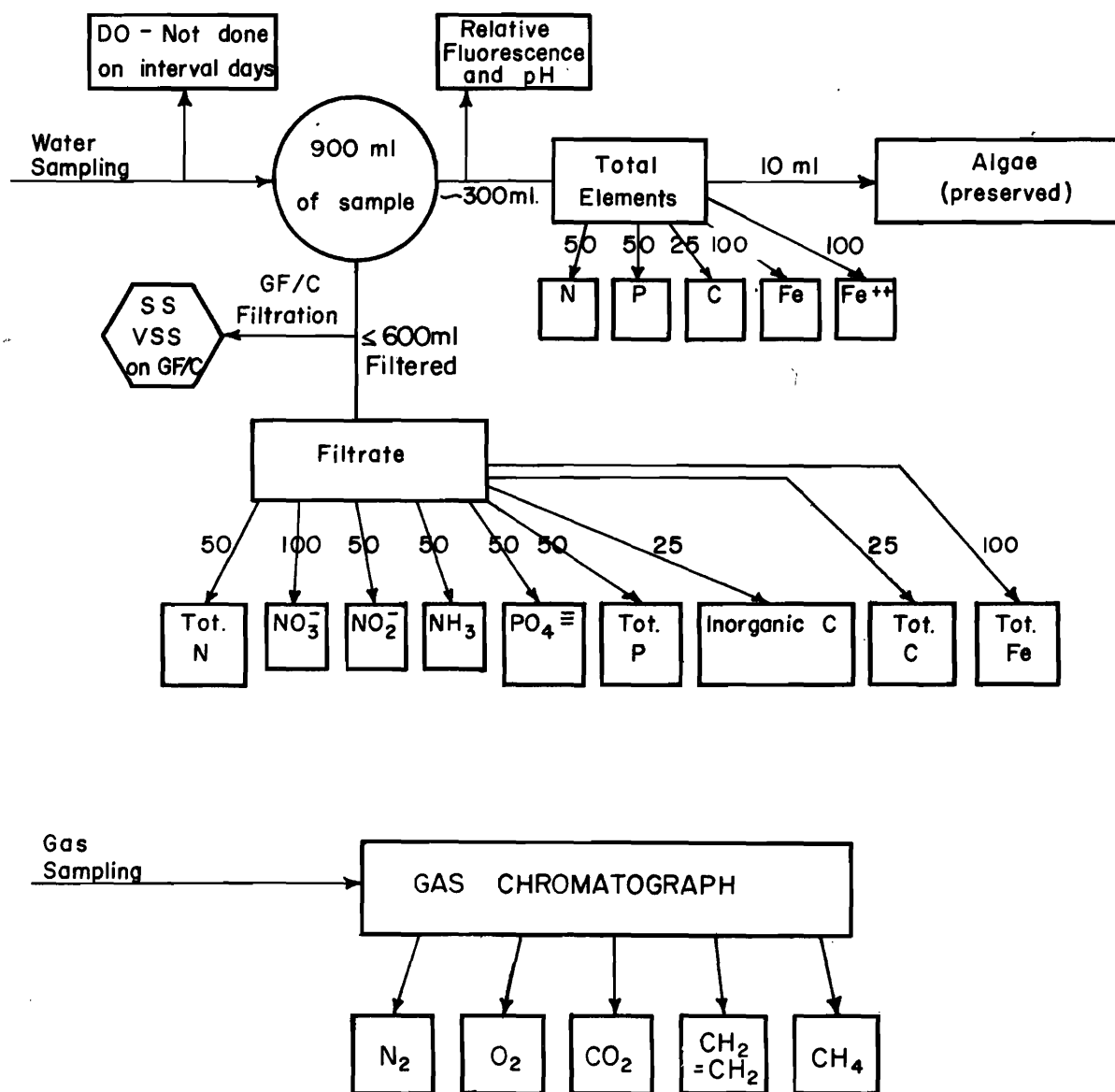
Figure 8. Phase II. Analysis flow sheet (numbers represent ml of sample).

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sparged with nitrogen gas for one half hour to attain anaerobic samples.

When DDW was used, boiling of samples did cause some reduction of Fe(III) to Fe(II) in anaerobic systems (DDW + 100 μg Fe(III)/l); however, the Fe(II) spikes showed higher recoveries of Fe(II) in boiled samples (both anaerobic and aerobic water).

Recoveries of Fe(II) were much lower in HRW. These low recoveries were due to the presence of greater quantities of organic matter, both particulate and soluble, in HRW than in DDW. Again, as was the case in DDW, some reduction of Fe(III) to Fe(II) did occur in the boiling process (HRW + 100 μg Fe(III)/l).

The presence of organic matter (present in HRW samples) reduced the Fe(II) concentration measured by this method. Even though boiling did not make any difference in the measurement of Fe(II) in anaerobic HRW, it did increase the yield of the 100 μg Fe(II)/l addition in aerobic HRW.

These results indicated that boiling had a beneficial effect in the Fe(II) analysis (both anaerobic and aerobic) in low organic systems (DDW), and a variable effect in the high organics systems. In this study, the boiling procedure was used in ferrous iron analyses. It was concluded from the experiments utilizing HRW that the estimate of Fe(II) obtained by boiling the sample of the microcosm effluent would be:
A.   Low: in Fe(II) system
B.   High: in Fe(III) system

Data and Statistical Analyses

Analyses of the results were performed on a Burroughs 6700 computer. Actual analytical measure-

ments made at the end of each interval period ($\simeq 14$ days) for nutrients (22 parameters for the first phase and 18 parameters for the second phase) and gases (five parameters for both periods) were transferred to IBM cards according to the format specified in Program Micro (Porcella et al., 1975). To determine gas production, measurements were made daily of the room temperature and pressure, the influent and effluent temperatures, the manometer (gas level, see Figure 5) readings prior to and after effluent changes, the amount of helium gas added to the gas leveling buret, and the variances in nutrient medium addition outside a predescribed range (0.870 liters to 0.930 liters).

Program Micro (Porcella et al., 1975) computed daily values and accumulated values for major nutrients and gases. The accumulated values represented mass balances for major elements of concern; negative cumulative flux values indicated accumulation of the element in the microcosm (input > output) and positive cumulative flux values indicated release of the element from the sediments and lost from the microcosm (input < output).

Accumulated nutrient values (total iron, total phosphorus, and total carbon) and accumulated gas values (net volume of gas, nitrogen, oxygen, carbon dioxide, methane and ethylene) were plotted utilizing plotting routines on an EAI 590 hybrid computing system.

Statistical analyses were performed on a Burroughs 6700 computer with a STATPAC (Hurst, 1972) program (STATPAC/FCTCVR) as described in Porcella et al. (1975). The program calculated average values for each parameter for each treatment and for all possible combinations of treatments. Statistical analysis of data was then performed by calculating

Table 2. Sample pretreatment: boiling vs. not boiling samples, Phase II. (Experiment performed twice, each with two replicates; mean values for iron, μg Fe/l.)

	Fe(II)				Total Fe
	Anaerobic		Aerobic		
	Boil	Not Boil	Boil	Not Boil	Boil
Distilled Deionized Water (DDW)					
DDW	14.	8.	6.	5.	17.
DDW + 100 μg Fe(II)/l	94.	77.	93.	61.	104.
DDW + 100 μg Fe(III)/l	31.	10.	8.	7.	88.
Hyrum Reservoir Water (HRW)					
HRW	20.	7.	27.	6.	37.
HRW + 100 μg Fe(II)/l	30.	33.	52.	14.	123.
HRW + 100 μg Fe(III)/l	49.	9.	47.	11.	91.

the mean square value for each treatment and for all possible combinations of treatments (mean square treatment, MST) and the mean square for the overall combination of all treatments (mean square error, MSE). F values were then determined (F = MST/MSE) and compared with F values from tables (Snedecor and Cochran, 1967) to determine the probability of rejecting the null hypothesis. The null hypothesis was that the parameter values from each different treatment and each combination of treatments were equal (not significantly different) at the 1 and 5 percent significance levels. If the calculated F values were greater than or equal to respective table F values the null hypothesis was rejected and the treatments were concluded to affect or change the value of that parameter.

Dark Microcosms

The dark units were operated to simulate the hypolimnetic regions of eutrophic lake or reservoir systems (absence of both light and dissolved oxygen (D.O.)). The results from Microcosm 2 will be discussed because it typifies dark microcosm units and its nutrient treatment (nitrate, no mercury) closely reflects conditions at Hyrum Reservoir. Results and discussion of all 16 units were given in Porcella et al. (1975).

The D.O. and pH conditions (Figure 9) were such that the reduced form of iron, Fe(II), was favored over the oxidized, Fe(III) form. Since the nutrient medium was buffered by the carbonate equilibrium system, the solubility of iron in the dark microcosms was governed by $FeCO_3$ (Figure 2). The hydrated ferrous iron ion, $Fe(H_2O)_x^{++}$ was the

dominate iron species in solution, however, the hydroxo-complex $FeOH^+$ was also present. Since organic matter (volatile suspended solids and soluble carbon) was present in the dark microcosms (Figure 10a,b), it was possible that iron existed in biologically bound or organically complexed forms.

Data at time zero represented the nutrient medium input concentration of the parameter. The first effluent analyses were performed on the 13th day of the experiment. The large increase in suspended solids, S.S. (day 13, Figure 10a) was reflected in comparable increases in inorganic carbon data (Porcella et al., 1975). High S.S. values resulted from mixing within the system and from the fact that by day 13 the material (mostly non-volatile solids), suspended when the mud and water were placed in the microcosms, had not settled. The suspended material contained inorganic carbon (not iron nor
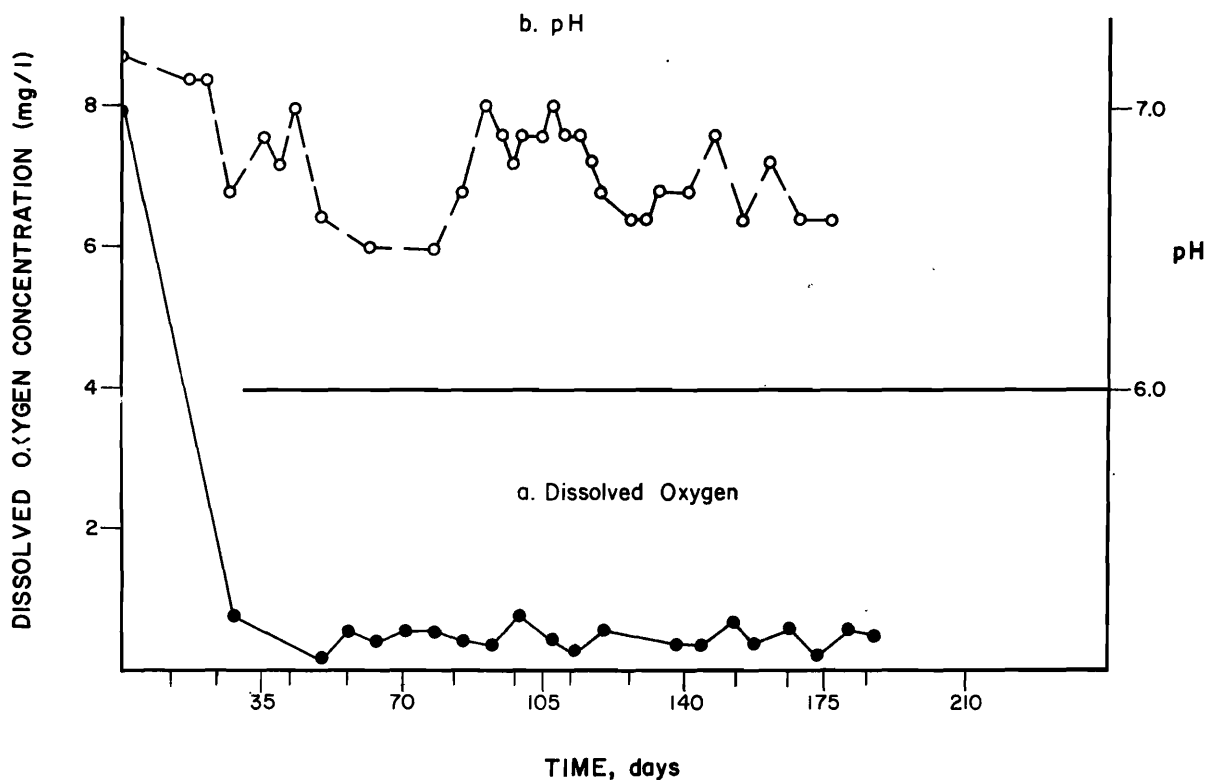
Figure 9. Phase I. Variation in dissolved oxygen and pH in the effluent of Microcosm 2.

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phosphorus, Figures 11 and 12). By day 27, the material had settled out onto the sediments, resulting in a S.S. value of 0.6 mg/l in the overlying liquid.

The large increase in soluble iron and phosphorus on day 27 (Figures 11 and 12) resulted from chemical conditions favoring the release of both elements from the sediments. By this time the D.O., which was at saturation with respect to the atmosphere at initial conditions, had reached equilibrium within the sealed system at $<$ 1 mg/l (Figure 9(a)), levels at which Fe(III) would be reduced to Fe(II) at the sediment surface thus increasing the soluble iron concentration considerably. The parallelism of iron and phosphorus concentrations (Figures 11 and 12) throughout the course of the experiment suggested a definite relationship between the iron and phosphorus flux in anaerobic sediment-water systems (flux towards the aqueous phase).

Based upon iron and phosphorus inputs (medium: 33 μg Fe/l and 93 μg P/l) and effluent concentrations, mass balances were performed (Figures 13 and 14). The total calculated iron input into the system was:

$$\left(\frac{0.033 \text{ mg Fe}}{1} \right) \left(\frac{0.9 \text{ l input}}{\text{day}} \right) (187 \text{ days}) =$$

5.6 mg Fe

The total calculated phosphorus input into the system was:

$$\left(\frac{0.093 \text{ mg P}}{1} \right) \left(\frac{0.9 \text{ l input}}{\text{day}} \right) (187 \text{ days}) =$$

16 mg P
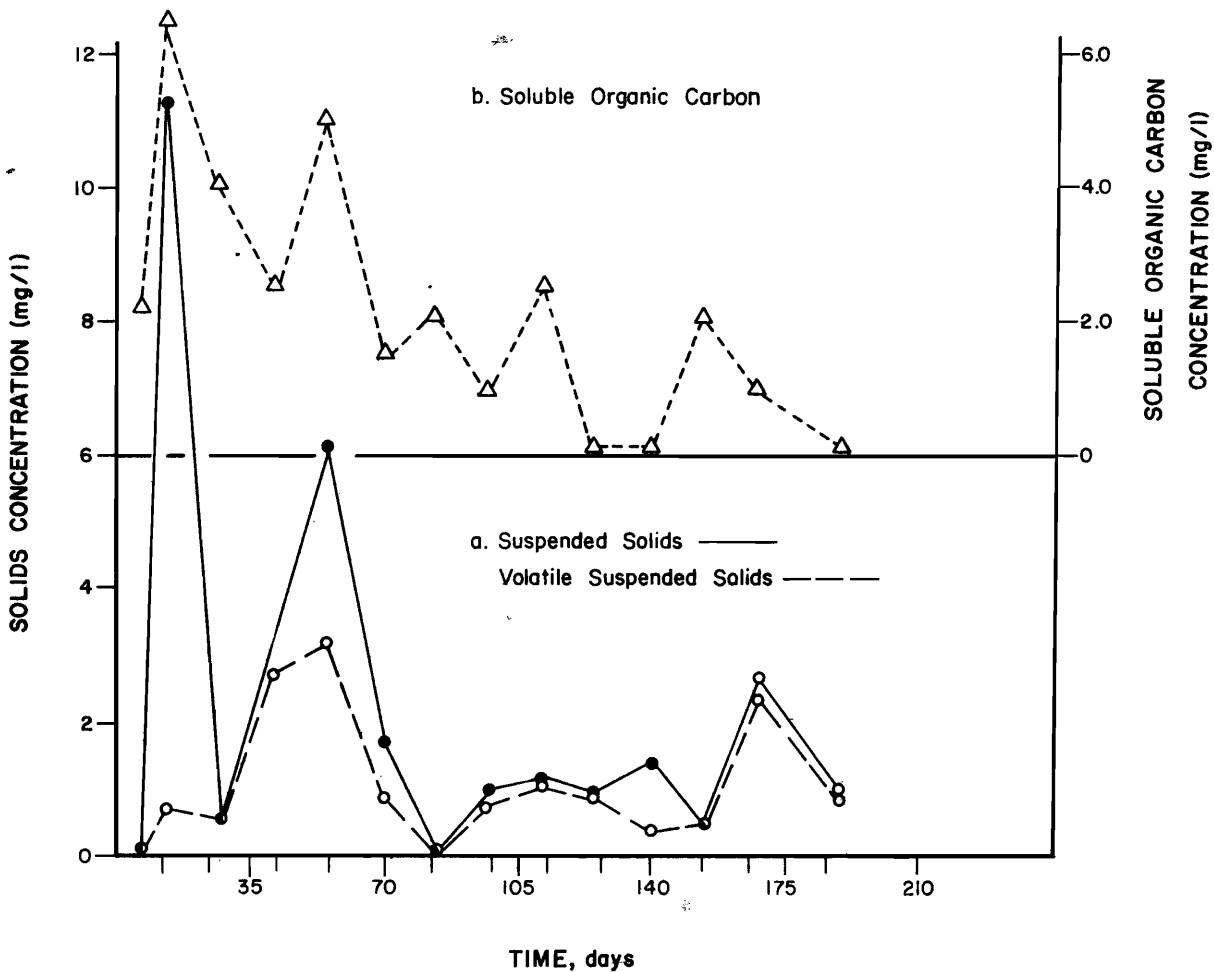
Figure 10. Phase I. Variation in suspended solids, volatile suspended solids and soluble organic carbon in the effluent of Microcosm 2.

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The anaerobic conditions in the dark units favored release of iron and phosphorus from the sediments. The net loss of iron (output > input) from Microcosm 2 was 16 mg Fe (Figure 13). The net loss of phosphorus from Microcosm 2 was 22 mg P (Figure 14). These fluxes are summarized in Table 3, and showed that a considerable output of carbon, nitrogen, phosphorus, and iron occurred in the dark anaerobic units.

To quantify this loss of iron and phosphorus from the system, the sediments were analyzed prior to and after the experimental run (Table 4). Sediment analysis was performed on thoroughly mixed sediment samples. The average mass of wet sediment placed in the microcosm was 2804 g, and the water content was 70.3 percent water (29.7 percent sediment dry weight). Initial masses of elements in the sediments were then calculated and are listed in Table 4:

$$\underbrace{(2804 \text{ g wet sediment}) (0.297)}_{(832.8 \text{ g dry sediment})} \left(\frac{\text{mg element}}{\text{g element}}\right) =$$

Initial mass of element, mg

Perchloric acid (60 percent $HClO_4$) digestion analysis of sediments for total iron yielded a change in sediment iron concentration from 22.4 mg Fe/g sediment to 19.8 mg Fe/g sediment for Microcosm 2 (Table 4). An iron loss of 2.6 mg/g sediment yielded a net flux of 2165 mg Fe [(832.8 g)(2.6 mg Fe/g)] out of the sediments. This extremely large flux does not match the 16 mg Fe flux determined in the mass balance of the aqueous phase. Mass balance calculations from influent and effluent concentrations represent the most accurate estimate of iron flux because sediment analysis was not sensitive to accurately determine such small (< 5 mg/g) changes in iron concentrations.

Persulfate digestion analysis of sediments for total phosphorus yielded a change in sediment phosphorus concentration from 1.16 mg P/g sediment to 1.08 mg P/g sediment for Microcosm 2 (Table 4). A phosphorus loss of 0.08 mg P/g sediment yielded a net flux of 66.6 mg P [(832.8 g) (0.08 mg P/g)]. This net flux resulting from sediment analysis was in approximate agreement with flux estimation from mass balance calculation. The difference in the phosphorus flux as estimated from mass balances (loss of 22 mg P) and from sediment analyses (loss of
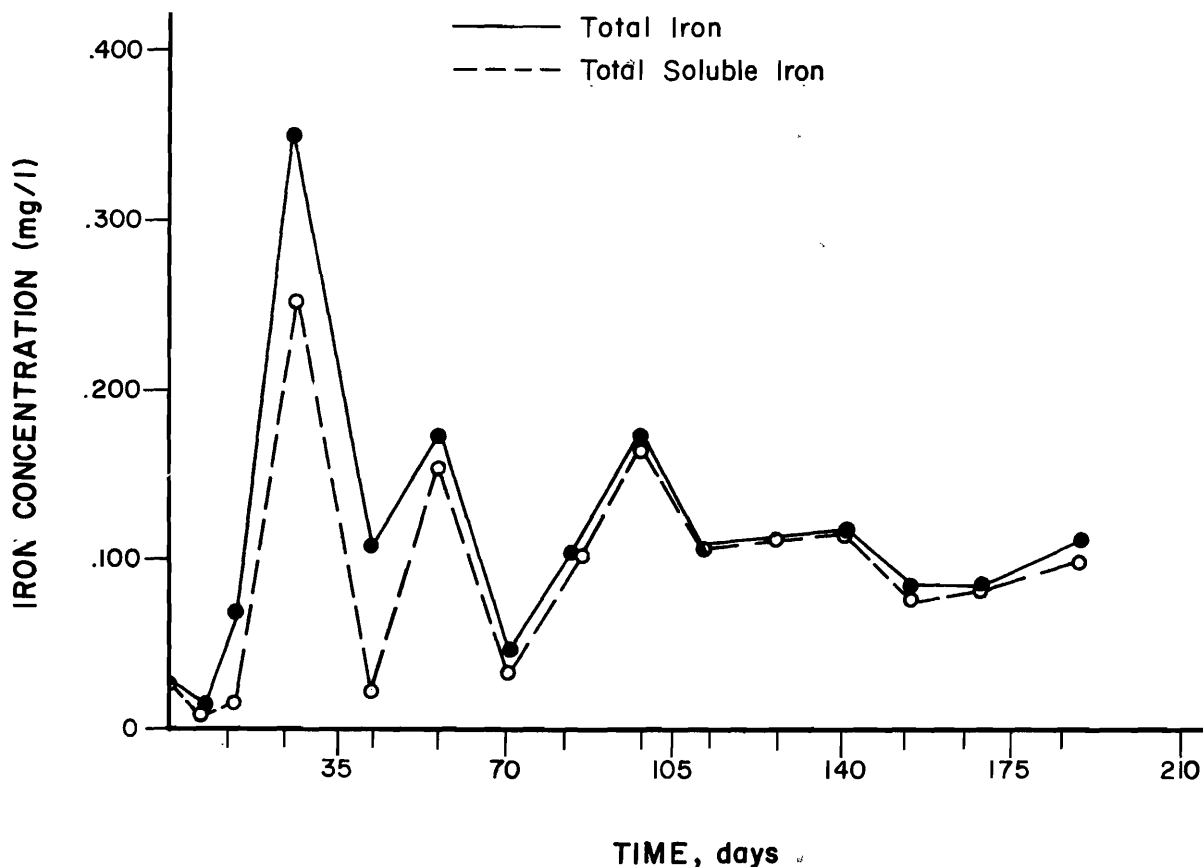
Figure 11. Phase I. Variation in total iron and total soluble iron in the effluent of Microcosm 2.

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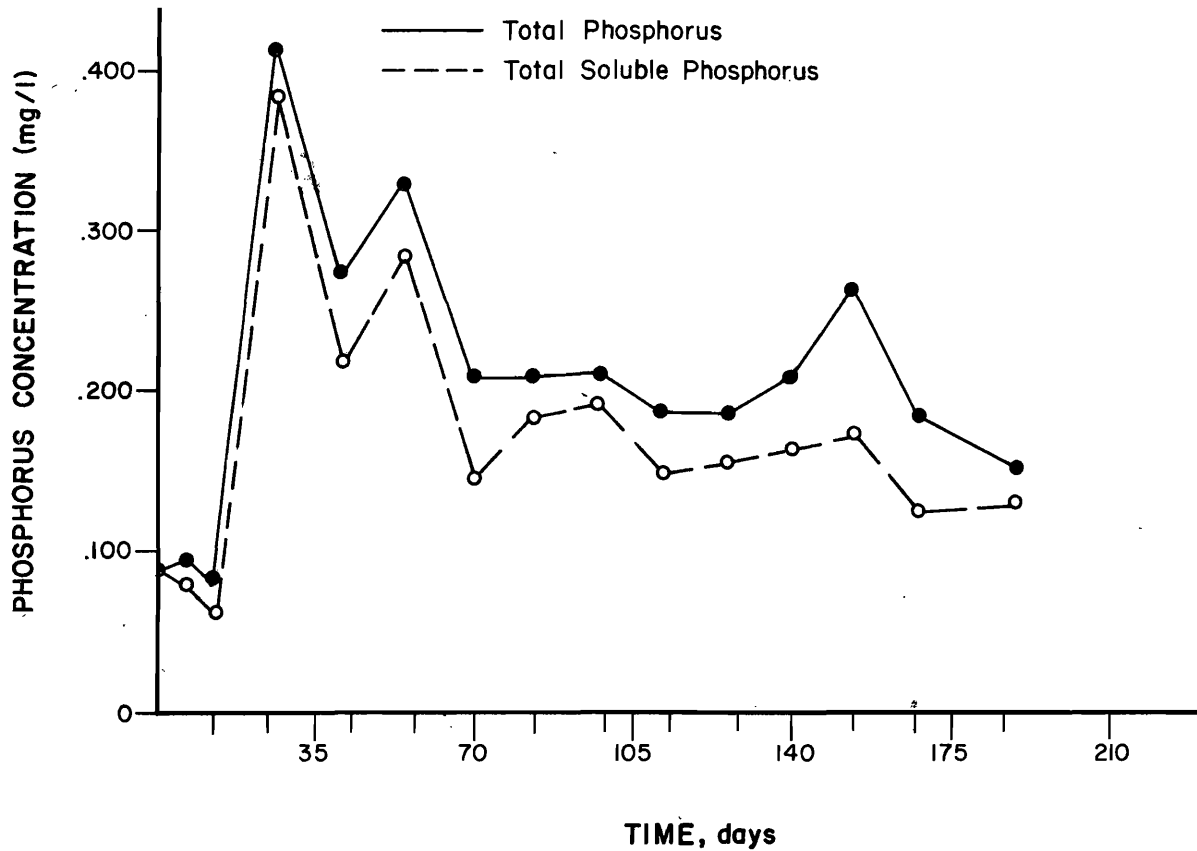
Figure 12. Phase I. Variation in total phosphorus and total soluble phosphorus in the effluent of Microcosm 2.
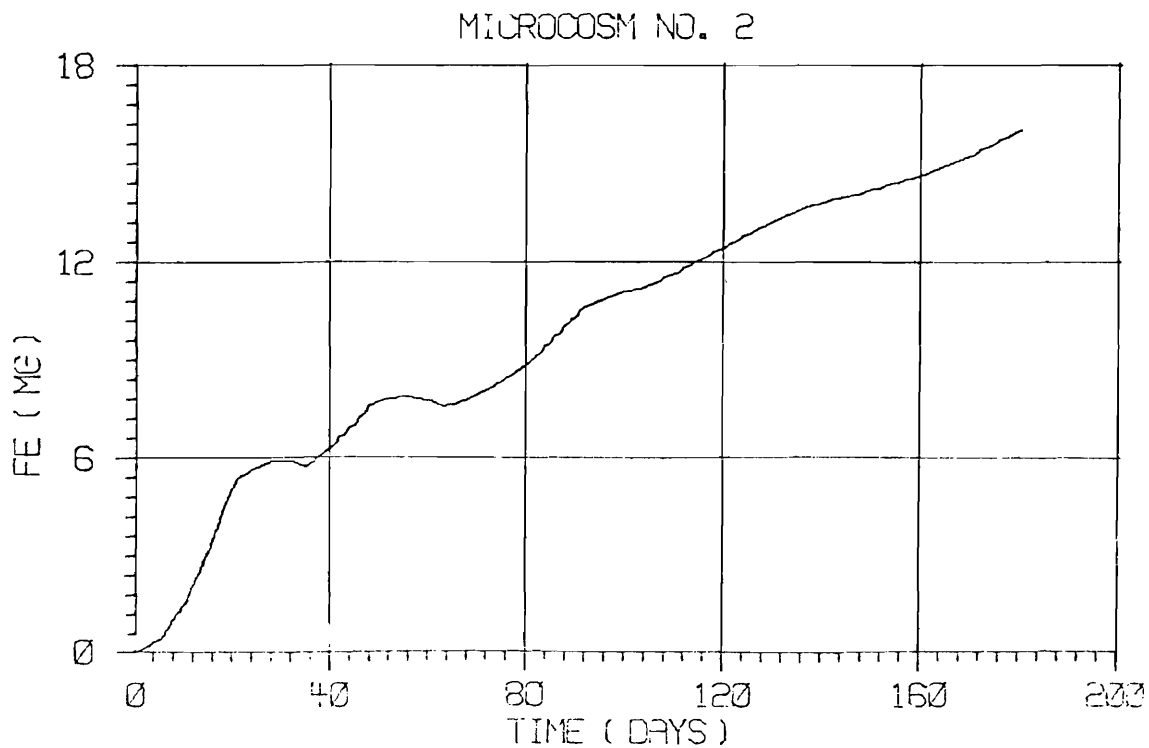
Figure 13. Phase I. Mass balance of total iron accumulated over the period of study in Microcosm 2.

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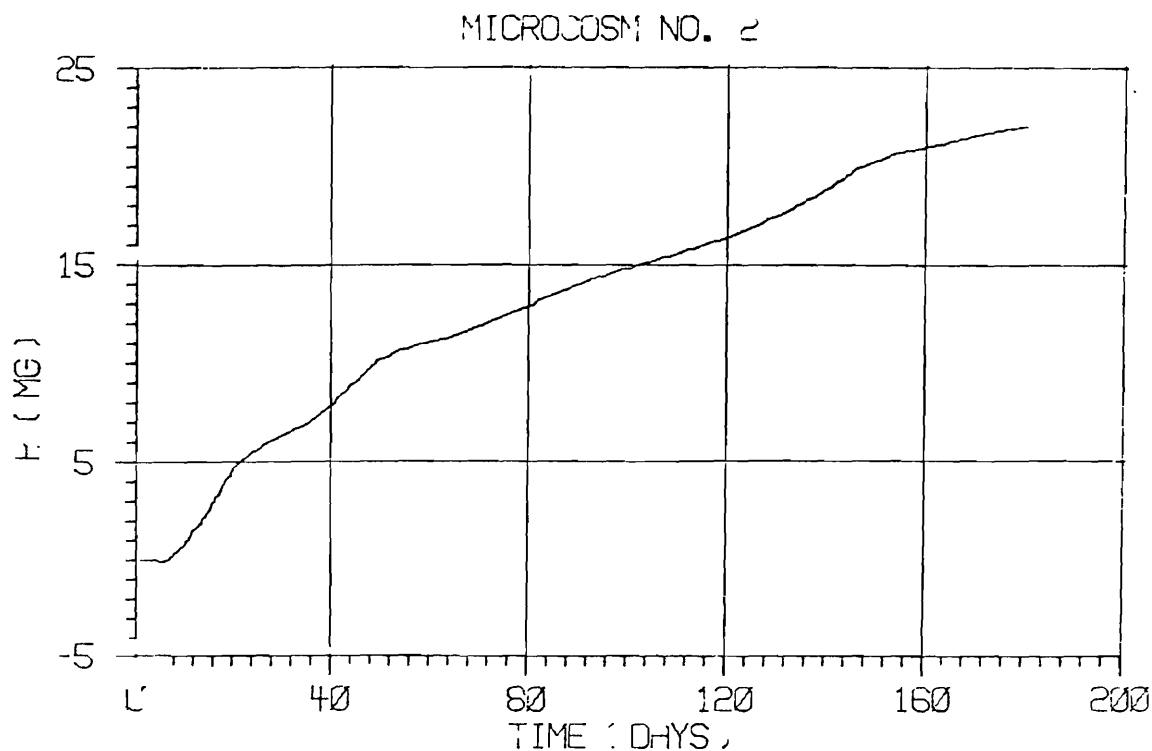
Figure 14. Phase I. Mass balance of total phosphorus accumulated over the period of study in Microcosm 2.

Table 3. Cumulative nutrient net flux, Phase I.

Nutrient Treatment	Microcosm Number	Carbon mg	Nitrogen mg	Phosphorus mg	Iron mg
No Nitrogen, No Mercury	1 Dark	1609	136	20	13.3
Nitrogen, No Mercury	2 Dark	2119	294	22	16
No Nitrogen, No Mercury	13 Light	394	84	-7.8	4.8
Nitrogen, No Mercury	14 Light	352	19	-7.2	6.9
	Total Input in	HCO_3-C	NO_3-N	P	Fe
	Liquid Media	808	0, 50.5	16	5.6

(-) Elements Accumulated (INPUT > OUTPUT) in the microcosm.
(+) Elements Released (OUTPUT > INPUT) from the microcosm.

Table 4. Sediment characteristics, Phase I.

	Initial Conditions (Initial Mass of Element, mg)		1	2	13	14
Total P, mg/g	1.16	(966)	1.18	1.08	1.09	1.03
Avail P, mg/g	0.057	(47)	0.058	0.049	0.054	0.053
Total N, mg/g	2.20	(1,832)	2.13	2.17	2.02	2.13
Org C, mg/g	22.7	(18,904)	16.	14.	12.	11.
Inorg C, as $CaCO_3$, mg/g	208.4	(173,553)	218.	228.	214.	218.
Total Fe, mg/g	22.4	(18,654)	20.	19.8	19.4	19.2

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66.6 mg P) could not be explained, but probably resulted from sediment analytical error as was apparently the case for iron.

Light Microcosms

The light units (Microcosms 13, 14) were operated to simulate the lighted littoral regions of eutrophic lake or reservoir systems (presence of light and extremely high D.O. concentrations). Algal growth in the light units resulted in elevated pH and D.O. levels (Figure 15). High D.O. levels favor the oxidized form of iron, Fe(III), thus Fe(III) solubility is totally dominated by solid phase $Fe(OH)_3$ (Figure 1). Organic matter (volatile suspended solids and soluble carbon) was present in the light microcosms (Figure 16a,b); it was possible that iron existed in biologically bound or organically complexed forms.

The dominance of $Fe(OH)_3$ over iron solubility presents the possibility of $Fe(OH)_3$ precipitate being suspended in the aqueous phase (the systems were mixed by water driven magnetic stirring bar mixing systems, Figure 5). The large amount of non-volatile

material in the suspended solids of the system (Figure 16a, area between the S.S. and V.S.S. curves) indicated the possible existence of such a suspended iron floc. These non-volatile suspended solids were not observed in the dark microcosm effluent (Figure 10(a)).

Total and total soluble iron data were variable (Figure 17), and in these complicated biological systems (lighted microcosms) the direct method of analyzing the samples for iron was inaccurate because of turbid samples. The influent iron concentration was constant at 0.033 mg Fe/l (total iron input to the system was 5.6 mg Fe). The effluent total iron concentration was consistently above this input value yielding a net loss of iron from the microcosm system.

The total and total soluble phosphorus data (Figure 18) were considered more reliable than the iron data because this analysis involved digestion and filtration steps which removed the turbidity problem. The influent phosphorus concentration was constant at 0.093 mg P/l (total phosphorus input to the system
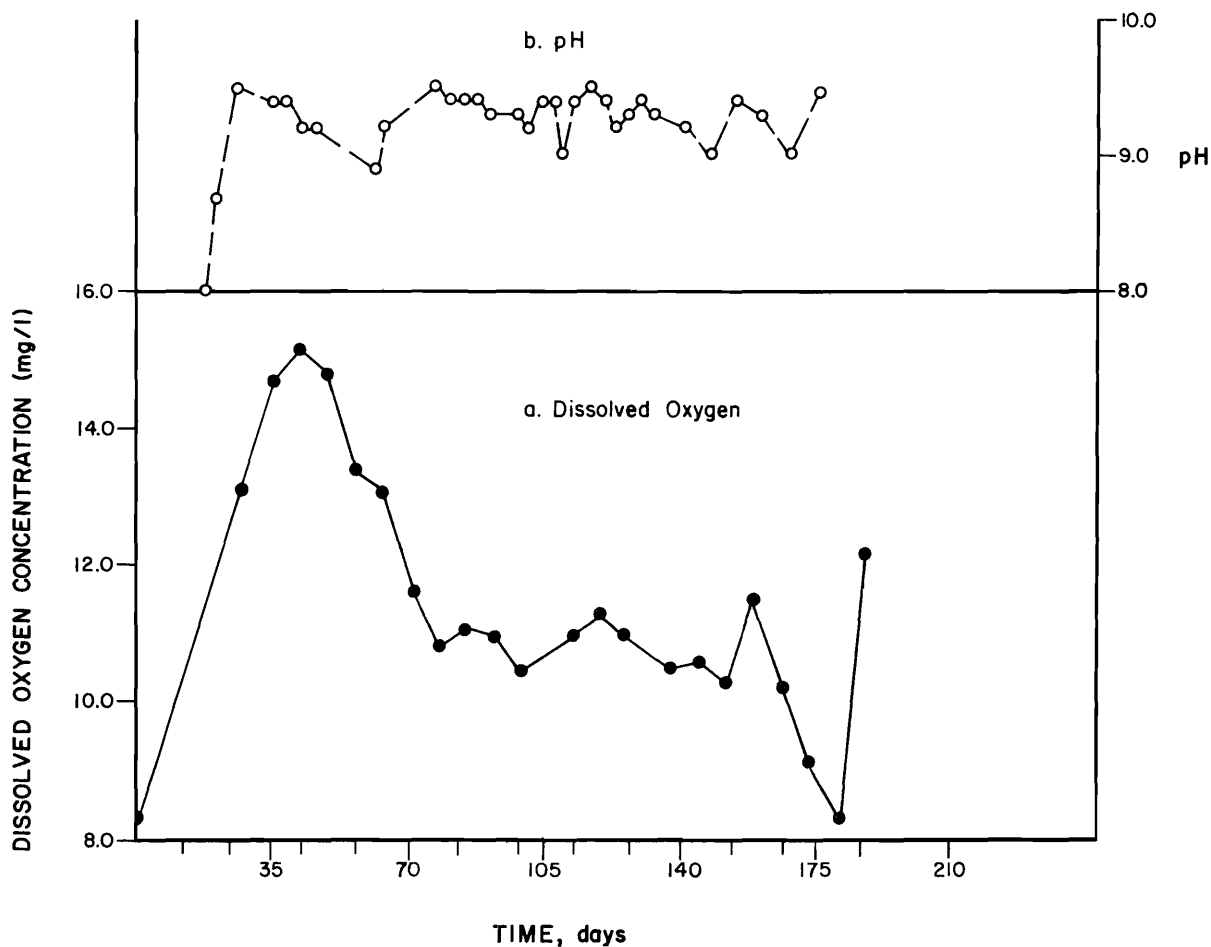
Figure 15. Phase I. Variation of dissolved oxygen and pH in the effluent of Microcosm 14.

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was 16 mg P). The effluent total phosphorus concentration was consistently below this input value yielding a net accumulation of phosphorus in the system.

Again, given the same iron and phosphorus inputs (33 μg Fe/l and 93 μg P/l) mass balances were performed (Figures 19 and 20). The net loss of iron was calculated to be 6.9 mg Fe (Table 3, Figure 19). The net accumulation of phosphorus within the system was determined by mass balance to be -7.2 mg P (Table 3, Figure 20). The opposite fluxes of iron and phosphorus indicated that in these biologically active aerobic systems, mechanisms for iron and phosphorus fluxes were unrelated.

Sediment analyses confirmed iron loss from the system; therefore, the sediments acted as the source for the iron which was lost from the system. Iron concentration decreased from 22.4 mg Fe/g sediment to 19.2 mg Fe/g sediment; this yielded a net loss from

the sediment of 2665 mg Fe [(832.8 g) (3.2 mg Fe/g)]. The extremely large flux is probably in error due to lack of analytical sensitivity.

Sediment analysis for phosphorus also indicated a loss of phosphorus from the sediments: 1.16 mg P/g sediment to 1.03 mg P/g sediment. This yielded a net loss from the sediments of 108.3 mg P [(832.8 g) (0.13 mg P/g)]. This sediment data does not negate the mass balance data because of the presence of periphyton (wall growth) in the lighted units (Table 5). Phosphorus accumulated within the microcosm (output < input) and phosphorus flux out of the sediment phase totaled 115.5 mg P (7.2 + 108.3). The periphyton from Microcosm 14 (11.62 g, Table 5) provided an adequate sink for the phosphorus flux. Stumm and Leckie (1970), in studying phosphate exchange with sediments, characterize algae as 1 percent phosphorus (by total weight). This 1 percent figure represents 116.2 mg P, which would account for the total phosphorus flux of 115.5 mg P.
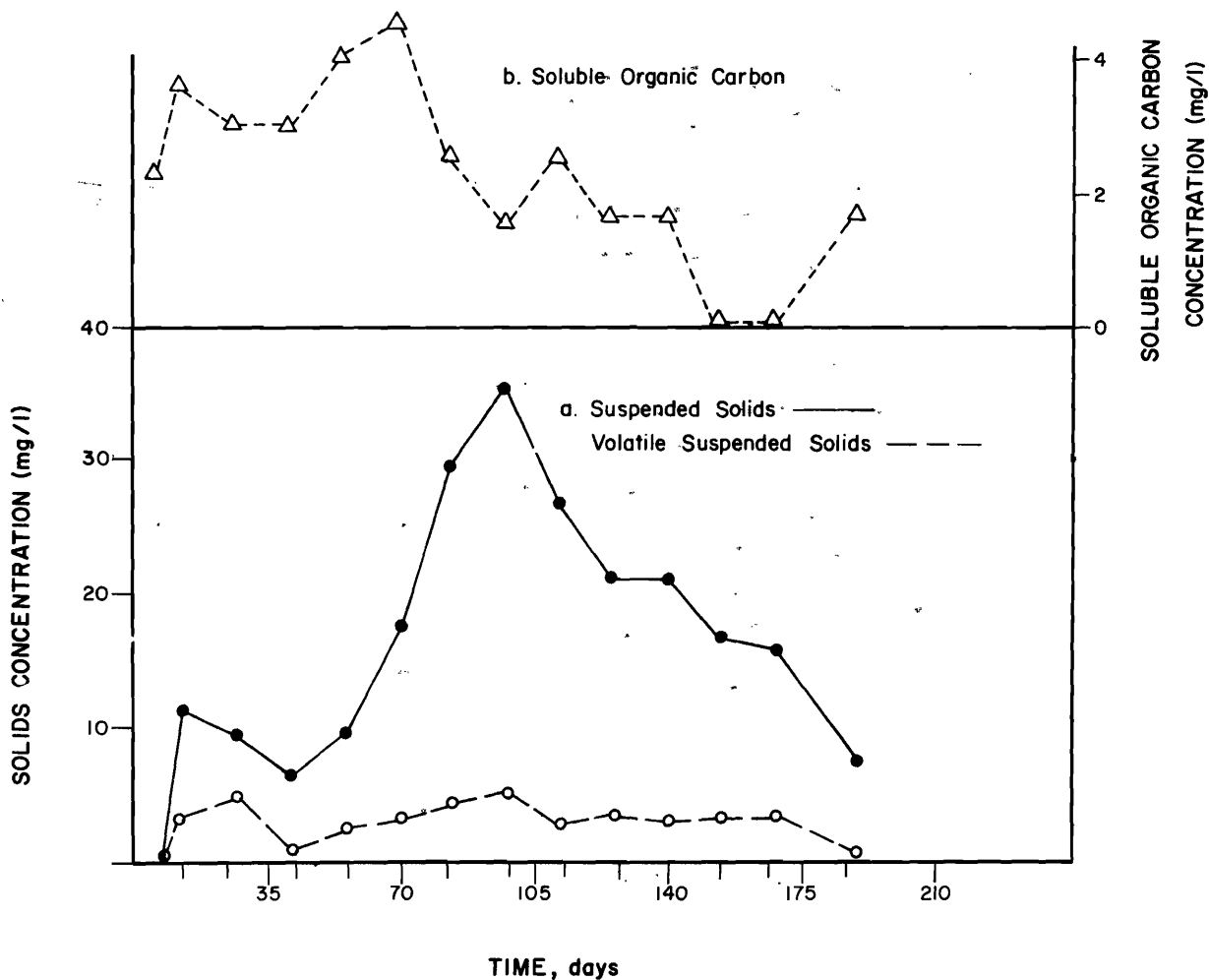
Figure 16. Phase I. Variation in suspended solids, volatile suspended solids and soluble organic carbon in the effluent of Microcosm 14.

25
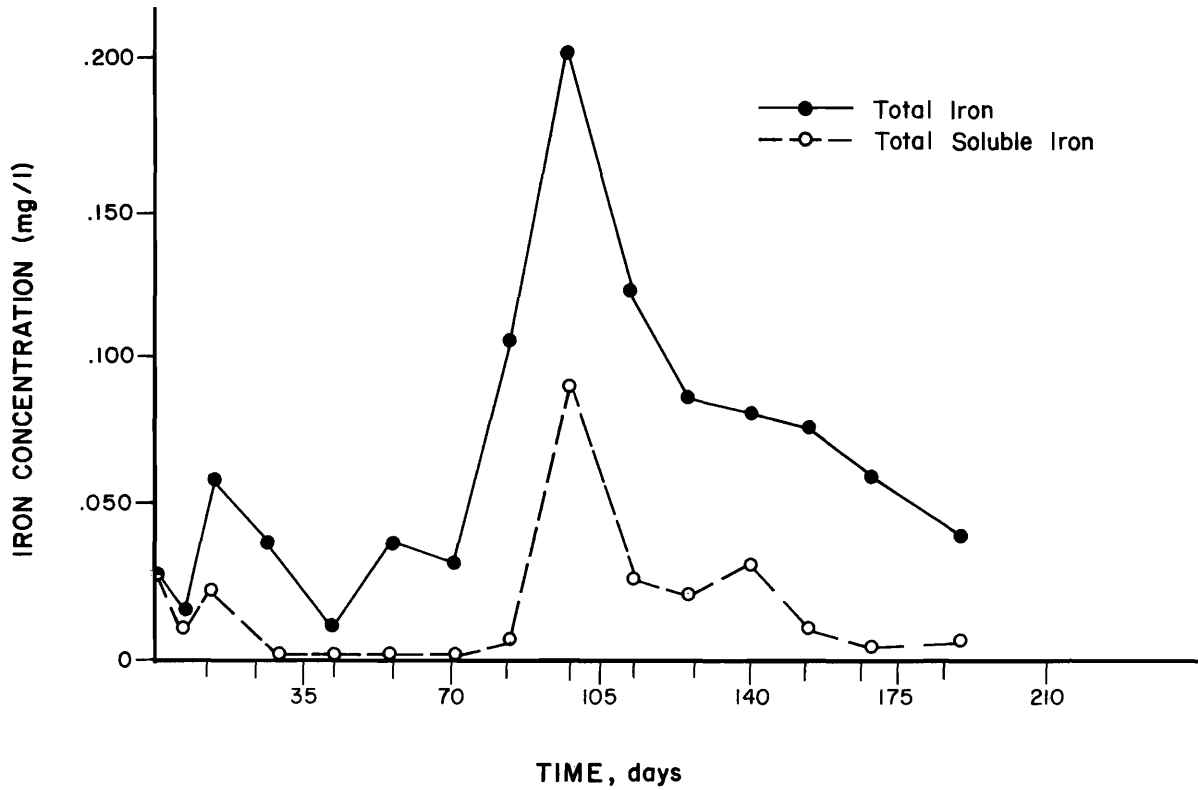
Figure 17. Phase I. Variation in total iron and total soluble iron in the effluent of Microcosm 14.
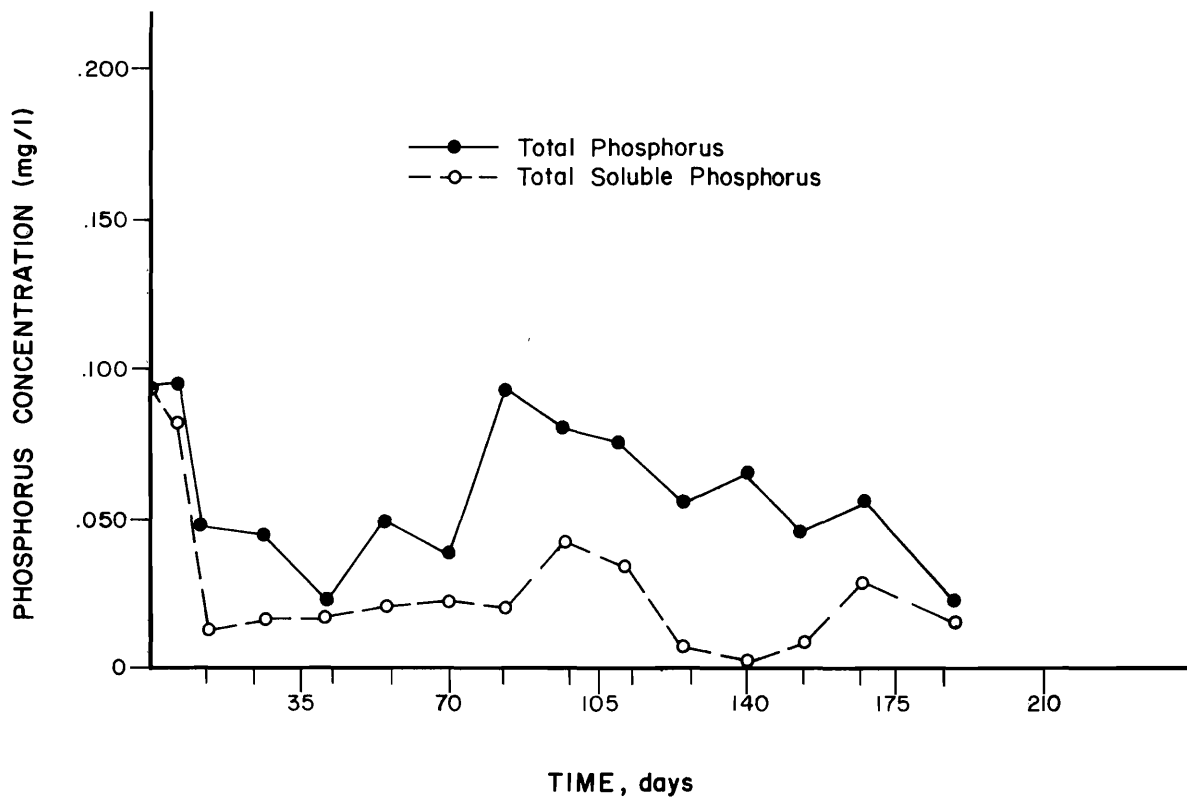
Figure 18. Phase I. Variation in total phosphorus and total soluble phosphorus in the effluent of Microcosm 14.

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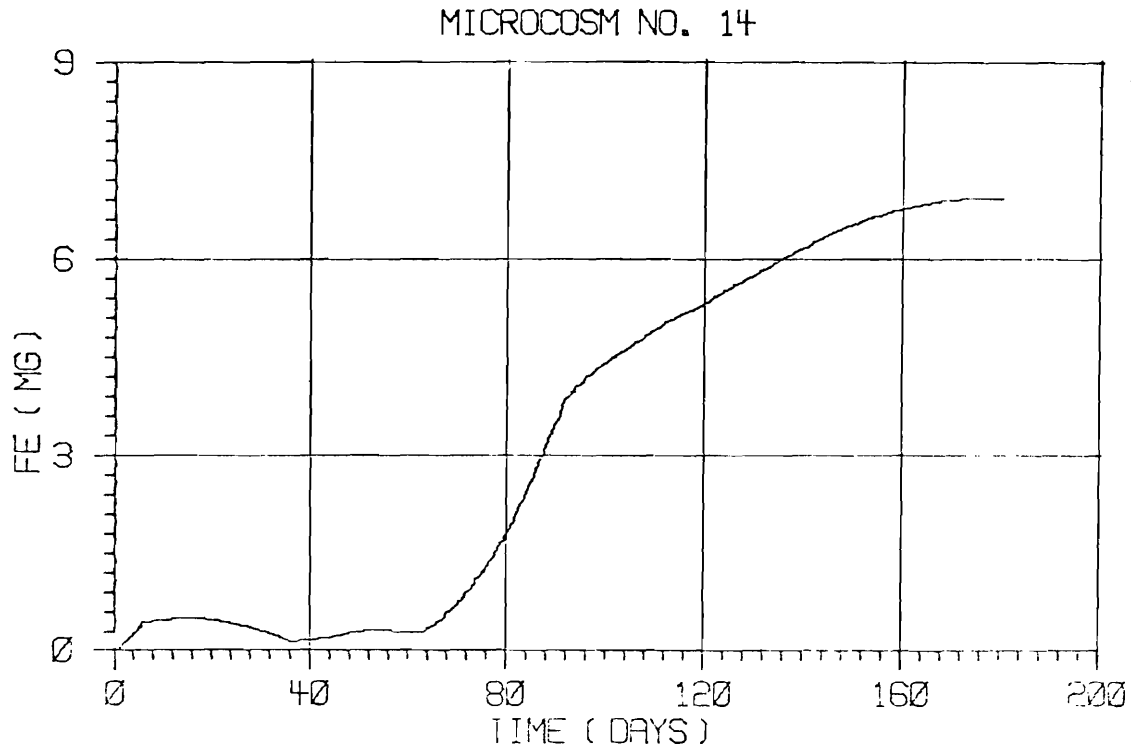
Figure 19. Phase I. Mass balance of total iron accumulated over the period of study in Microcosm 14.
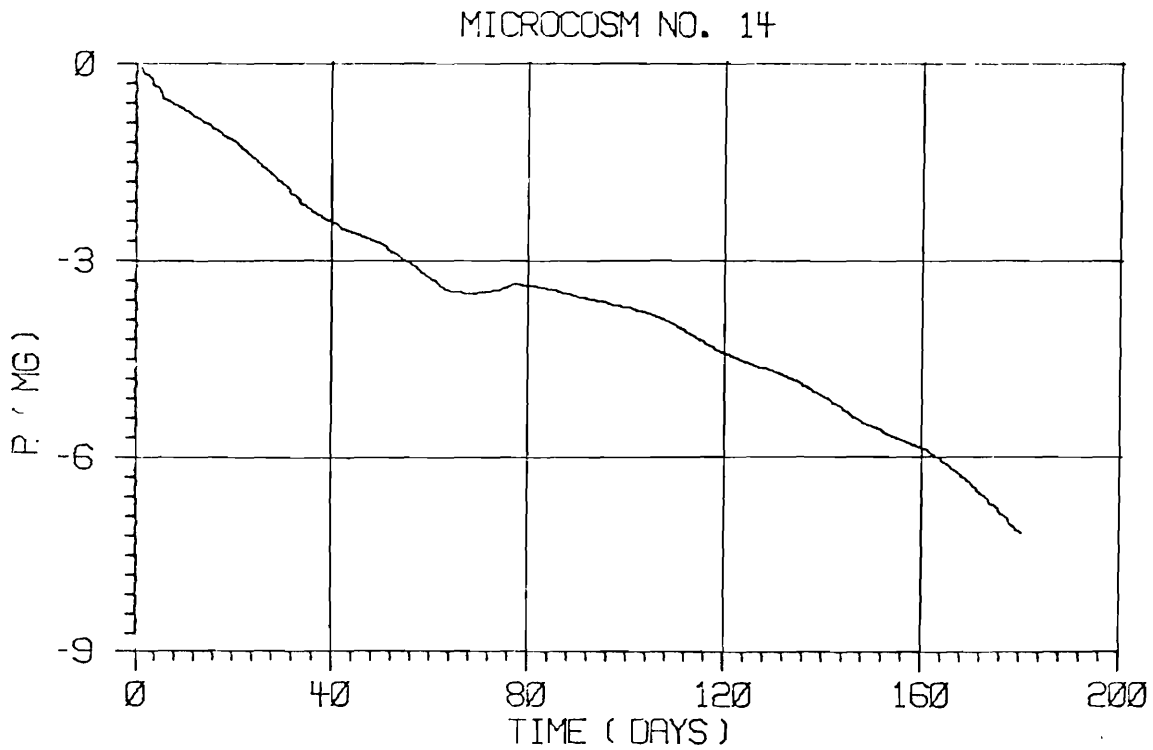
Figure 20. Phase I. Mass balance of total phosphorus lost over the period of study in Microcosm 14.

Table 5. Dry weight of periphyton after Phase I.

Microcosm Dark	Grams Dry Weight	Microcosm Light	Grams Dry Weight	Microcosm Light	Grams Dry Weight	Microcosm Light	Grams Dry Weight
1	0.42	5	10.82	9	3.42	13	15.24
2	0.53	6	0.24	10	20.08	14	11.62
3	0.40	7	9.16	11	17.59	15	14.05
4	0.56	8	8.48	12	5.71	16	8.96

DISCUSSION: PHASE I

Factorial analysis of variance (Hurst, 1972) of the iron data showed that higher total iron concentrations existed in the dark microcosm effluent (0.131 mg Fe/l) than in the light microcosm effluent (0.092 mg Fe/l). If the data from Microcosms 6, 8, and 9 (light microcosms having high turbidity and/or low wall growth) are omitted, the average effluent total iron concentration would be even less (0.052 mg Fe/l).

The fact that the dark microcosm effluent was higher in iron was expected because:

1. Chemical and physical conditions of light microcosms favored deposition of iron onto the sediments.
2. Iron taken up by organisms in the lighted microcosms was extracted predominantly by the periphyton, which did not show up in the effluent.
3. Dark microcosm with low pH values and low D.O. favored iron solubility as hydrated Fe(II).

Iron in the dark microcosm effluent had a greater soluble fraction (64 percent soluble) than the iron in the light microcosm effluent (29 percent soluble). This was expected because:

1. The dark microcosm effluent iron concentration was composed of inorganic and soluble forms (hydrated $Fe(H_2O)_x^{++}$ and Fe^{++}-hydroxo complexes) with only small quantities of Fe(II)-organic and Fe(III)-organic, and a portion of the organically complexed iron was also soluble.
2. The iron in the effluent from the light microcosm was associated with organic matter, with a portion of the particulate fraction undoubtedly being the suspended inorganic precipitate $Fe(OH)_3$.

Mass balances yielded a positive flux (output > input) of iron for the light microcosms, and iron was therefore released from the sediments. Conditions existed in these biologically active systems favoring extraction of iron from the sediments by microorganisms present within the system. Since analytical technique was unable to differentiate between the organic and inorganic (precipitate) particulate, the amount of $Fe(OH)_3$ could not be quantified. The particulate fraction is defined as that portion of the sample which would be retained upon a Whatman (GF/C) glass fiber filter.

Iron, under natural conditions, plays a major role in controlling the distribution of phosphorus between the solid (sediment) and liquid (aqueous) phases (Stumm and Morgan, 1970). Iron and phosphorus are sometimes found in nature in comparable concentrations, and any extensive complex formation involving iron and phosphorus would have a significant effect upon the distribution of iron, phosphorus, or both.

Factorial analysis of variance indicated that the total phosphorus concentration was significantly higher in the effluent from the dark microcosms (0.235 mg P/l) than in the effluent from the light systems (0.091 mg P/l). If the data from Microcosms 6, 8, and 9 of the light units were to be omitted, the average effluent concentration would be 0.048 mg P/l. Phosphorus follows almost exactly the same pattern as iron. Much lower phosphorus concentrations in the light microcosm effluent were apparently caused by:

1. Phosphorus complexing with Fe(III) iron and precipitating onto the sediments (pk_{sp} of $FePO_4$ is 23, Stumm and Morgan (1970)).
2. Phosphorus being taken up by the periphyton and thus removed from the aqueous phase.
3. Phosphorus forming insoluble compounds with other elements and precipitating.

As with the case of iron, the fraction of total phosphorus which is soluble is much higher in the dark microcosms (74 percent) than in the light microcosms (27 percent). In the dark microcosm effluent, essentially all of the soluble total phosphorus was inorganic (97 percent orthophosphate). In the light microcosm effluent, the phosphorus (again as was the case with iron) was associated mostly with the particulate phase.

Phosphorus and iron have a molecular weight ratio of P:Fe of 0.56:1. The average influent P:Fe ratio to all the microcosms was 2.8:1 (0.093 mg P/l to 0.033 mg Fe/l). The effluent from the dark microcosms had a P:Fe ratio of 1.79:1 (0.235 mg P/l to 0.131 mg Fe/l) and the effluent from the light microcosms had a P:Fe ratio of 0.99:1 (0.091 mg P/l to 0.092 mg Fe/l). Both phosphorus and iron were released from the sediments in the dark microcosms; this release was at a lower ratio than was present in the input medium. Phosphorus was sorbed to the sediments and iron was released from the sediments in the light microcosms.

Considering the light microcosms only, no definite pattern of iron and/or phosphorus could be attributed specifically to one of the variables (NO_3-N, Hg, or lighting scheme). The only apparent pattern seemed to be the equal effluent concentrations of iron and phosphorus. The effluent total iron concentration was significantly higher in Microcosms 6,

8, and 9 (0.176, 0.204, 0.260 mg Fe/l) than the average of all other light units (0.052 mg Fe/l). The effluent total phosphorus concentrations (0.172, 0.210, 0.280 mg P/l) were also higher than the average of the other light units (0.048 mg P/l). The consistent one to one relationship (Figure 21) between total iron and total phosphorus in the lighted microcosms indicated an interaction between these elements. Since a large portion of iron and phosphorus was particulate (71 percent iron and 73 percent phosphorus), the particulate phase was examined and an approximate one to one relationship was found (Figure 22). Because of the high productivity of the light units, it is concluded that the causes for these similar mechanisms for distribution of iron and phosphorus were primarily biologically instigated.

The other phases (dark: total, particulate, soluble; and light: soluble) exhibited different patterns of distribution (Figure 23), with iron found at higher concentrations than phosphorus.
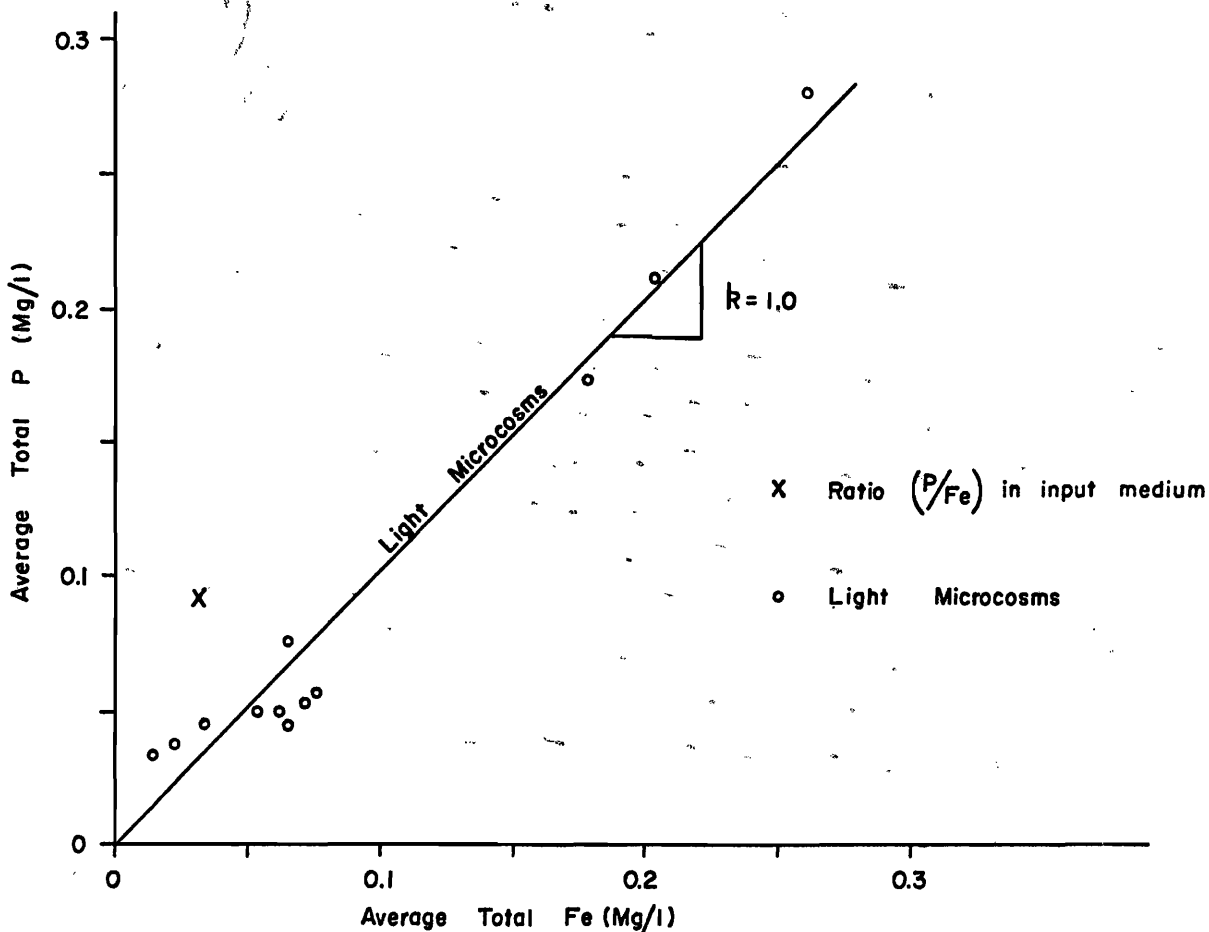
Figure 21. Phase I. Relationship of total iron and total phosphorus in the light microcosms.
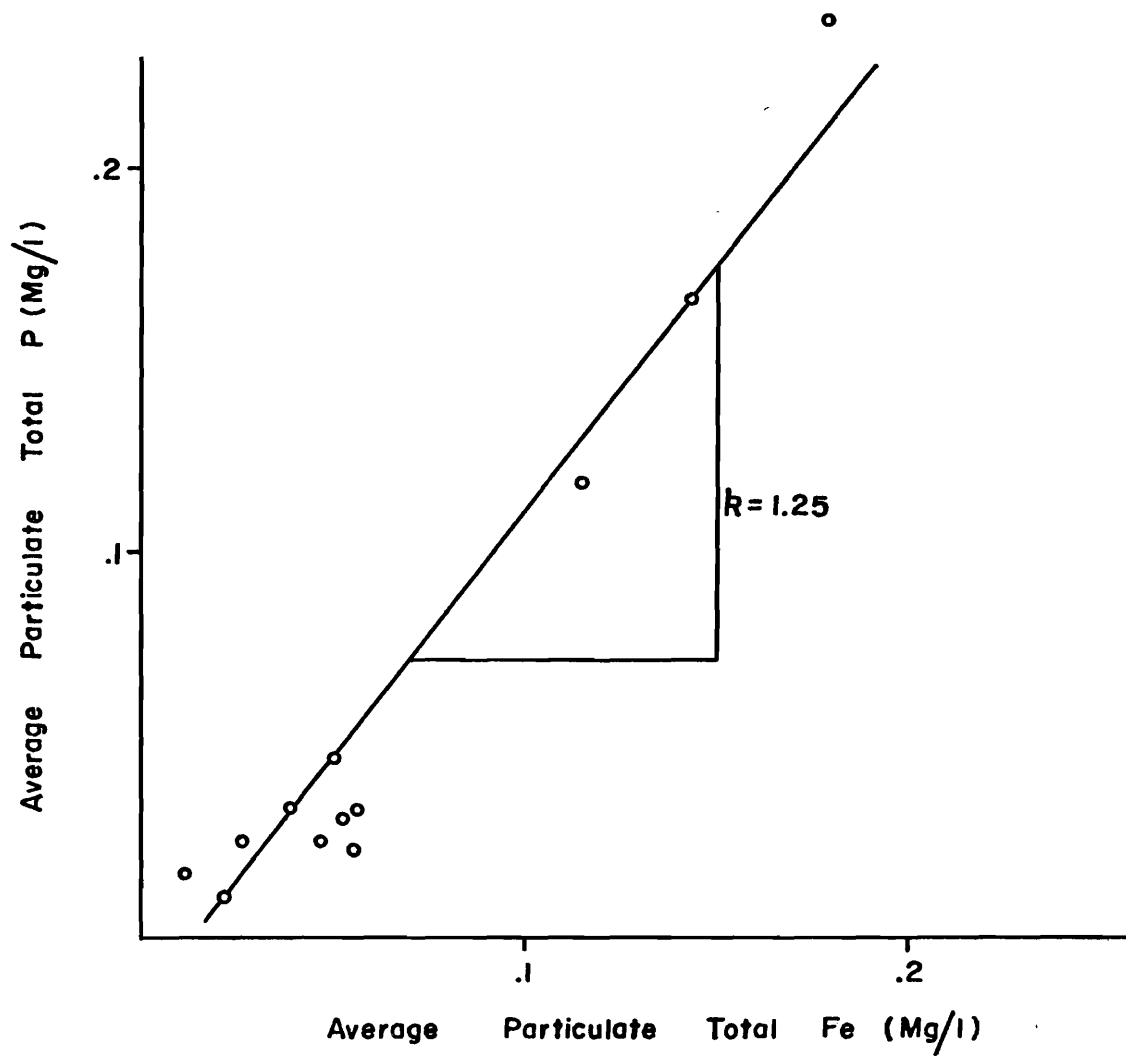
Figure 22. Phase I. Relationship of particulate iron and phosphorus of the light microcosms.
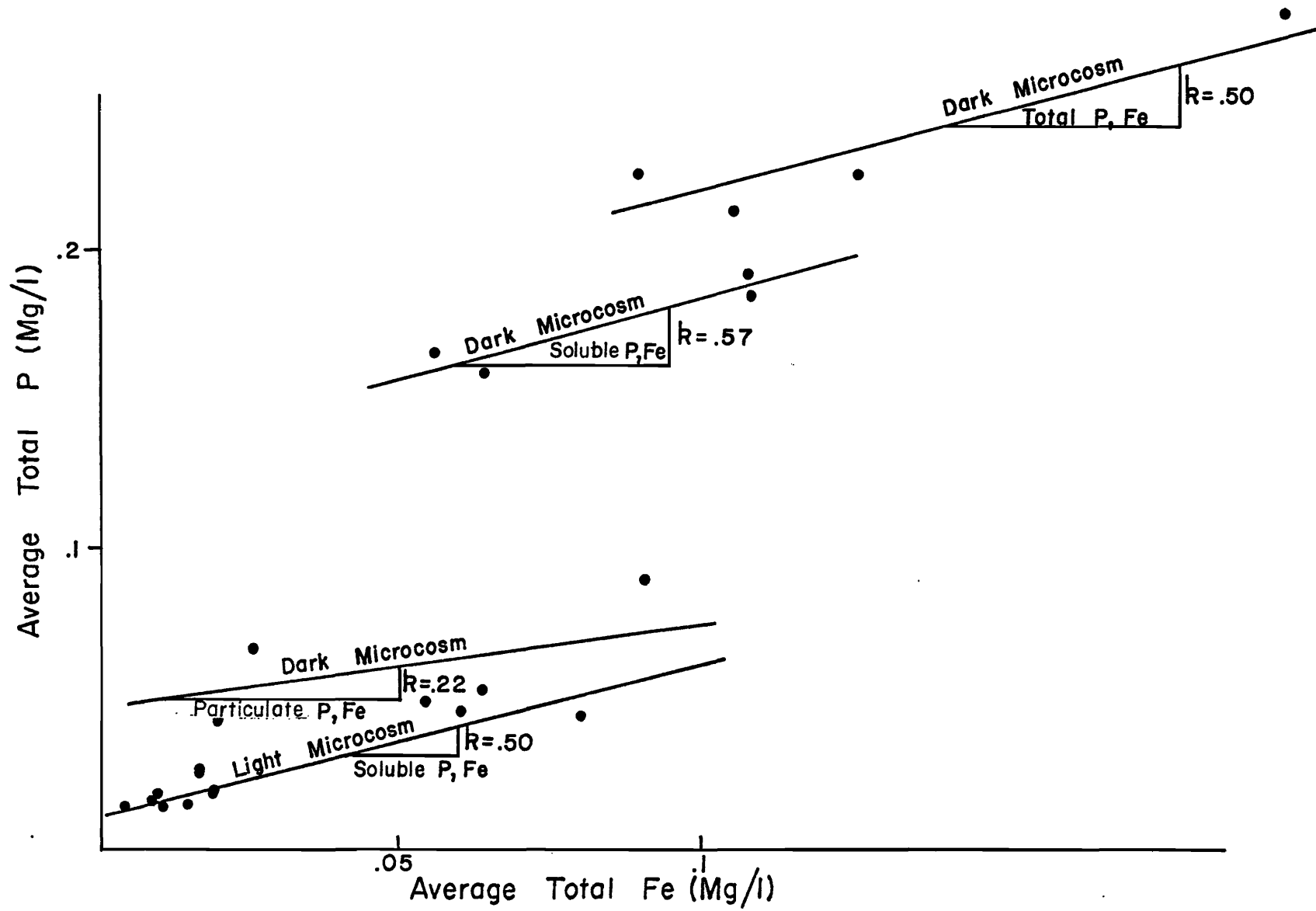
Figure 23. Phase I. Relationship between iron and phosphorus which did not exhibit distribution.

During the second phase of the study the microcosms were operated 175 days and the second phase was designed specifically to make iron a variable (variables: light, iron, and time). Notes and observations for Phase II are listed in Table 6. There was no visible growth in the dark units, therefore, observations concerning algae and microcosm appearance were limited to the light units unless otherwise noted. Two accidents occurred, both concerning dark units: (1) day 60, Microcosm 1: a major acid spill (acid from gas trap siphoned backwards into the unit) which required four days to get back into operation, (2) day 78, Microcosm 3: minor acid spill, neutralization was not required. On day 115 NO_3-N (10 mg N/l) was eliminated from the nutrient feed medium.

Nitrate Nitrogen Perturbation

Average effluent nitrate nitrogen concentrations were plotted vs. time for the dark units (Figure 24) and the light units (Figure 25) in order to assess the effects that the nitrogen perturbation had upon the microcosms. The nitrate nitrogen input was stopped on day 115 and the concentration of nitrate nitrogen in the microcosms dropped off dramatically. Hydraulically, the nitrate nitrogen decrease in concentration was calculated according to the formula:

$$\ln \frac{C}{C_o} = -0.1 \, t$$

in which

C_o = nitrate nitrogen concentration at day 115, mg NO_3-N/l

C = nitrate nitrogen concentration after t days, mg NO_3-N/l

t = time, days

This decrease in concentration is shown on Figures 24 and 25. The correspondence of the curves showed that the microorganisms in both the light and dark units had no effect upon the nitrate nitrogen concentration until the concentration was below 2 mg NO_3-N/l (approximately day 127).

In the dark microcosms (Figure 24, after day 127), the concentration of aqueous phase NO_3-N was lower than was predicted. This difference could be

rationalized by denitrification (a biochemical reduction where nitrate or nitrite is converted to a gaseous form of nitrogen, i.e. N_2) occurring in the dark units.

In the light microcosms (Figure 25, after day 127), the concentration of aqueous phase NO_3-N was also lower than was predicted. This difference could be rationalized by nitrogen assimilation (incorporation of nitrogen compounds into the cells of living organisms) occurring in the light units.

Statistical Analysis of Variance

Factorial analysis of variance (Hurst, 1972) isolated each response parameter (nutrient, gas, accumulated nutrient and accumulated gas measurements) and compared them for the different treatments and the different possible combinations of treatments (Table 7). For example, treatment "light only" represented a comparison of the parameter means for the two light schemes: all dark vs. all light. The treatment "time only" would be a comparison of all microcosms for the initial and the 12 interval analytical measurements. The treatment "light + iron" represented a comparison of the parameter averages for dark, 0 Fe/l; dark, 9.9 μg Fe/l; dark, 33 μg Fe/l; light, 0 Fe/l; light, 9.9 μg Fe/l; light, 33 μg Fe/l. Significant differences at the 1 percent and 5 percent levels were indicated (Table 7).

As in the first experiment (Phase I), the most responsive parameters were the gas and nutrient mass balances. The treatments involved had significant effects upon these parameters and results were significantly different at least at the 5 percent level. In addition to these parameters, total iron and total phosphorus also showed responsiveness, and, along with solids data (S.S. and V.S.S.) and oxygen gas results, gave at least six out of seven significant occurrences. It will be the responsive parameters which will be emphasized in the results section.

Algal Community Dynamics

In order to identify and quantify organisms in the microcosms, algal counts were made (on regular analysis days) on the microcosm effluents. Counts were made using a Sedgwick-Rafter counting cell (APHA, 1971). The genera and phyla identified are

Table 6. Notes and observations, Phase II.

	1974 Date	Day No.	Observation[a]
	Jan. 17	1	Start of Experimental Run II.
	25	9	Appearance of insect burrows in sediment.
	Feb. 1	13	Starting to show green tint. No. 7, No. 8 growth on walls. No. 7 filamentous algae near stirring bar.
	20	32	Stirring bar dishes completely covered by an algal mat, filamentous.
	Mar. 12	55	Two outside horizontal lights replaced.
	13	56	Streaks of lighter colored algae on walls.
	14	57	No. 6 darkest green of all units; No. 7 exhibited light green splotches.

Acid Spill━━━━━▶	17	60	No. 1 acid spill into unit from gas trap.
			  A. Effluent pH 2.52.
			  B. Unit No. 1 opened to the atmosphere; neutralized with 63 ml 1 N NaOH; buffered with a 100 ml solution of distilled water containing 4 g NaHCO$_3$.
			  C. Allow unit to stabilize.

	18	61	No. 1
			  A. Neutralize again with 360 ml 1 N NaOH.
			  B. Buffer with 100 ml solution of distilled water containing 10 g NaHCO$_3$.
			  C. Close system to atmosphere.

	19	62	No. 1
			  A. Open system, add 170 ml 1 N NaOH.
			  B. Buffer with 100 ml solution containing 10 g NaHCO$_3$.

	20	63	No. 1 A. Close system to atmosphere.
	21	64	No. 1 unit gas tube disconnected during the night, reconnect and level. No. 5 small sheets of algae in effluent No. 6 blotches becoming very dark green. No. 8 band of brown growth near top.
	22	65	Light units darker in color towards the top; lighter in color in the bottom third of the units.
	24	67	No. 1 effluent has brown-yellowish tinge.
	25	68	No. 1 gas tube slipped off; reconnected.
	29	72	No. 1 leak in gas tube; plugged.
	31	74	Electricity to microcosm room off; switched on. No. 1 pH check 6.29.
Acid Spill━━━▶	Apr. 4	78	No. 3 small quantity of acid into unit while changing medium.
	16	90	No. 3 gas tube disconnected during the night; reconnected. No. 3 gas tube off again; reconnected.
	17	91	No. 3 gas tube off again; reconnected.
	22	96	Electricity off; reset circuit breakers.
	May 6	110	Temperature differences between effluent of microcosms; heaters and fans adjusted.
N input changed━━━▶	11	115	10 mg N/l feed discontinued.
	June 17	152	No. 3 liquid level low; added additional medium; 90 ml gas taken out of unit to level gas bulb.
	July 10	175	End of experimental run II.

[a]General observations made on lighted microcosms (Nos. 5-8) unless otherwise noted.
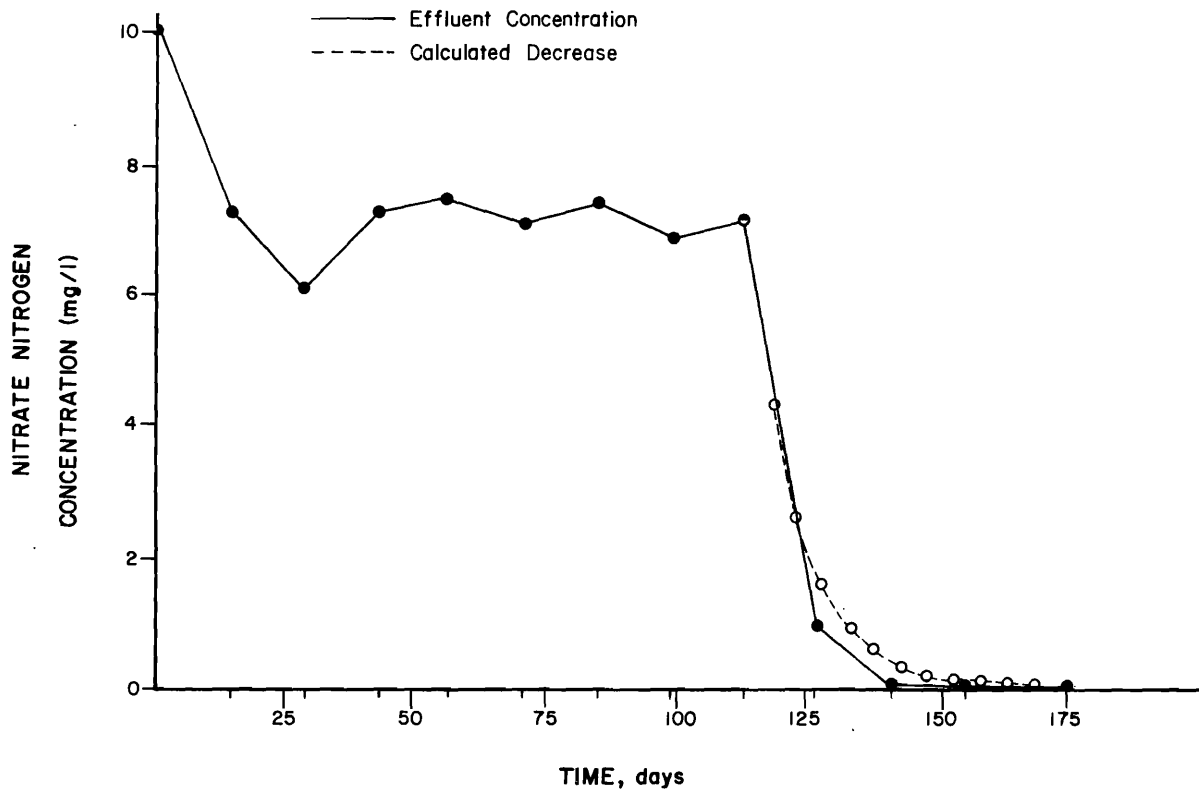
Figure 24. Phase II. Average variation of nitrate nitrogen in the effluent of the dark microcosms (fluxes due to acid spills into the units not included).
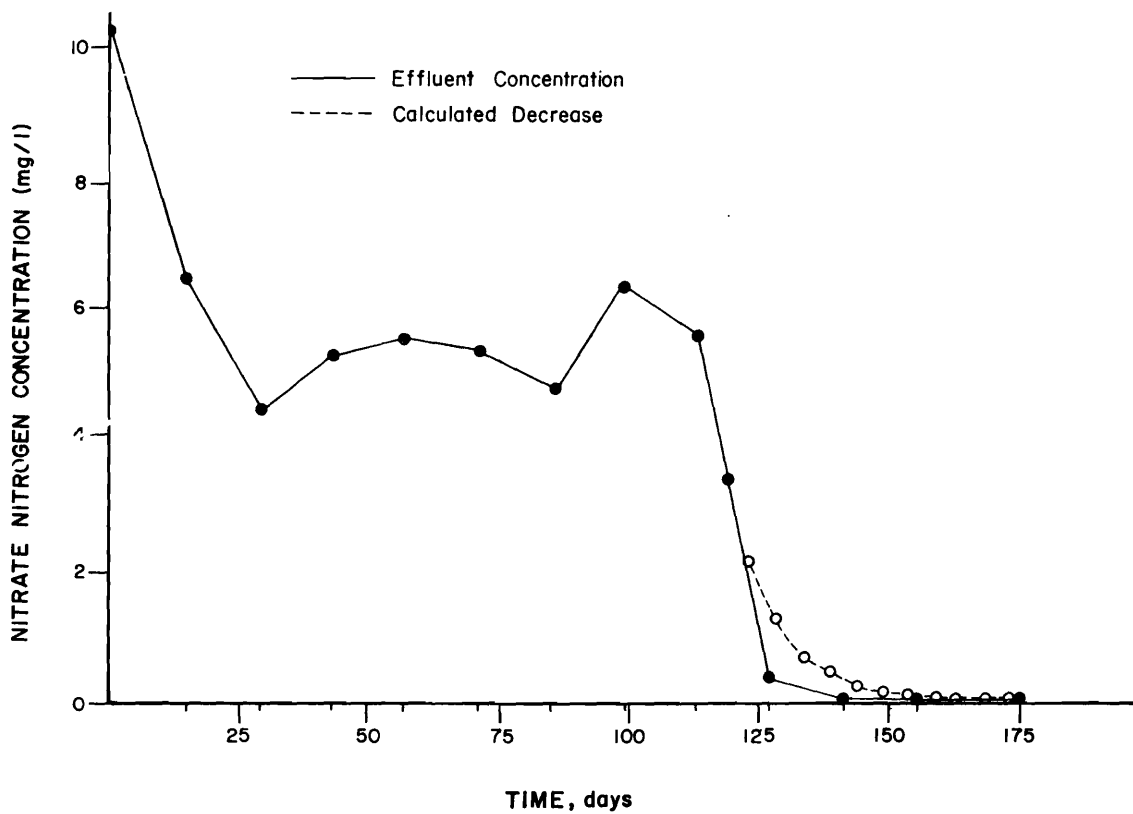
Figure 25. Phase II. Average variation of nitrate nitrogen in the effluent of the light microcosms.

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listed in Table 8. All counts from the light units and those counts from the dark units which were > 100 cells/ml are listed in Table 9. The distilled water which was used for preparation of the input media was filtered through 0.45 μ MF Millipore filters, and periodic counts of input media yielded zero counts. Initial conditions in the aqueous portion of each unit were zero cells/ml. Any algae which appeared in the aqueous phase and, therefore, in the microcosm effluent came from the sediments.

Algal growth in the dark units was small and was limited to the blue-green algae *Lyngbya sp.*, *Merismopedia sp.*, and *Microcystis incerta*. These algae, being obligate photoautotrophs, require light for growth (Prescott, 1968, 1973). Reasons for the appearance of these algae in the dark units were: (1) light entered the dark units daily when the nutrient medium was changed; therefore, limited light was available; (2) there was an error of algal cell carry-over on the Sedgwick-Rafter counting cell from

Table 7. Significant effects and interactions on response parameters as affected by the experimental treatments: Phase II.

Response Parameters	Number of Significant Occurrences	All Experimental Combinations (103)	Light Only (1)	Iron Only (3)	Time Only (12)	Light + Iron (3)	Light + Time (12)	Iron + Time (36)
Unfiltered								
Total Phosphorus	7	1	1	1	1	5	1	1
Total Carbon	1		1					
Total Iron	6	1	1	1	5	5	1	
Suspended Solids	6	1	1	1	1	5	1	
Volatile Suspended Solids	6	1	1	5	1	5	1	
Total Ferrous Iron	4	5	1		5		1	
Inorganic Carbon	1		1					
Total Organic Carbon	4	5		5	1	5		
Filtered								
Orthophosphate-P	5	1	1	1	5		1	
Total Phosphorus	6	1	1	1	1	5	1	
Nitrite-N	1				5			
Nitrate-N	4	1	1		1		1	
Ammonia-N	5	1	5		1	1	1	
Total Carbon	1		1					
Inorganic Carbon	1		1					
Total Organic Carbon	1				5			
Total Iron	3	5			5		1	
Nitrogen Gas	4	1	1		1		1	
Oxygen Gas	6	1	1	5	1	1	1	
Carbon Dioxide Gas	1		1					
Methane Gas	4	1	1		1		1	
		(95)			(11)		(11)	(33)
Phosphorus Balance	7	1	1	1	1	1	1	1
Iron Balance	6	1	1	1	1	1	1	
Carbon Balance	6	1	1	1	1	1	1	
Total Gas Volume Balance	7	1	1	1	1	1	1	5
Nitrogen Gas Weight Balance	7	1	1	1	1	1	1	5
Oxygen Gas Weight Balance	6	1	1	1	1	1	1	
Carbon Dioxide Gas Weight Balance	6	1	1	1	1	1	1	
Methane Gas Weight Balance	5	1	1	5	1		5	

[a]1,5 percent levels of significance. Greater than 5 percent are left blank.

Table 8. Algal identification, Phase II.

Count Type: Sedgwick-Rafter Counting Cell
(APHA, 1971, p. 734)
Magnification: 200X

Organism Algal Genera	Phylum
Ankistrodesmus convolutus	Chlorophyta
Ankistrodesmus falcatus	Chlorophyta
Ankyra sp.	Chlorophyta
Asterionella formosa	Chrysophyta
Chlamydomonas globosa	Chlorophyta
Chlamydomonas sp.	Chlorophyta
Chlorella sp.	Chlorophyta
Diatoms	
Navicula sp.	Chrysophyta
Rhopalodia sp.	Chrysophyta
Unknown Pennate	Chrysophyta
Unknown Centrate	Chrysophyta
Lyngbya sp. (trichomes)	Cyanophyta
Merismopedia sp.	Cyanophyta
Microcoleus sp. (trichomes)	Cyanophyta
Microcystis incerta (small colonies)	Cyanophyta
Planktosphaeria sp.	Chlorophyta
Scenedesmus abundans	Chlorophyta
Scenedesmus dimorphus	Chlorophyta
Scenedesmus obliquus	Chlorophyta
Unknown Euglenoid	Euglenophyta

previous algal counts. The large population of *Merismopedia sp.* in Microcosm 3 on day 71 (9,977 cells/ml) could not be explained. The algal counts before and after this date for Microcosm 3 were < 100 cells/ml (day 57) and 253 cells/ml (day 85).

Cell counts (Table 9) and generic diversity (Table 9, Figure 26) increased sharply in the light units until the period between the 15th and 43rd days. Diversity, as defined by the Shannon-Wiener Index (Margalef, 1968), was calculated:

$$D = -\Sigma \ \frac{n_i}{N} \ \ln \ \frac{n_i}{N}$$

in which
n_i = number in genus i
N = total number of organisms

After the 43rd day a steady decrease in effluent algal populations occurred. This decrease coincided with increases in attached algae (periphyton) on the walls and stirring apparatus (Table 6) of each light unit.

Generic diversity showed an uneven but definite decline after day 15, with all light units going from diverse algal communities (diversity ≅ 2) to communities with almost no diversity (diversity < 1). The algal populations in the light microcosms were diverse communities of green and blue-green algae

Table 9. Algal counts and generic diversity, Phase II.[a]

1974 Date	(Day)	Microcosm Number	Diversity	Dominant Organism	Number of cells/ml	Total Number of cells/ml
1/31	(15)	5	1.9	*Scenedesmus obliquus*	8,092	21,701
		6	1.6	*Scenedesmus obliquus*	11,933	23,235
		7	1.9	*Scenedesmus obliquus*	6,085	22,650
		8	1.7	*Scenedesmus obliquus*	12,157	28,262
2/14	(29)	5	1.6	*Scenedesmus dimorphus*	18,533	49,580
		6	1.6	*Planktosphaeria sp.*	36,089	76,510
		7	2.0	*Ankistrodesmus convolutus*	10,296	35,318
		8	1.3	*Scenedesmus dimorphus*	72,204	159,932
2/28	(43)	5	1.3	*Scenedesmus obliquus*	11,246	18,108
		6	1.1	*Scenedesmus dimorphus*	20,698	43,744
		7	1.7	*Scenedesmus obliquus*	3,089	9,082
		8	0.61	*Scenedesmus dimorphus*	192,614	247,869
3/14	(57)	5	1.9	*Scenedesmus obliquus*	2,323	6,558
		6	0.91	*Scenedesmus obliquus*	6,758	8,737
		7	2.0	*Ankistrodesmus convolutus*	752	2,639
		8	1.1	*Scenedesmus obliquus*	7,286	13,173
3/28	(71)	3	0.00	*Merismopedia sp.*	9,977	9,978
		4	0.61	*Microcystis incerta*	197	282
		5	1.7	*Microcystis incerta*	1,742	4,220

Table 9. Continued.

1974 Date	(Day)	Microcosm Number	Diversity	Dominant Organism	Number of cells/ml	Total Number of cells/ml
		6	1.2	*Scenedesmus obliquus*	2,402	3,575
		7	1.3	*Microcystis incerta*	2,165	4,738
		8	1.3	*Scenedesmus obliquus*	1,056	1,887
4/11	(85)	1	0.13	*Microcystis incerta*	174	179
		3	0.72	*Merismopedia sp.*	253	324
		4	1.0	*Merismopedia sp.*	169	300
		5	1.6	*Microcystis incerta*	964	2,705
		6	1.6	*Scenedesmus obliquus*	898	1,966
		7	1.4	*Microcystis incerta*	1,214	1,926
		8	1.4	*Lyngbya sp.*	198	608
4/25	(99)	2	0.88	*Microcystis incerta*	66	114
		5	1.7	*Microcystis incerta*	120	406
		6	1.3	*Scenedesmus obliquus*	195	461
		7	1.5	*Lyngbya sp.*	99	264
		8	1.3	*Microcystis incerta*	98	226
5/9	(113)	5	0.92	*Microcystis incerta*	77	111
		6	0.53	*Lyngbya sp.*	1,288	1,518
		7	0.91	*Microcystis incerta*	206	370
		8	0.78	*Lyngbya sp.*	174	275
5/23	(127)	5	0.33	*Microcystis incerta*	181	202
		6	0.49	*Lyngbya sp.*	528	656
		7	0.69	*Lyngbya sp.*	156	300
		8	0.64	*Lyngbya sp.*	206	308
6/6	(141)	1	0.65	*Microcystis incerta*	99	152
		2	0.41	*Microcystis incerta*	253	295
		3	0.95	*Microcystis incerta*	306	544
		5	0.38	*Microcystis incerta*	422	475
		6	0.63	*Lyngbya sp.*	549	765
		7	0.78	*Lyngbya sp.*	227	389
		8	0.80	*Lyngbya sp.*	190	370
6/20	(155)	3	0.54	*Microcystis incerta*	133	173
		5	0.32	*Microcystis incerta*	338	372
		6	0.7	*Lyngbya sp.*	364	543
		7	0.79	*Lyngbya sp.*	152	290
		8	0.78	*Microcystis incerta*	136	273
7/10	(175)	2	0.51	*Microcystis incerta*	106	134
		3	0.22	*Microcystis incerta*	216	229
		4	0.08	*Microcystis incerta*	252	256
		5	0.78	*Lyngbya sp.*	42	74
		6	0.69	*Microcystis incerta*	63	117
		7	0.64	*Lyngbya sp.*	226	340
		8	0.59	*Microcystis incerta*	161	225

[a]All light microcosms (Nos. 5-8) and dark microcosms (Nos. 1-4) when counts > 100 cells/ml.

[a]All light microcosms (Nos. 5-8) and dark microcosms (Nos. 1-4) when counts > 100 cells/ml.

and diatoms (by day 15). The green algae were dominate because of high light intensities which existed (Figure 7). Diatoms, which were present in small numbers, disappeared from the aqueous phase by day 43. As wall growth progressed, light penetration into the light microcosms decreased and the blue-greens, which require less light, began to dominate by day 95. *Microcystis incerta* and *Lyngbya sp.*, blue-green obligate photoautotrophs, were the predominate algae in the light unit by this time. *Microcystis incerta*, a small alga covered by a gelatinous sheath and *Lyngbya sp.*, a filamentous alga surrounded by a non-gelatinous sheath, do not contain heterocysts and are not capable of nitrogen fixation (Prescott, 1968, 1973).

Periphyton measurements were made at the termination of the experiment, and the total dry weight $9(103°C)$ of periphyton and Sedgwick-Rafter identification of attached algae are listed in Table 10. Although fungal and bacterial populations were

present, the largest proportion of biomass was algal biomass. The dominant organism present was the blue-green alga *Microcystis incerta*, whose small colonies comprised 90 - 95 percent of the material present.

Gas Analyses and Mass Balances

The microcosm units were sealed systems (Figure 5) and included a gas phase. In the first experimental run of the microcosms (Porcella et al., 1975) total gas volume gave the highest number of significant responses (14 out of 15 total combinations) of all response parameters tested. Since microorganisms react with and greatly affect the gas phase of such a closed system, microbial activity can be monitored by gas phase measurements.

The results of gas analyses (Appendix B: Analytical Results) are summarized as mass balances in Figures 27-34. In order to perform mass balances,
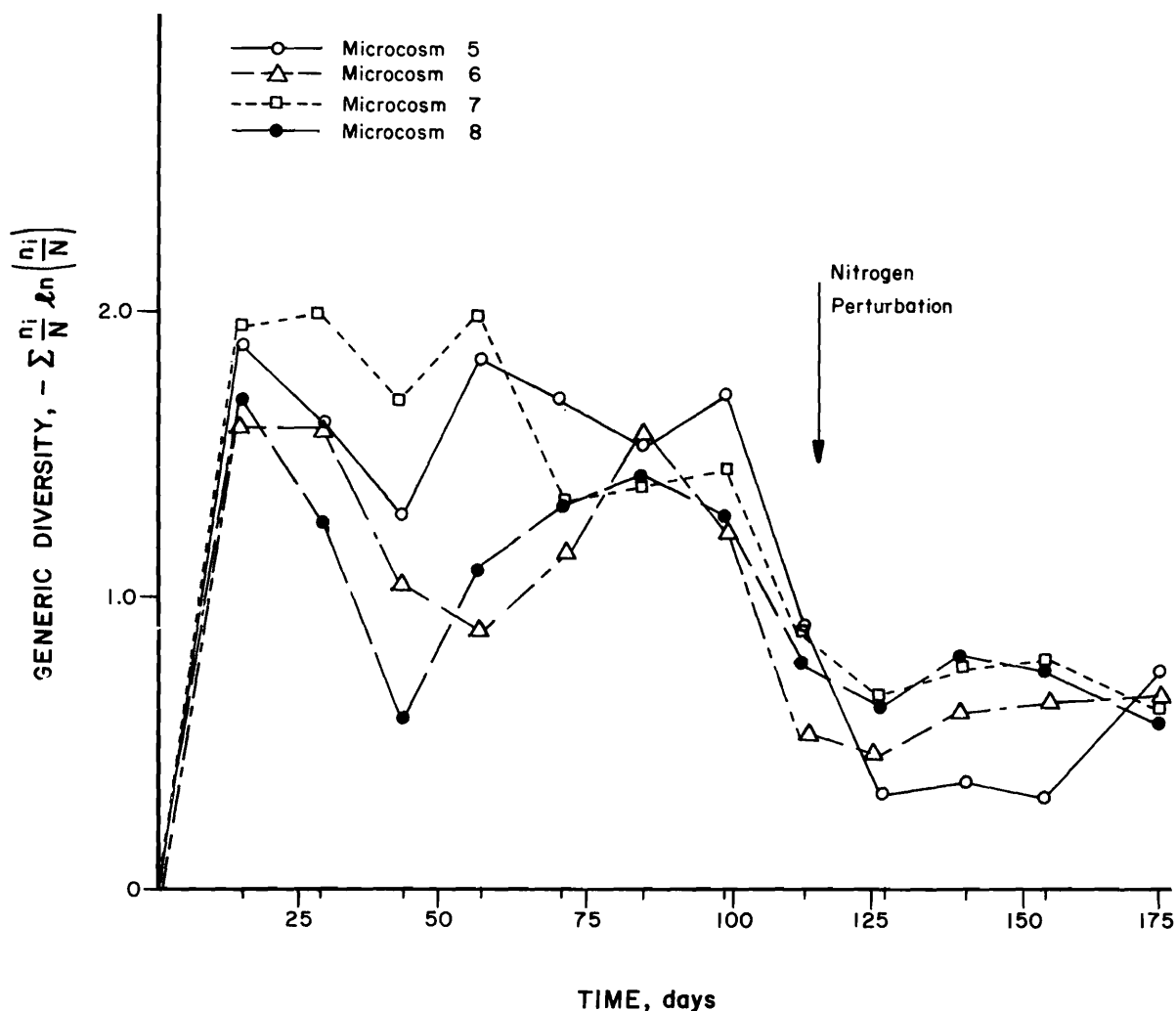
Figure 26. Phase II. Generic diversity of algal populations in the light microcosms.

input (medium) and output (effluent) water must be considered. As with the first run, the input medium was aerated for 24 hours prior to nutrient addition in order to air saturate the input medium with atmospheric gases. Gas analyses were performed on regular analysis days (every 2 weeks) by gas chromatography. Given gas input in the medium, Program Micro (Porcella et al., 1975) then utilized gas data to determine the amount of gas leaving in the effluent and therefore the amount of gas (V_{NET} ml at S.T.P., other gases in mg) accumulated in or leaving the system. Positive slopes on mass balance graphs indicated gas production within the microcosm (output > input), while a negative slope indicated utilization of gas within the system (input > output).

Dark microcosms

Total gas production was similar in all four dark units until day 60 when the acid spill occurred in No. 1 (Table 6, Figure 27). The subsequent opening up of No. 1 to the atmosphere and neutralization of the acid with NaOH severely upset the system and negated any further total gas measurements in No. 1. The high total gas production in No. 2 (Figure 28) cannot be explained. Microcosms 3 (Figure 29) and 4 (Figure 30) produced (same iron treatment) exactly the same volume of gas.

Oxygen utilization, which began immediately, was the same in all four dark units (Figures 27-30) with respect to rates and amounts. Oxygen utilization was expected in the dark microcosms, where the

absence of light prevented any algal growth. D.O. in the influent (O_2 saturated with respect to the atmosphere) was utilized by bacteria to stabilize (oxidize) any organic matter present within the system. The primary source of organic material was the sediments; however, it must be noted that the distilled water used in the daily feeds contained 1-2 mg organic carbon/l, and therefore a second source of organic material was available to bacteria in the microcosms.

The acid spills in Microcosm 1 on day 60 (Figure 27) and Microcosm 3 on day 78 (Figure 29) did not appear to affect the oxygen balances of these nearly anaerobic systems.

Carbon dioxide production proceeded at the same rate for all four dark units. Since CO_2 is an end product of bacterial stabilization of organic matter ($C(H_2O) + O_2 \xrightarrow{bacteria} CO_2 + H_2O$), CO_2 production was expected in dark units containing bacteria and organic matter. The effect of the acid spills was apparent in the carbon dioxide balances. Microcosm 1 showed a large rise in CO_2 production between days 85 and 99, over 25 days after the acid spill. This lag cannot be explained. The increase in CO_2 production in Microcosm 3 (between day 71 and day 85) coincided with the acid spill date (day 78). Disregarding these two intervals for Microcosms 1 and 3, CO_2 production (slopes on mg CO_2 vs. time graphs of Figures 27-30) proceeded at the same rate for all four dark units.

Table 10. Periphyton identification, Phase II.

Sample Date: 11 July 1974
Count Type: Sedgwick-Rafter Counting Cell (APHA, 1971)
Magnification: 200X
Concentration Factor: 1X

Organism Algal Genera	Microcosm, % of Field								Phyla
	1	2	3	4	5	6	7	8	
Anabaena sp.					Occ.[a]				*Cyanophyta*
Chlamydomonas sp.					3%		Occ.	Occ.	*Chlorophyta*
Fragilaria sp.						0.5%			*Bacillariophyta*
Lyngbya sp. (trichomes)					1.5%	2.5%	5%	10%	*Cyanophyta*
Microcystis incerta (small colonies)					95%	95%	95%	90%	*Cyanophyta*
Scenedesmus obliquus					0.5%	2%		Occ.	*Chlorophyta*
Dried Weight (103°C), grams Wall Scrapings After Phase II	0.24	0.46	0.52	1.13	11.01	9.69	10.62	11.31	

[a]Occ. = Occasional.
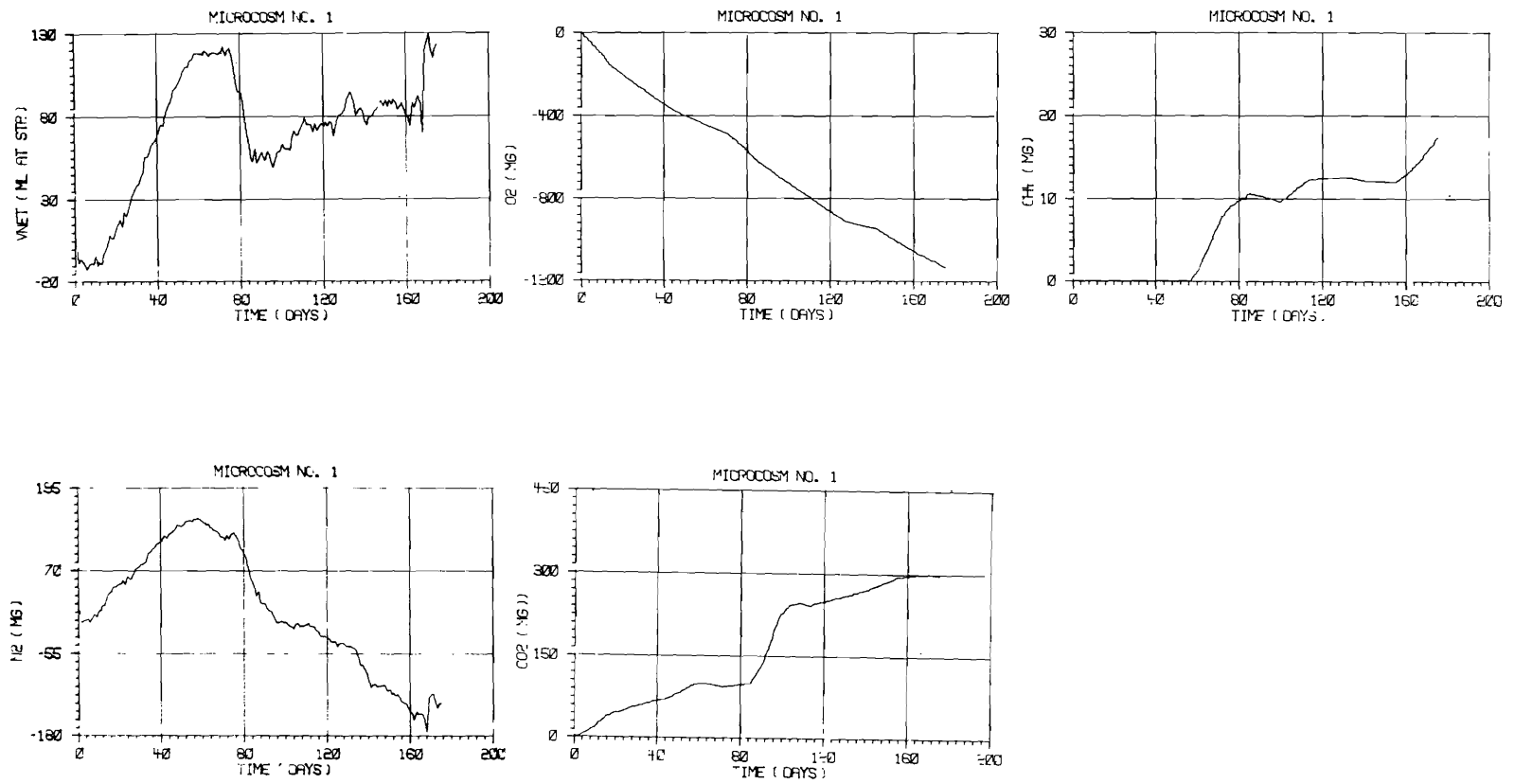
Figure 27. Mass balances of gases detected in Microcosm 1 accumulated over Phase II. V_{NET} is total gas production (ml at S.T.P.); other gases in mg.

MICROCOSM NO. 2

MICROCOSM NO. 2

MICROCOSM NO. 2

MICROCOSM NO. 2

MICROCOSM NO. 2

VNET (ML AT STP)

CO2 (MG)

CH4 (MG)

N2 (MG)

CO2 (MG)

TIME (DAYS)
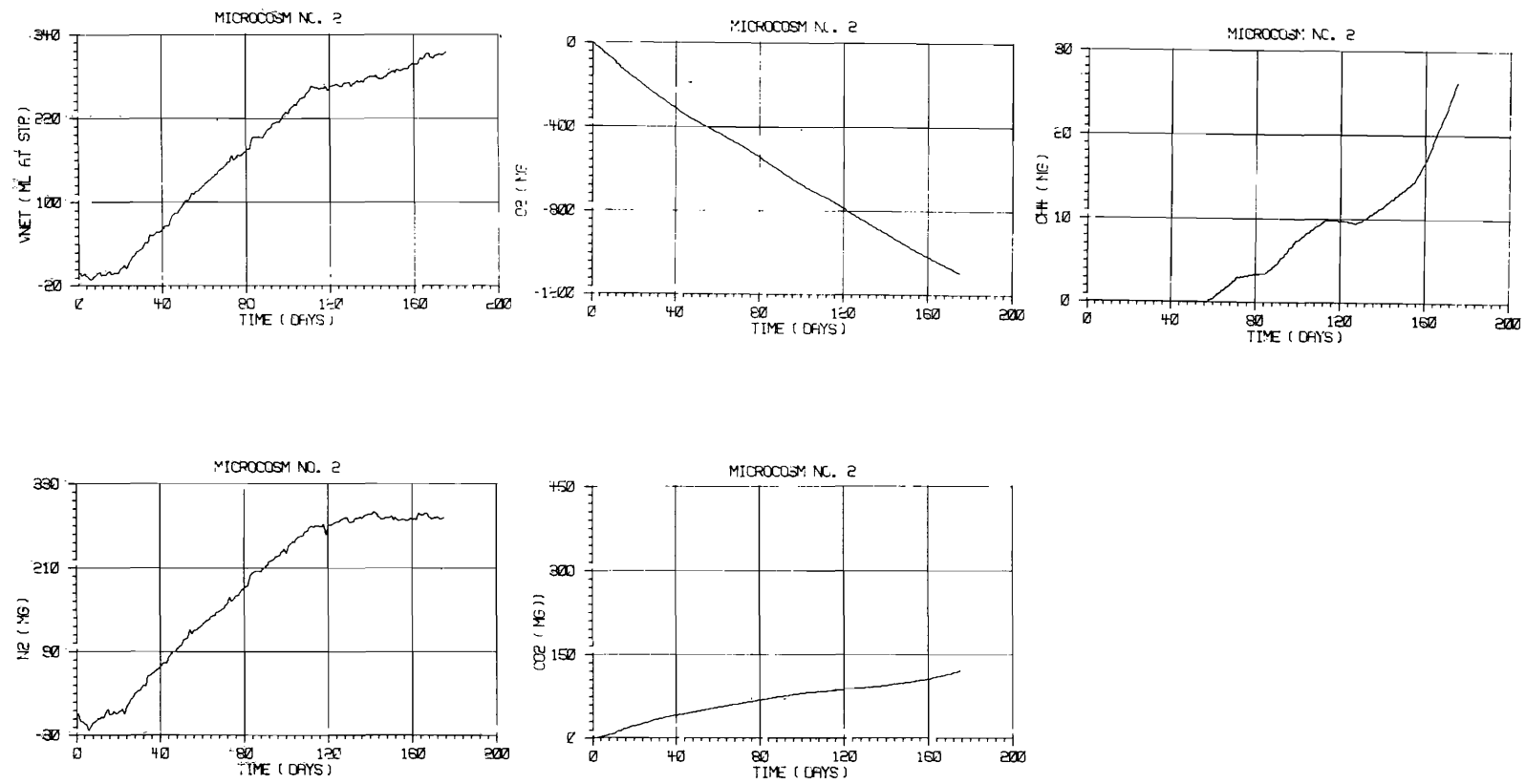
Figure 28. Mass balances of gases detected in Microcosm 2 accumulated over Phase II. V_{NET} is total gas production (ml at S.T.P.); other gases in mg.
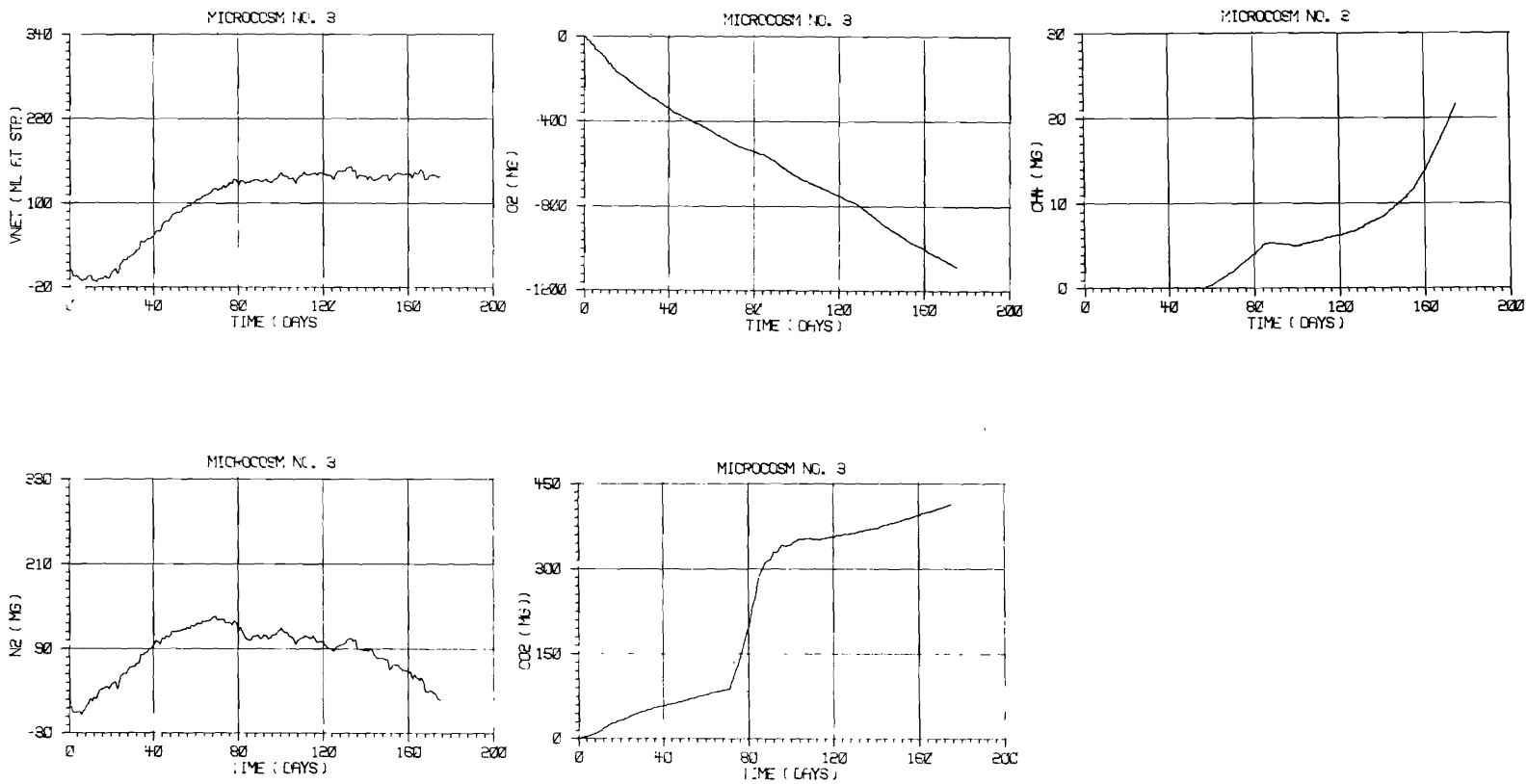
Figure 29. Mass balances of gases detected in Microcosm 3 accumulated over Phase II. V_{NET} is total gas production (ml at S.T.P.); other gases in mg.

MICROCOSM NO. 4

MICROCOSM NO. 4

MICROCOSM NO. 4

340

220

VNET (ML AT STP)

100

-20

0   40   80   120   160   200
TIME (DAYS)

0

-400

O2 (MG)

-800

-1200

0   40   80   120   160   200
TIME (DAYS)

30

CH4 (MG)

20

10

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 4

MICROCOSM NO. 4

330

210

N2 (MG)

90

-30

0   40   80   120   160   200
TIME (DAYS)

450

300

CO2 (MG)

150

0

0   40   80   120   160   200
TIME (DAYS)
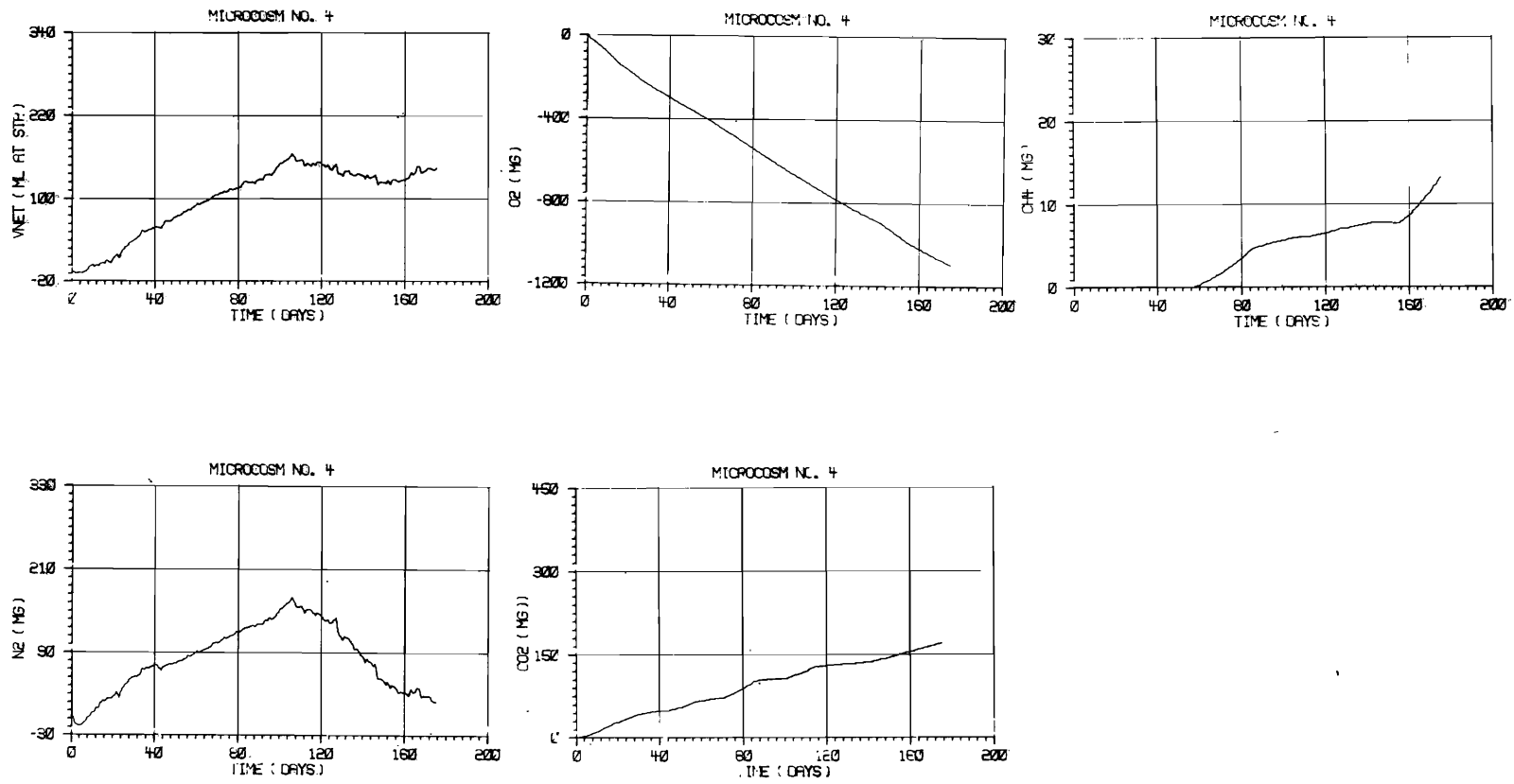
Figure 30. Mass balances of gases detected in Microcosm 4 accumulated over Phase II. V_{NET} is total gas production (ml at S.T.P.); other gases in mg.

Table 11. Summary of wall growth and oxygen production in the light microcosms, Phase II.

Average Cell Count After Day 70 cells/ml	Microcosm Number	O_2 Production mg	Dry Weight (103°C) Wall Scrapings g	Iron Treatment μg Fe/l
1200	6	1865	9.69	9.9
1077	7	1782	10.62	33.
1071	5	1487	11.01	0
522	8	1316	11.31	33.

Methane (CH_4) production occurred in all the dark units but did not begin until approximately day 60. The quantities of CH_4 produced were much less than that reported by Porcella et al. (1975) and were unlike Phase I in that CH_4 production began by approximately day 20 in the first experiment.

Ethylene (C_2H_4) was not detected in the Phase II dark microcosms; again, unlike Phase I where C_2H_4 was detected on approximately day 80.

It may be that the higher NO_3-N inputs in Phase II accounted for these observations.

Light microcosms

Total gas production was an order of magnitude greater when light was added to each iron treatment (Figures 31-34). The light systems had the only significant production of algae (Table 9) and this microbial activity was expected to increase gas production to these high levels. O_2 was produced and CO_2 was utilized in each light unit, and only Microcosm 5 showed a production of 23 mg of CO_2 between days 57 and 71 (Figure 31). This production was contradicted by the fact that algal populations increased approximately 3400 cells/ml during that same interval for Microcosm 5 (Table 9). Again, except for this interval, comparison of mg CO_2 vs. time slopes yielded identical rates and amounts of CO_2 utilization for all four light units.

O_2 production showed a short lag ($\simeq$ 16 days) at the beginning of operation, indicating that the algal populations were not high enough to affect the D.O. levels in the newly established closed systems until two weeks after the systems were sealed. Cumulative O_2 production was higher in Microcosms 6 (9.9 μg Fe/l) and 7 (33 μg Fe/l) and was lower in Microcosms 5 (0 μg Fe/l) and 8 (33 μg Fe/l) (Table 11). The low O_2 production in Microcosm 8 could only be explained by the increase in algal population to 247,869 cells/ml by day 43 (Table 9). This large population of cells was three to seven times the maximum cell count in any of the other light units. After day 71 (and therefore for the last 104 days of the experiment) the counts in No. 8 were the lowest of all the light units (Table 9), and could have

accounted for Microcosm 8 producing the least amount of O_2.

Periphyton measurements as dry weight (103° C) of wall scrapings were higher in Microcosms 8 and 5 and lower in Microcosms 7 and 6 (Table 11).

Microcosm 6 with the lowest wall growth yielded the highest O_2 production while Microcosm 8 with the highest wall growth yielded the lowest O_2 production. High wall growth reduced light penetration into the units and decreased aqueous phase populations; therefore, playing a major role in determining levels of O_2 production. Different iron treatments did not affect gas parameters in the microcosms.

Methane (CH_4) production occurred in all the light microcosms but did not begin until approximately day 80. The quantities of CH_4 produced were less than in the study by Porcella et al. (1975). In Phase I, CH_4 production began almost immediately.

Ethylene (C_2H_4) was not detected in the Phase II light microcosms. This differed from Phase I where C_2H_4 was detected on approximately day 80. As was the case in the dark microcosms, the high NO_3-N input may have been responsible for these observations.

Water Chemistry

Dark microcosms

As was the case in the first experiment, the dark units simulated the hypolimnetic region of a eutrophic impoundment. D.O. (Figure 35(a)) and pH (Figure 35(b)) conditions were similar to the first run and the reduced form of iron, Fe(II), was favored. Organically bound iron was possible due to the presence of biological material (V.S.S., Figure 36(a)) and soluble organic material (S.O.C., Figure 36(b)). Figures 35-38 represent the average values for all dark units, but these values do not include data which resulted from acid spills (Microcosm 1, day 60, and in Microcosm 3, day 78, Table 6). Data points at time zero represent the average nutrient medium input
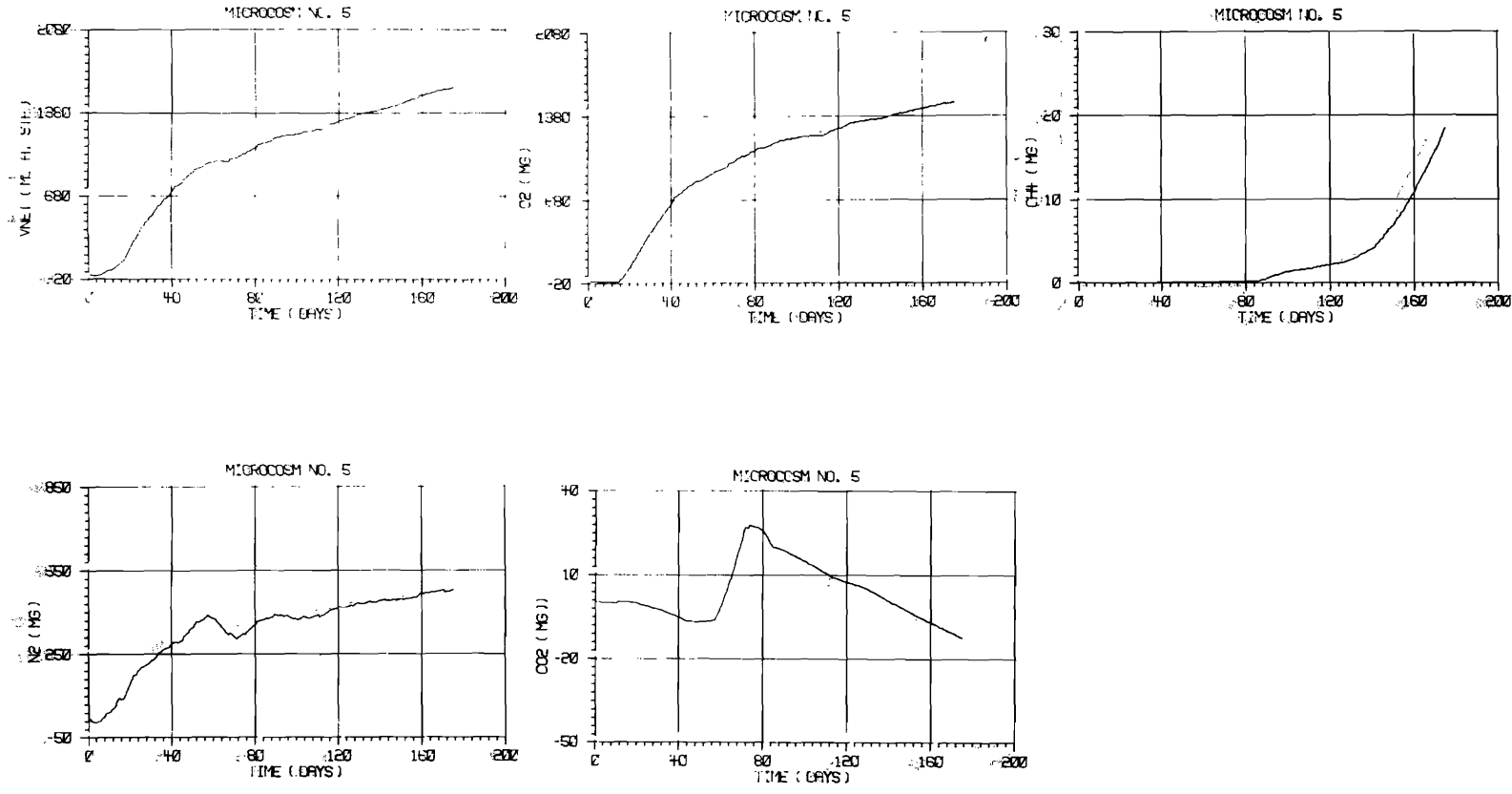
Figure 31. Mass balances of gases detected in Microcosm 5 accumulated over Phase II. V_{NET} total gas production (ml at S.T.P.); other gases in mg.
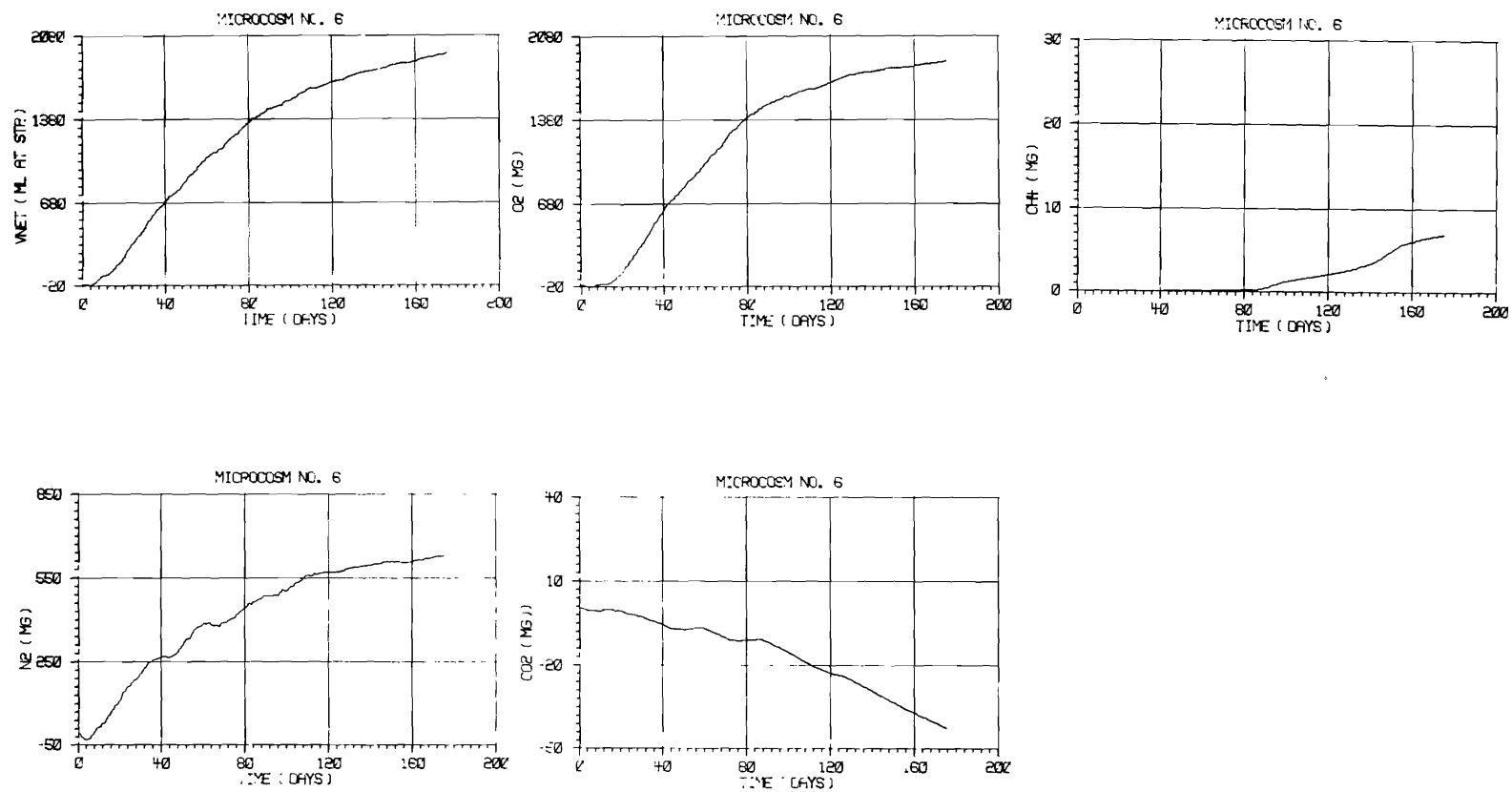
Figure 32. Mass balances of gases detected in Microcosm 6 accumulated over Phase II. V_{NET} total gas production (ml at S.T.P.); other gases in mg.
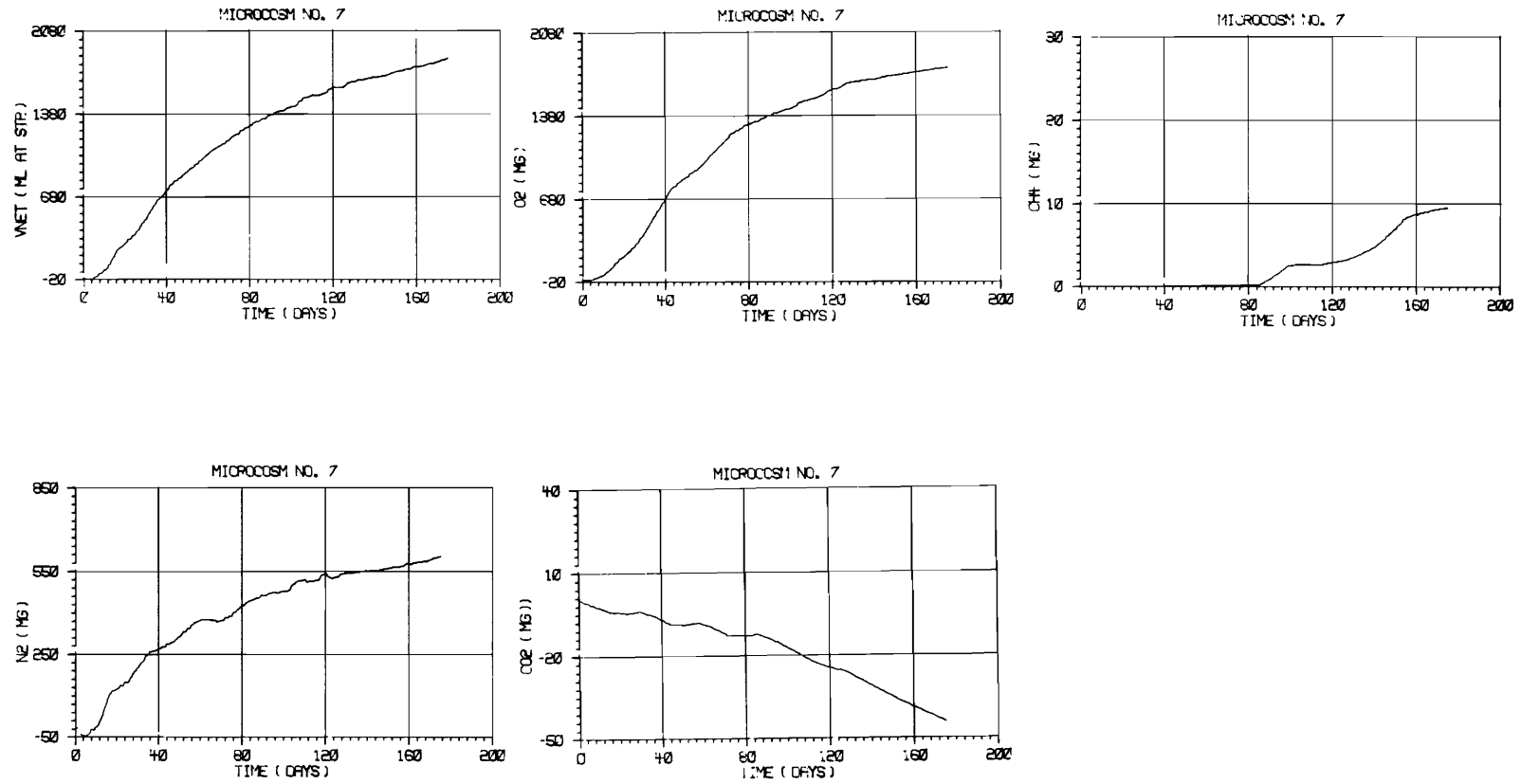
Figure 33. Mass balances of gases detected in Microcosm 7 accumulated over Phase II. V_{NET} total gas production (ml at S.T.P.); other gases in mg.
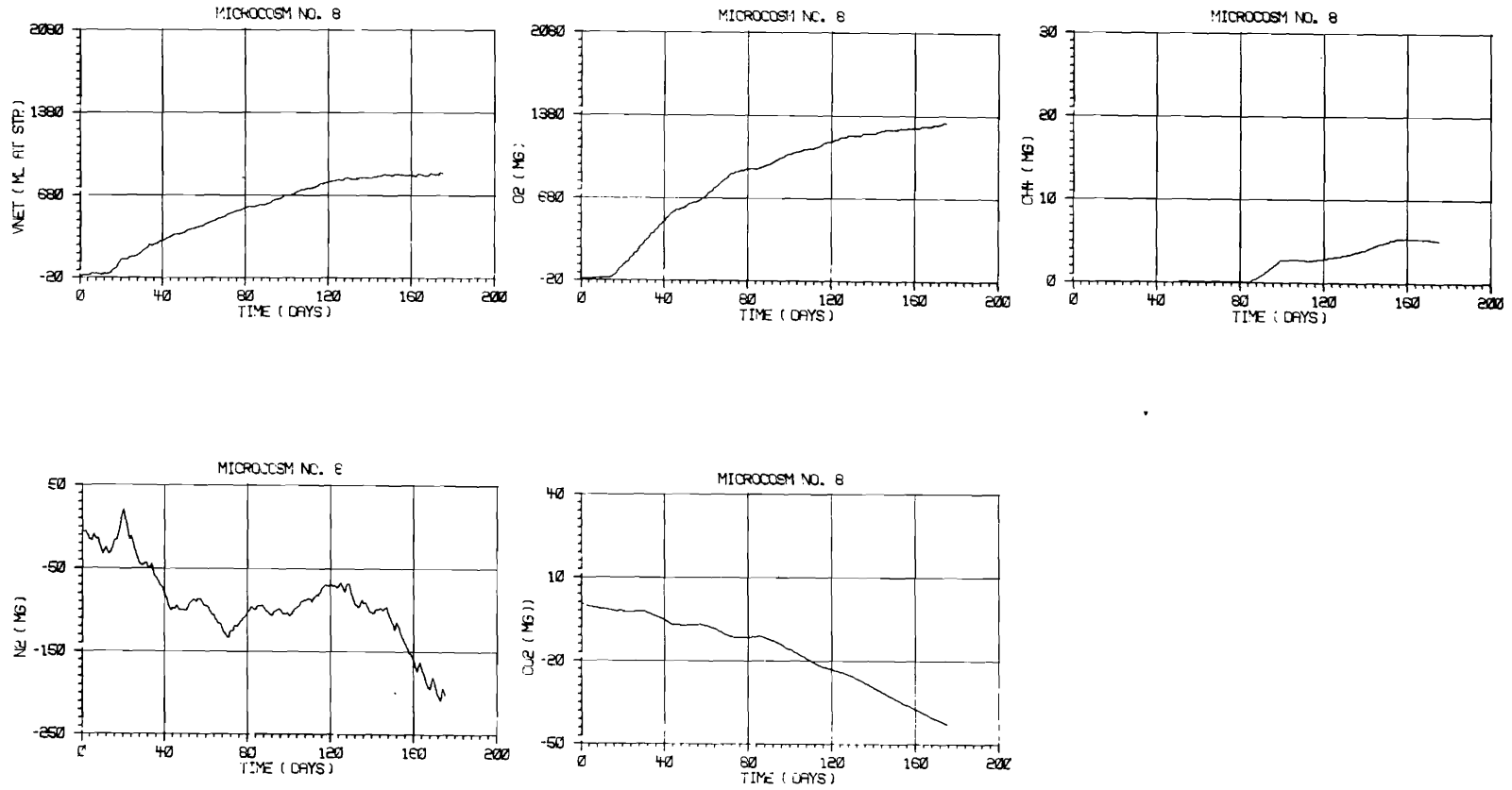
Figure 34. Mass balances of gases detected in Microcosm 8 accumulated over Phase II. V_{NET} total gas production (ml at S.T.P.); other gases in mg.

concentration and are not effluent concentrations. The first effluent analysis was performed on day 15.

As was the case in Phase I, high S.S. values resulted from mixing within the system and from the fact that by day 15 the material (mostly non-volatile solids) suspended when the mud and water were placed in the microcosms had not settled. The trend in the dark units (no algal growth) was a steady decrease in S.S. to levels < 4 mg/l.

The nitrogen perturbation on day 115 (indicated by an arrow) had no effect upon D.O., pH or the solids content of the dark units (Figures 35 and 36).

Average concentrations for iron (Figure 37) and phosphorus (Figure 38) species were plotted vs. time to obtain a general view of these parameters in the dark units. The nitrogen perturbation at day 115 resulted in the steady increase of the aqueous phosphorus concentration over the next two intervals. A possible new equilibrium value was reached at 3-4 times its previous equilibrium value of approxi-

mately 80 μg P/l (Figure 38). Iron lagged phosphorus by two interval periods and began to increase in concentration between day 141 and 155 to levels approaching 40 times its previous equilibrium level of 15 μg Fe/l total iron (Figure 37). This increase in iron concentration coincided with a decrease in the nitrate nitrogen concentration which went from approximately 1 mg NO_3-N/l on day 127 to < 40 μg NO_3-N/l on day 141.

Light microcosms

Again, as was the case in Phase I, the light units simulated the lighted littoral regions of a eutrophic impoundment. Figures 39-42 represent the average values for all the light units. Algal growth in the light units resulted in high D.O. and pH conditions (Figure 39). Greatest algal growth in the effluents was observed only up to day 43 (Table 9). This followed exactly the parameters of D.O. and pH, both of which peaked on day 43. The lower effluent algal populations after day 43 occurred simultaneously with a decrease in both D.O. and pH (Figure 39). The effect of periphyton (wall growth) on oxygen
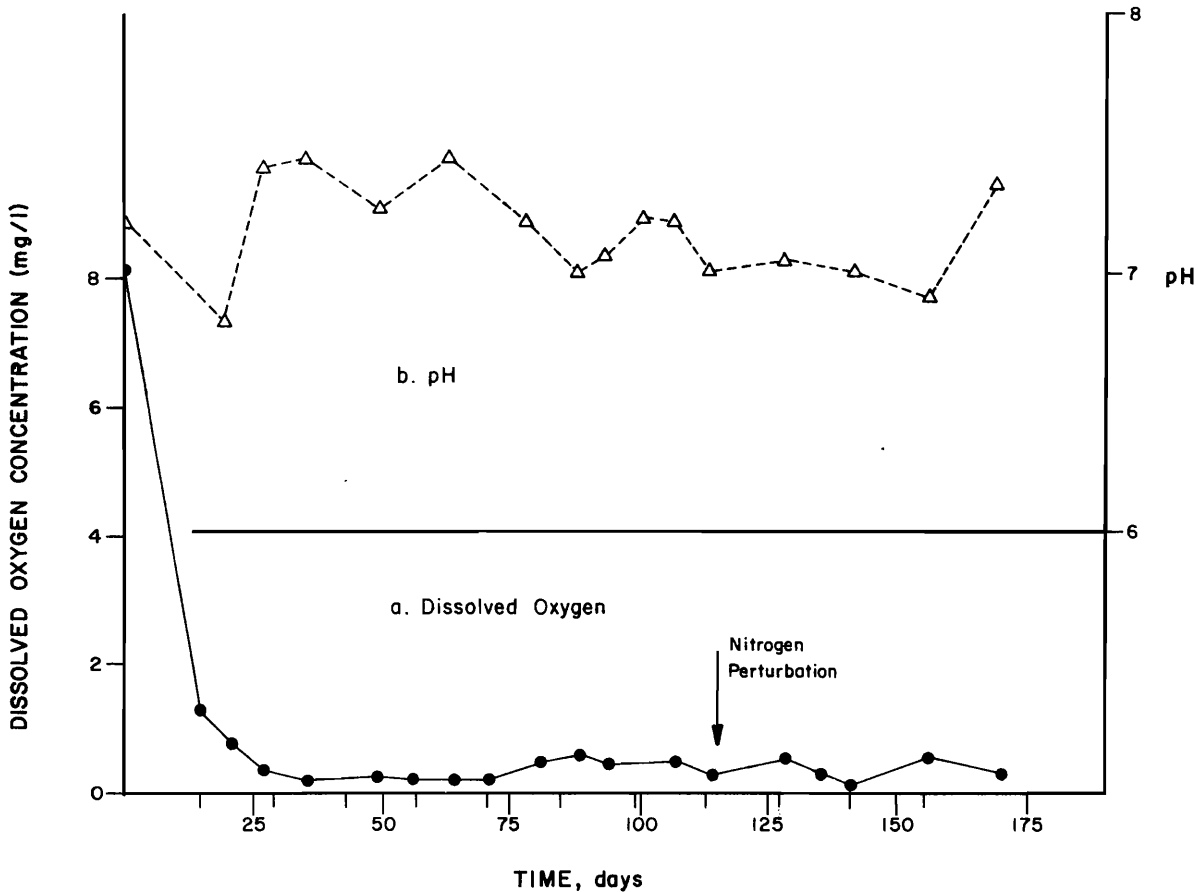
Figure 35. Phase II. Average variation in dissolved oxygen and pH in the effluent of the dark microcosms (fluxes due to acid spills into units not included).

50

dynamics apparently was less than that produced by the algae in the aqueous phase.

Organically bound iron was possible due to the presence of biological material (algae, Table 9; V.S.S., Figure 40(a)) and soluble fractions (S.O.C., Figure 40(b)). Initial condition data (day zero) were influent (input) while the other data were effluent concentrations. The non-volatile portion of the suspended fraction (S.S.-V.S.S.) remained constant throughout the experiment except for the period between day 57 and 71. The large increase in S.S. values during this period was reproduced in the total iron concentration (Figure 41) and to a lesser extent in the total phosphorus concentrations (Figure 42). Increases of these magnitudes showed up in no other parameter (Appendix B, Table B-2). The lack of increase in inorganic carbon during this period (Appendix B, Table B-2) indicated that CaCO₃ was not contained in the suspended material. Iron was more important

than CaCO₃ in regulating phosphorus content of material suspended during this period.

The nitrogen perturbation on day 115 had the effect of introducing nitrogen limitation to the complicated microbial populations in the lighted microcosms. The D.O. concentration, which had begun to level at approximately 13 mg/l by day 110, decreased steadily during the period, day 131 to day 156. This decrease in D.O., accompanied by a decrease in pH (pH decreased steadily from day 80 to day 156) resulted from decreased biological activity introduced by the nitrogen limitation. By day 156 biological activity, as indicated by D.O. and pH, began to increase again. The algal populations in the light microcosms were predominately blue-green algae at this time (aqueous phase algae, Table 9; periphyton, Table 10). Anabaena is capable of fixing nitrogen when NH₃-N and NO₃-N concentrations are low. The nitrogen perturbation had no observable
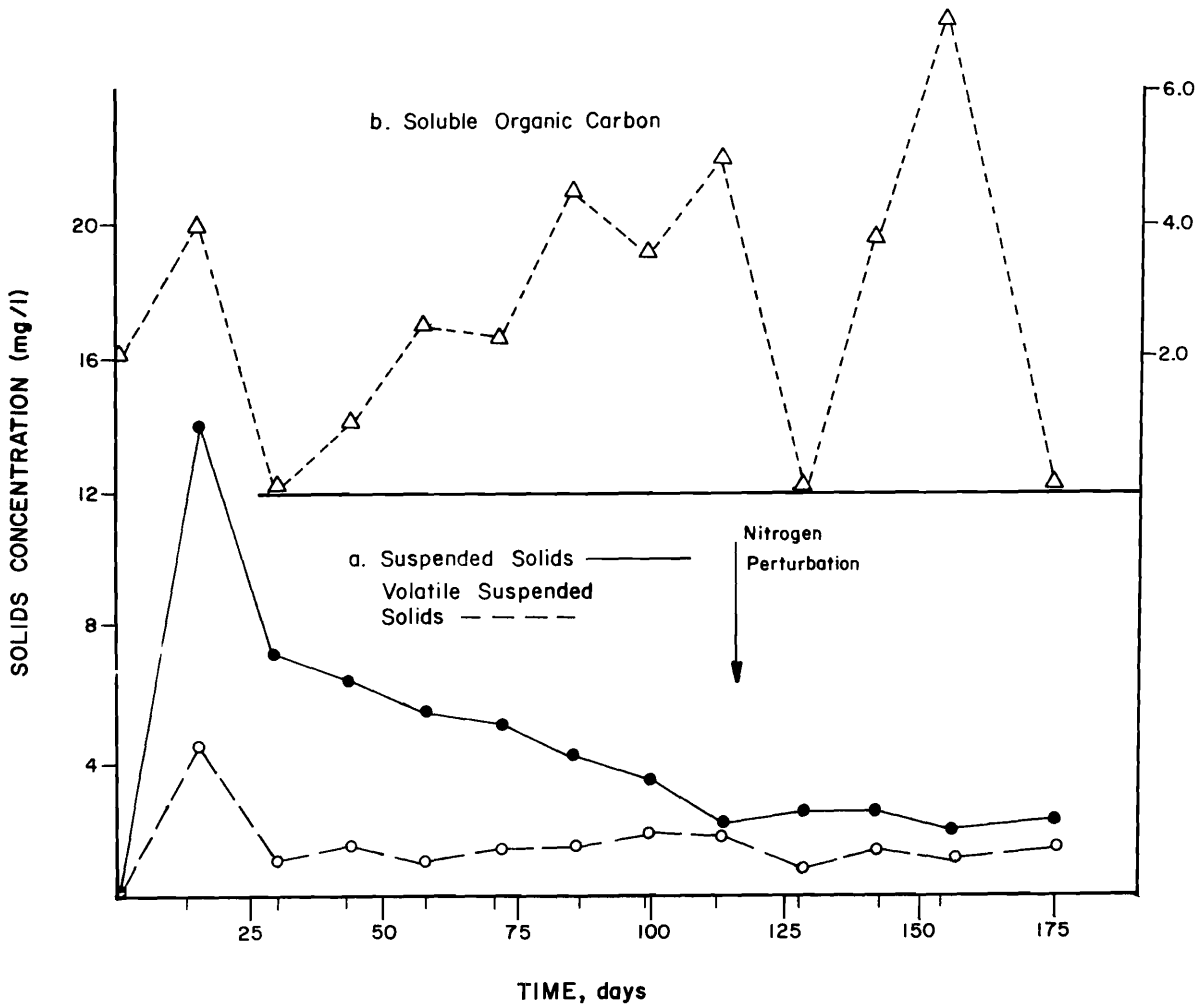
Figure 36. Phase II. Average variation in suspended solids, volatile suspended solids and soluble organic carbon in the effluent of the dark microcosms (fluxes due to acid spills into units not included).

51
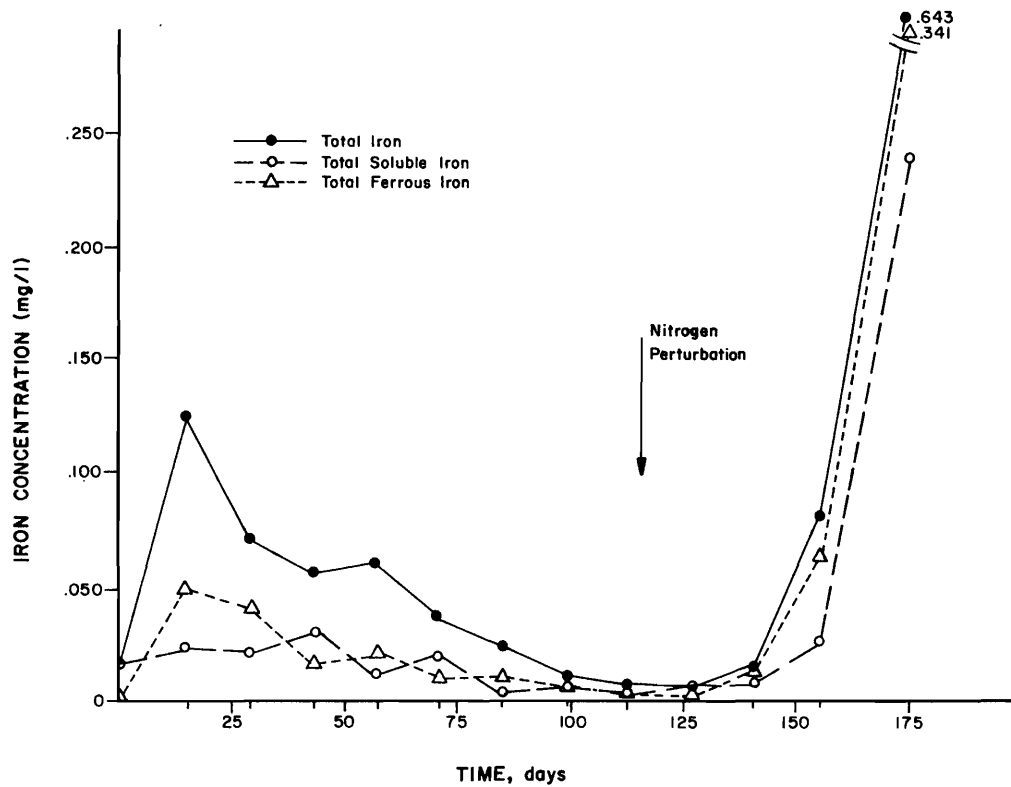
Figure 37. Phase II. Average variation in total iron, total soluble iron, and total ferrous iron in the effluent of the dark microcosms (fluxes due to acid spills into units not included).
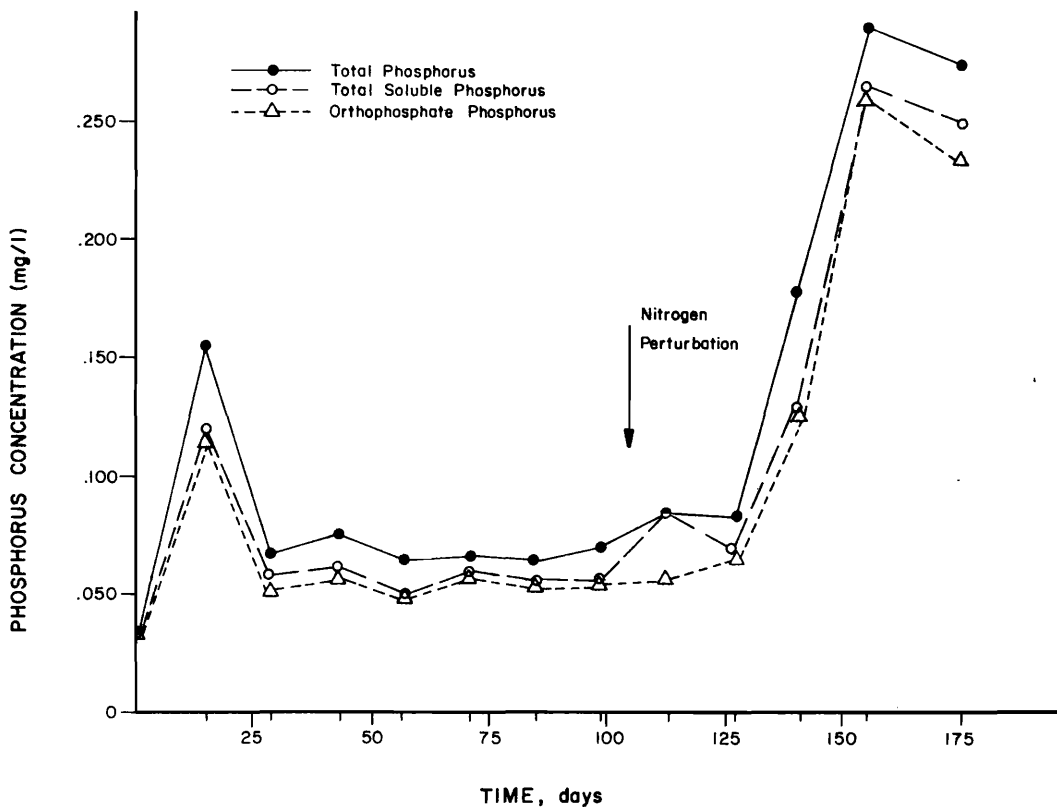
Figure 38. Phase II. Average variation in total phosphorus, total soluble phosphorus and orthophosphate phosphorus in the effluent of the dark microcosms (fluxes due to acid spills into units not included).

52
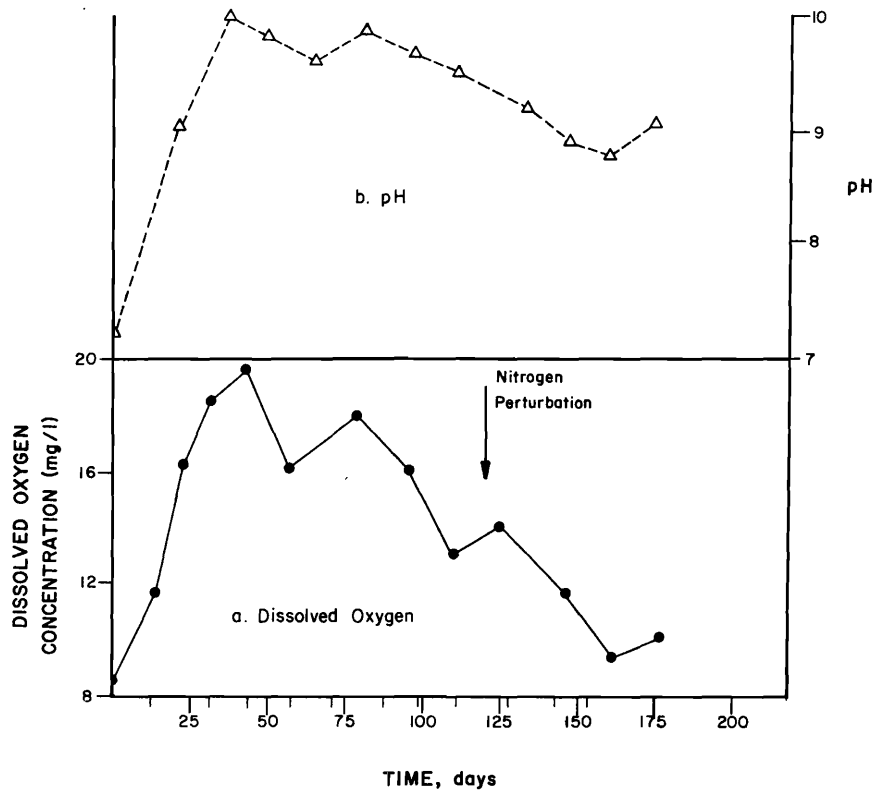
Figure 39. Average variation in dissolved oxygen and pH in the effluent of the light microcosms.
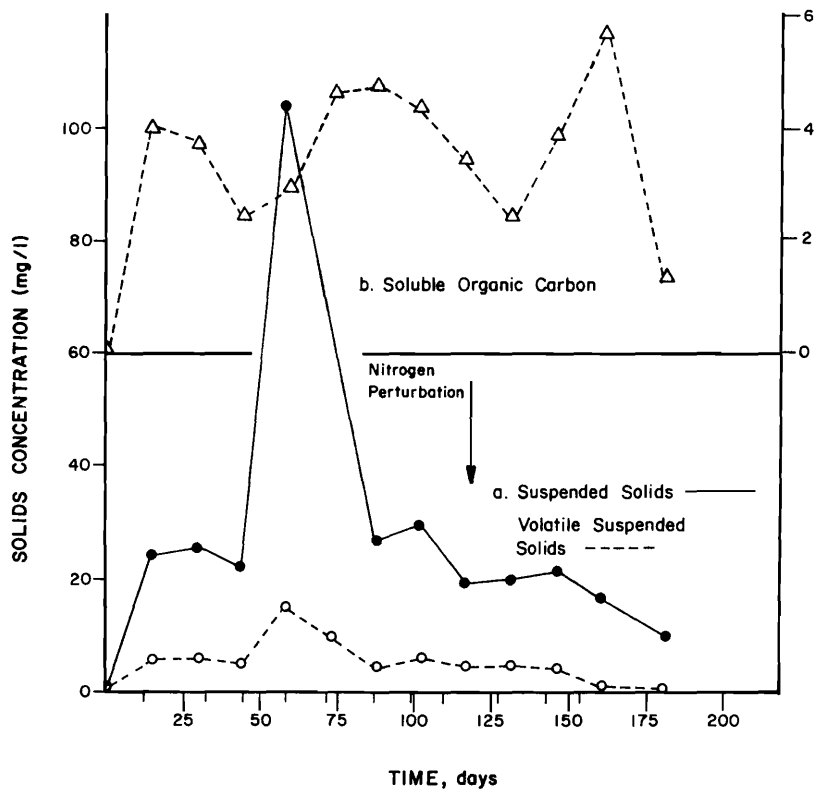
Figure 40. Phase II. Average variation in suspended solids, volatile suspended solids and soluble organic carbon in the effluent of the light microcosms.

53
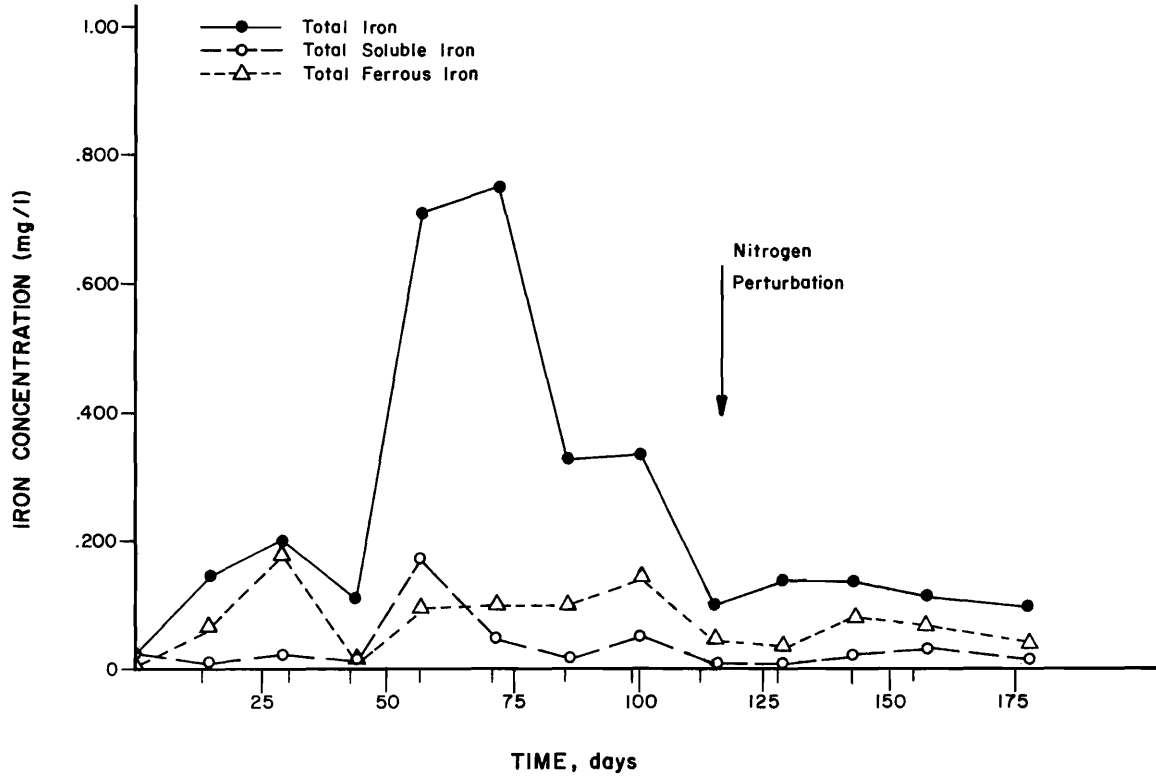
Figure 41. Phase II. Average variation in total iron, total soluble iron, and total ferrous iron in the effluent of the light microcosms.
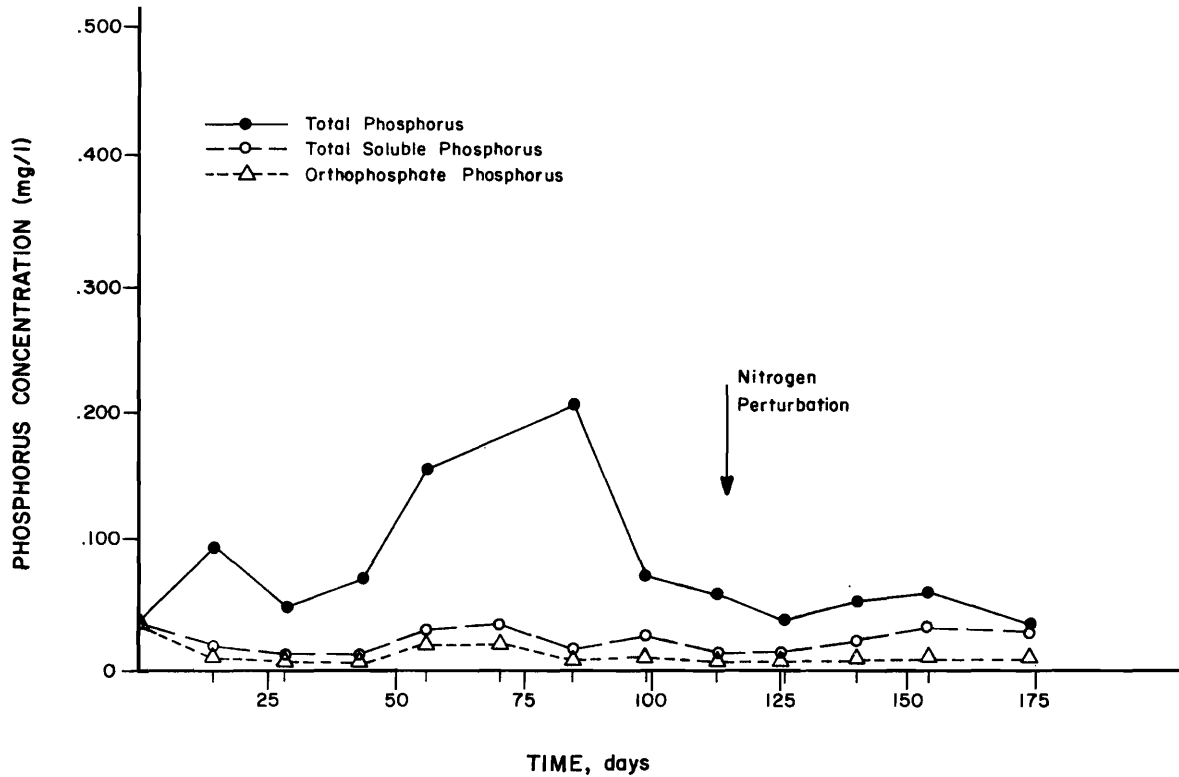
Figure 42. Phase II. Average variation in total phosphorus, total soluble phosphorus, and orthophosphate phosphorus in the effluent of the light microcosms.

effect upon any of the iron or phosphorus species in the lighted microcosms (Figures 41 and 42).

Nitrogen Fixation

Because no measurable acetylene reduction was detected in the effluent samples in the experiment by Porcella et al. (1975), it was assumed in Phase II that nitrogen fixation occurred in the cells attached to the microcosm walls (periphyton). Due to the fact that the microcosms were sealed systems, it was not possible to measure nitrogen fixation until the termination of the experiment. The acetylene reduction method (Stewart et al., 1967; Hardy et al., 1973) as described in Porcella et al. (1975) was utilized to measure nitrogen fixation (Table 12).

Blue-green algae capable of fixing nitrogen *(Anabaena sp.)* were observed but represented < 1 percent of algae present on the walls of the light microcosms (Table 10). Bacteria, present in the periphyton and/or in the sediments (not quantified) could provide other sources of nitrogen fixation. However, the nitrogen fixation values were at least an order of magnitude less than the Phase I experiments, and these values indicated essentially negligible fixation. However, because nitrogen was just beginning to be limiting (Figure 25), fixation may have become more important if the Phase II experiment had continued.

Iron as a Variable

In order to assess the impact of iron as a variable upon the aqueous phase iron and phosphorus concentrations, each microcosm was plotted separately for iron (Figures 43 and 45) and phosphorus (Figures 44 and 46). Varying the concentration of iron in the input medium had variable effects upon the aqueous concentrations of both iron and phosphorus depending upon light conditions.

Dark microcosms

Comparing Microcosms 2, 3, and 4 for the duration of the experiment and Microcosm 1 prior to the acid spill (day 60), the units had comparable effluent concentrations of iron (Figure 43) and phosphrous (Figure 44). The high iron concentrations on day 15 occurred in all four dark units (Figure 43). The acid spill in No. 1 had the effect of increasing the concentration of iron above the levels found in the other dark units by releasing bound iron. Lowering the pH value increases the solubility of iron (Figures 1 and 2), and iron concentrations, both soluble and particulate, were higher than those measured in any of the other dark units. This remained so even after the low pH was neutralized, and iron concentrations in Microcosm 1 did not reach equality with the other dark units until day 141. It was at this time that the N perturbation in the input medium caused drastic increases in the iron concentrations in Microcosms 2, 3, and 4. This increase did not occur in Microcosm 1.

The high phosphorus concentrations in Microcosm 1 on day 15 (Figure 44) resulted from the high S.S. value on that day (Appendix B, Table B-2) and represented phosphorus from the mostly non-volatile particulate matter which had not settled and was therefore carried over in the effluent.

The drastic increase in phosphorus concentrations in No. 1 during the period day 57-71 showed direct response to the acid spill. The phosphorus, like the iron, was both soluble and particulate, with a higher proportion of the phosphorus being soluble. By day 141 the units all had comparable phosphorus concentrations, but because of the high phosphorus concentrations in No. 1 prior to day 141, it did not show as sharp a response to the N perturbation as did units 2, 3, and 4. Total and total soluble phosphorus concentrations in all four units were comparable by day 175; however, the orthophosphate phosphorus

Table 12. Estimated nitrogen fixation rate for light microcosms, Phase II (mg N_2/microcosm·day).[a]

Microcosm Number	Dry Weight (103°C) Wall Scrapings	Nitrogen Fixation $\dfrac{\text{mg } N_2 \, [C_2 H_2]}{\text{Microcosm·Day}}$	Iron Treatment $\mu g \, Fe/l$
8	11.31	0.021	33.
5	11.01	0.009	0.
7	10.62	0.006	33.
6	9.69	0.003	9.9

[a]Microcosm·day = 16 hours; light on 16 hours and off 8 hours.

MICROCOSM 1

Total Iron
Total Soluble Iron
Ferrous Iron

Acid Spill

Nitrogen Perturbation

IRON CONCENTRATION, mg/l

TIME, days

MICROCOSM 3

Acid Spill

Nitrogen Perturbation

.585
.356

IRON CONCENTRATION, mg/l

TIME, days

MICROCOSM 2

Nitrogen Perturbation

.401

IRON CONCENTRATION, mg/l

TIME, days

MICROCOSM 4

Nitrogen Perturbation

.885
.409
.390

IRON CONCENTRATION, mg/l

TIME, days

56

Figure 43. Phase II. Variation of total iron, total soluble iron, and total ferrous iron in the effluent of the dark microcosms.
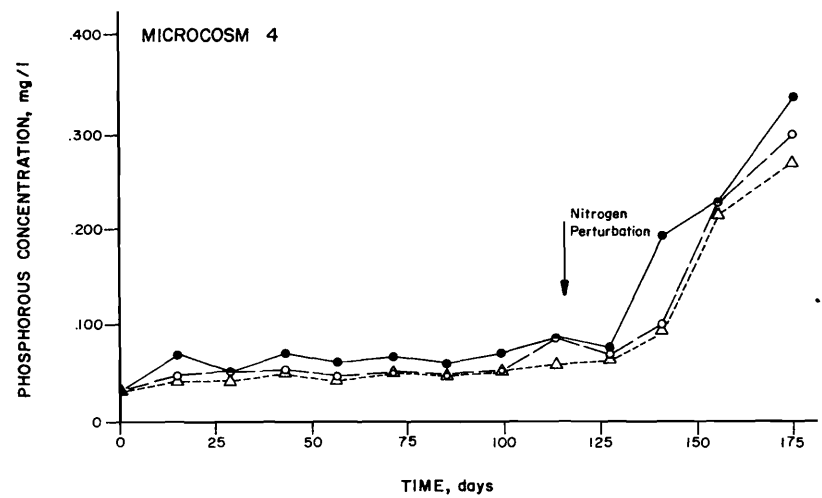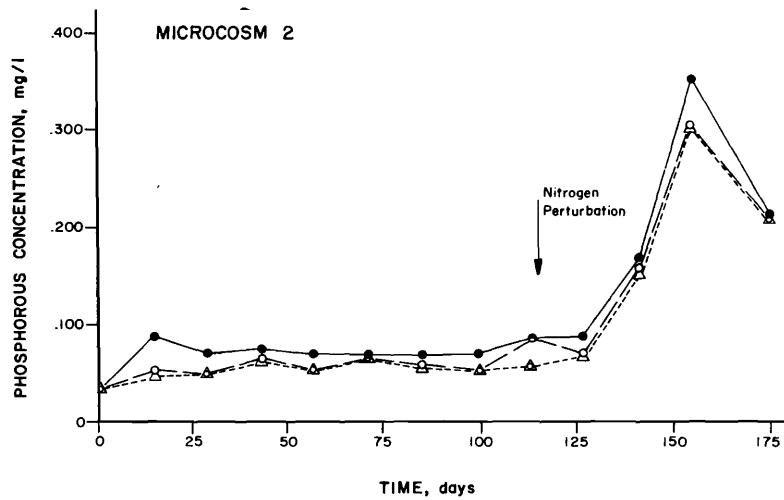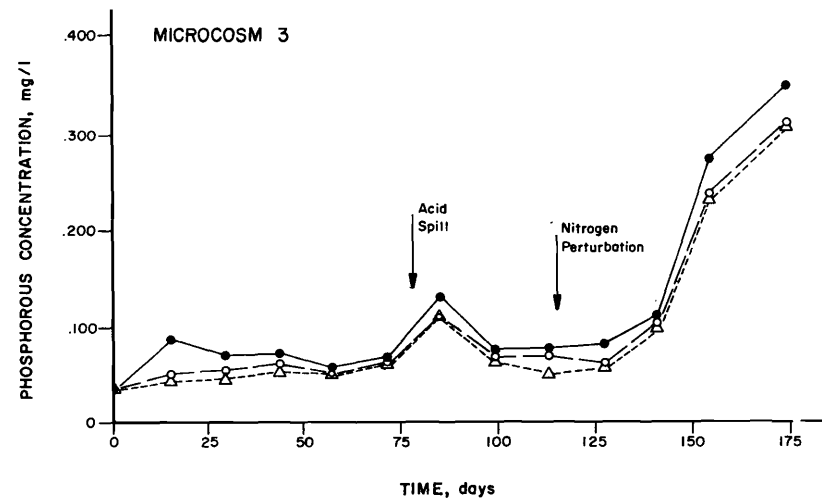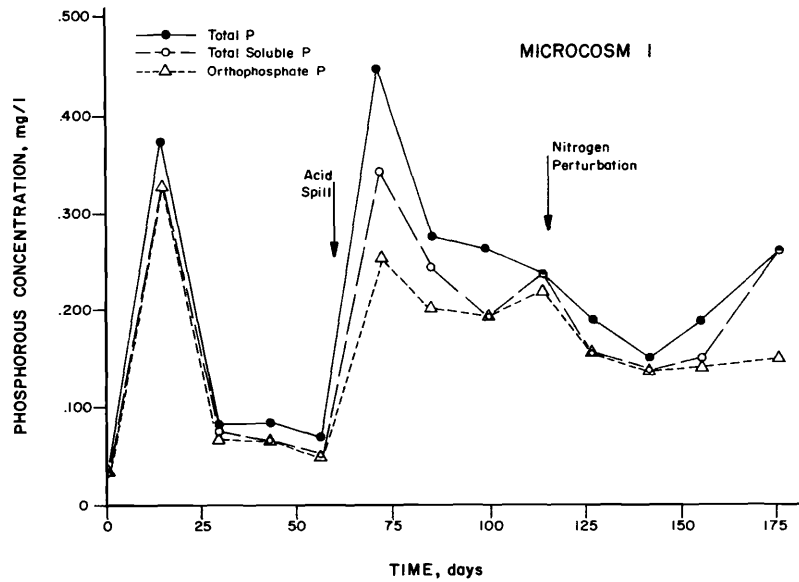
Figure 44. Phase II. Variation of total phosphorus, total soluble phosphorus, and orthophosphate phosphorus in the effluent of the dark microcosms.
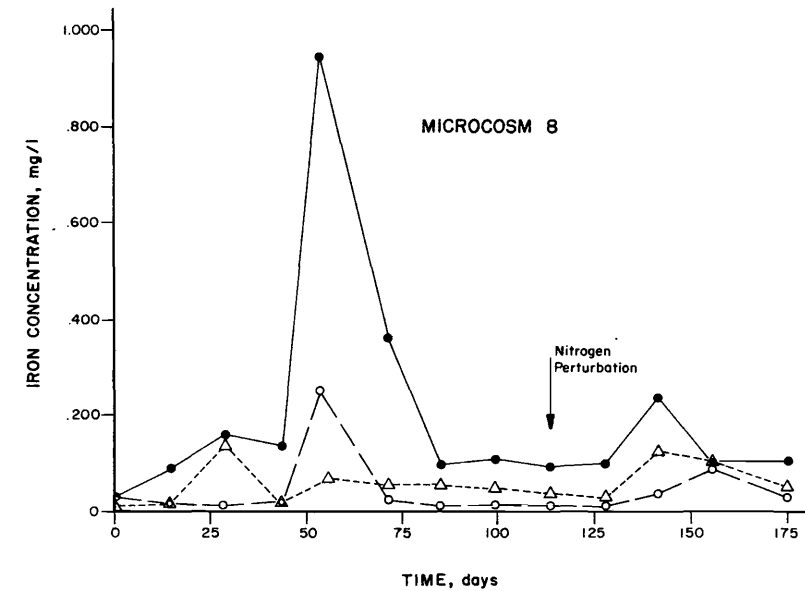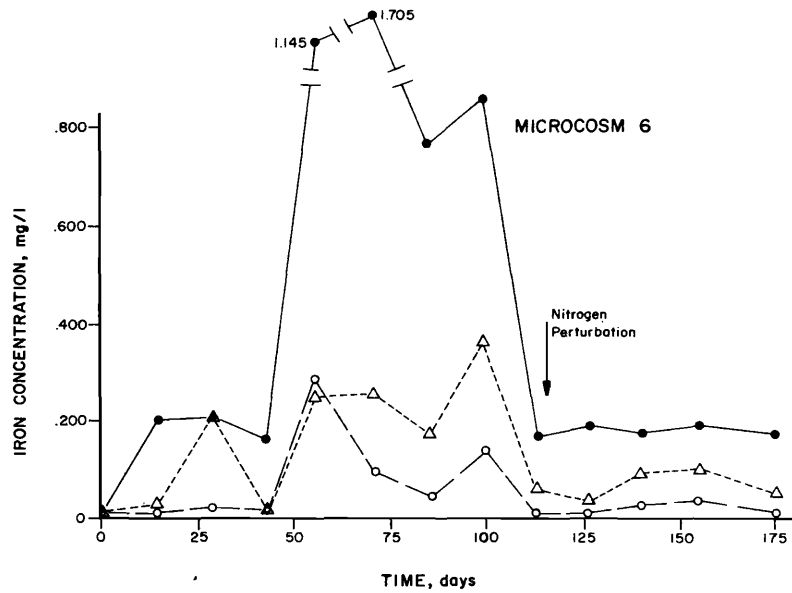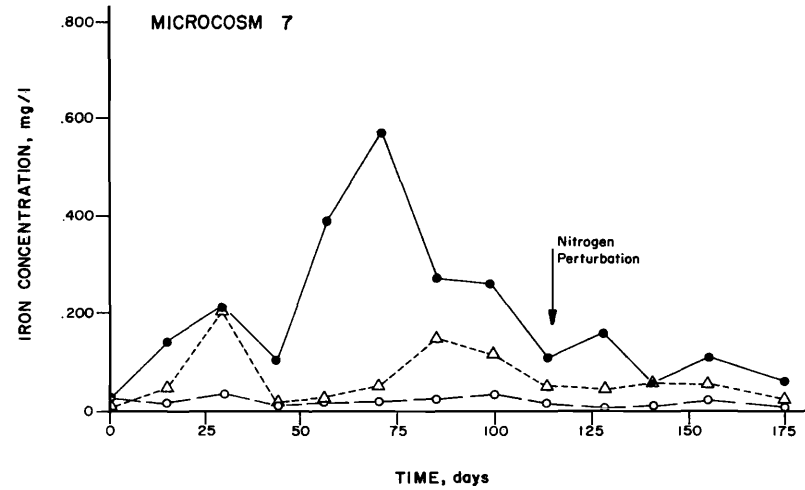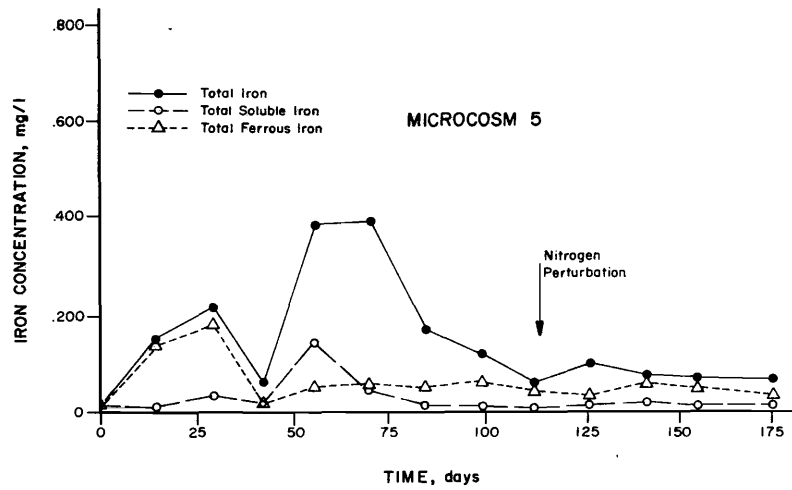
Figure 45. Phase II. Variation of total iron, total soluble iron, and total ferrous iron in the effluent of the light microcosms.
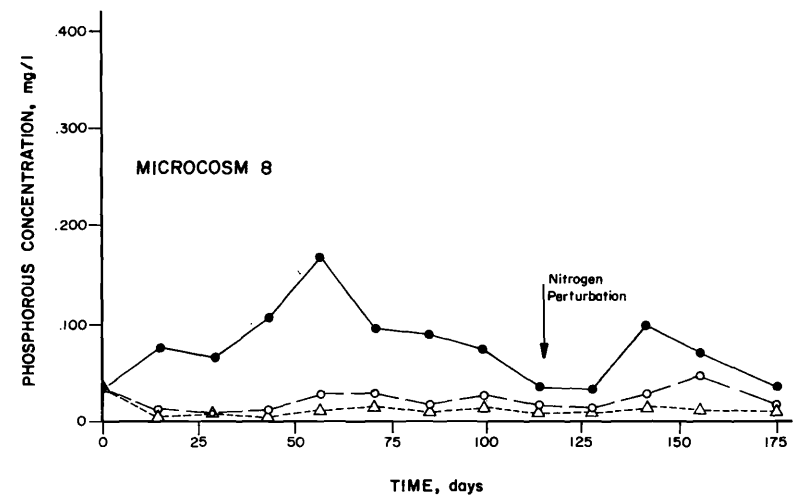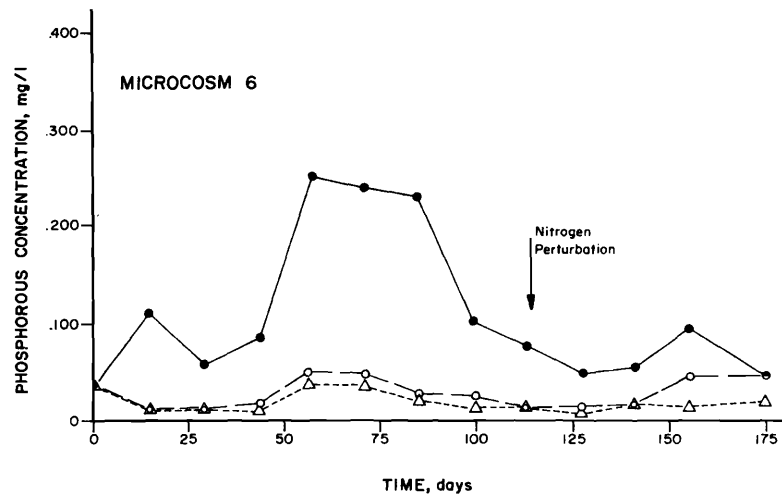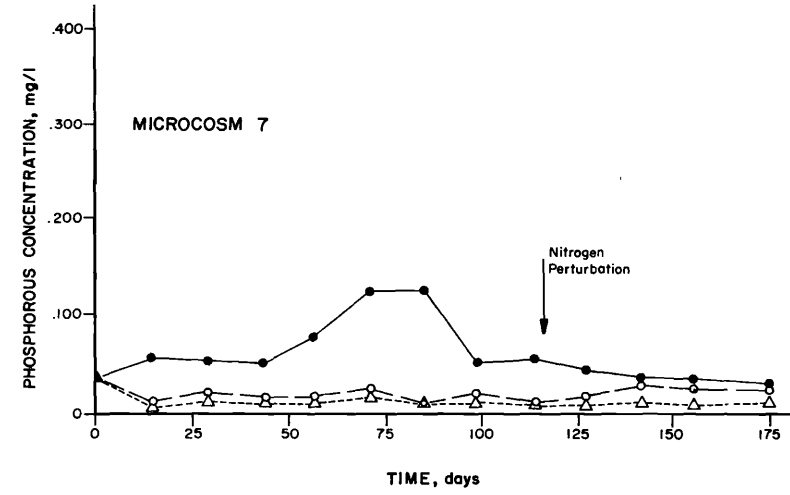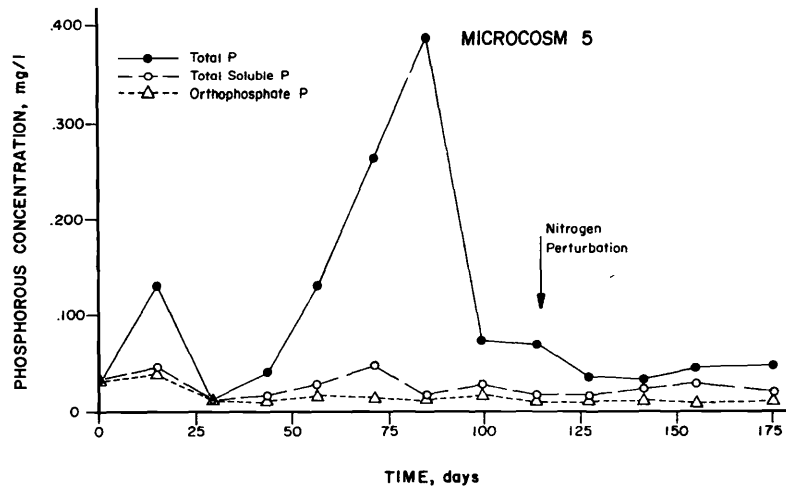
Figure 46. Phase II. Variation of total phosphorus, total soluble phosphorus, and orthophosphate phosphorus in the effluent of the light microcosms.

concentration in No. 1 was lower indicating the presence of polyphosphate and organic phosphorus in No. 1 as a result of the acid spill.

Light microcosms

Because of the presence of algal populations, the light microcosms were more complicated systems than the dark units, making data interpretation relative to iron variation more difficult.

In every case where total iron concentration increased (Figure 45), total phosphorus concentration increased (Figure 46) as well. Increases in total iron and phosphorus were mostly in the particulate fraction (total-total soluble) and were mostly non-volatile (S.S.-V.S.S.). The sharp decline in aqueous phase algal populations after day 43 (Table 9) and the sharp increase in both total iron and total phosphorus during the period day 43 to day 99 (Figures 45 and 46) substantiate the observation that at this time the iron and phosphorus in the aqueous phase were not biologically bound to or found within micro-organisms. The iron and phosphorus came either from the sediment or from biodegraded algal biomass and appeared only after aqueous phase algae decreased in population. The decreases in algal populations could be accounted for in two ways: first, increased attached algae (periphyton); second, bacterial decomposition of algae, with subsequent increase in bacterial populations. The decreased light within the lighted units because of increased wall growth and the increased bacterial populations created conditions for dramatic increases in particulate non-volatile iron and phosphorus. Therefore, in these aerobic biologically active systems, it appears to be either the sediments

or the bacteria which were responsible for the large fluxes in iron and phosphorus.

These fluxes in total iron and in total phosphorus did not seem to be related to the iron concentrations in the influent media (Figures 45 and 46).

Nutrient Mass Balances

Dark microcosms

In the dark microcosms, the nutrient mass balances showed that iron had no effect upon the phosphorus and carbon mass balances, a small effect upon the iron mass balances, and that the acid spill had a drastic impact upon Microcosm 1. Mass balances are summarized in Table 13 and plotted in Figures 47 and 48.

Table 13 and Figures 47 and 48 illustrate that given iron input medium concentrations of 9.9 and 33 μg Fe/l, Microcosms 2, 3, and 4 released the same amount of phosphorus and at approximately the same rates (Figures 47 and 48). Microcosm 2 (9.9 μg Fe/l) released more iron in the first 15 days than did the replicates No. 3 and No. 4 (33 μg/l); however, after, day 15, the release rates were the same for the three units (approximately zero release, zero slopes, Figures 47 and 48).

The effects of the acid spill (day 60, Microcosm 1, Table 13 and Figure 47) showed immediately in the phosphorus and carbon mass balances. Acid solutions favored the release of phosphorus from the sediments (Figure 47). Given anaerobic conditions,

Table 13. Cumulative nutrient net flux, Phase II.

Nutrient Treatment		Microcosm Number	Carbon mg	Phosphorus mg	Iron mg	
0 μg Fe/l	Dark	1	4301.	29.7	11.7	
9.9 μg Fe/l	Dark	2	1708.	14.3	13.4	
33 μg Fe/l	Dark	3	1975.	15.1	10.9	
33 μg Fe/l	Dark	4	1937.	13.8	15.8	
0 μg Fe/l	Light	5	923.0	10.8	24.0	
9.9 μg Fe/l	Light	6	1025.	12.5	74.7	
33 μg Fe/l	Light	7	753.2	3.99	26.6	
33 μg Fe/l	Light	8	881.0	6.78	26.9	
			HCO_3-C	NO_3-N[a]	PO_4-P	Fe
Total Input in		1,5	337.5	1035.	4.725	0
Liquid Media, mg		2,6	337.5	1035.	4.725	1.418
		3,4,7,8	337.5	1035.	4.725	5.198

[a] 10 mg NO_3-N/l feed for 115 days.
(+) Elements Released (OUTPUT > INPUT) from microcosm.
(-) Elements Accumulated (INPUT > OUTPUT) in the microcosm.
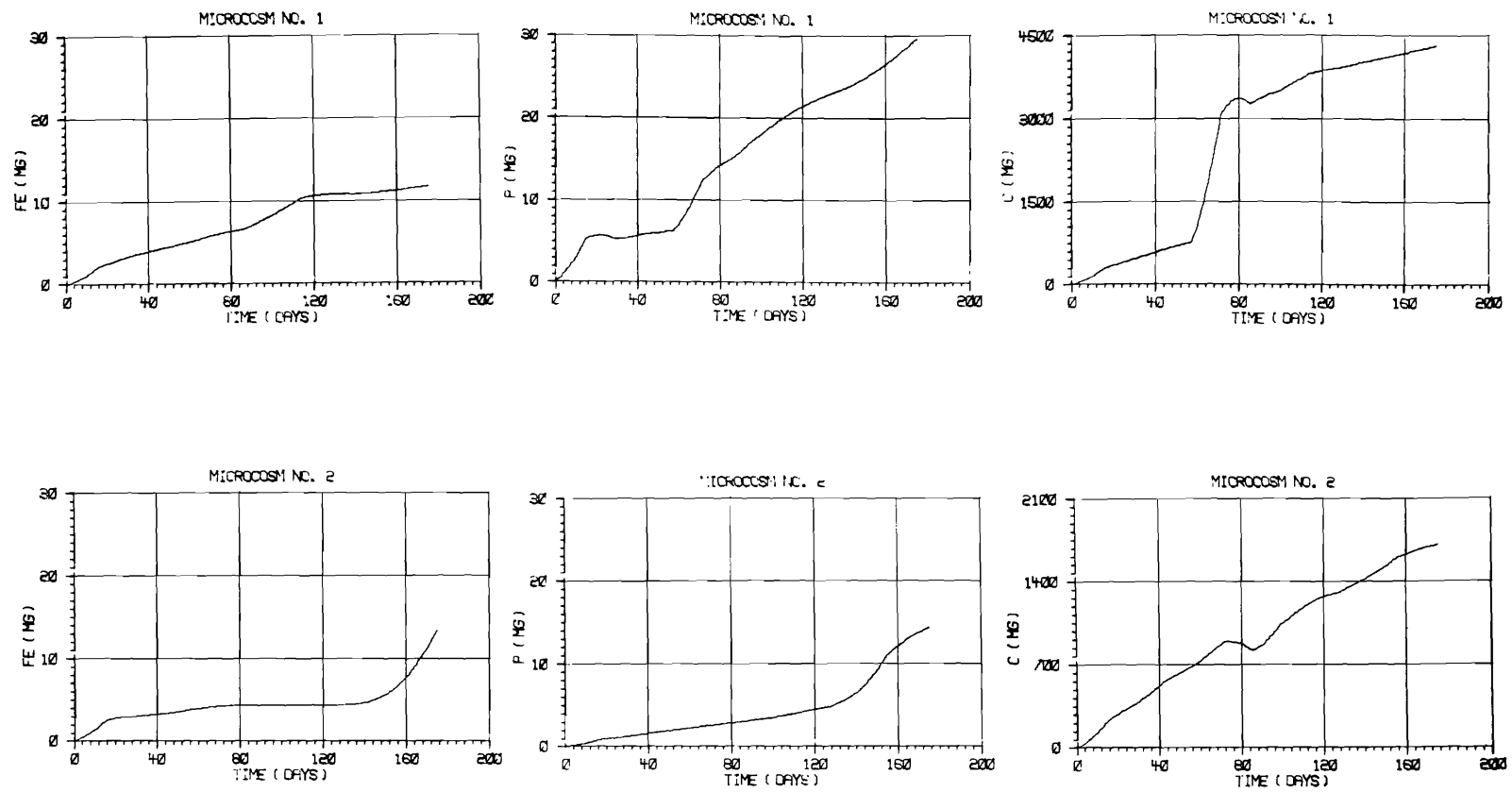
Figure 47. Phase II. Mass balances of nutrients detected in Microcosms 1 and 2 accumulated over Phase II, in mg.

MICROCOSM NO. 3

FE (MG)

30

20

10

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 2

F (N)

30

20

10

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 3

C (MG)

2100

1400

700

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 4

FE (MG)

30

20

10

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 4

F (N)

30

20

10

0

0   40   80   120   160   200
TIME (DAYS)

MICROCOSM NO. 4

C (MG)

2100

1400

700

0

0   40   80   120   160   200
TIME (DAYS)
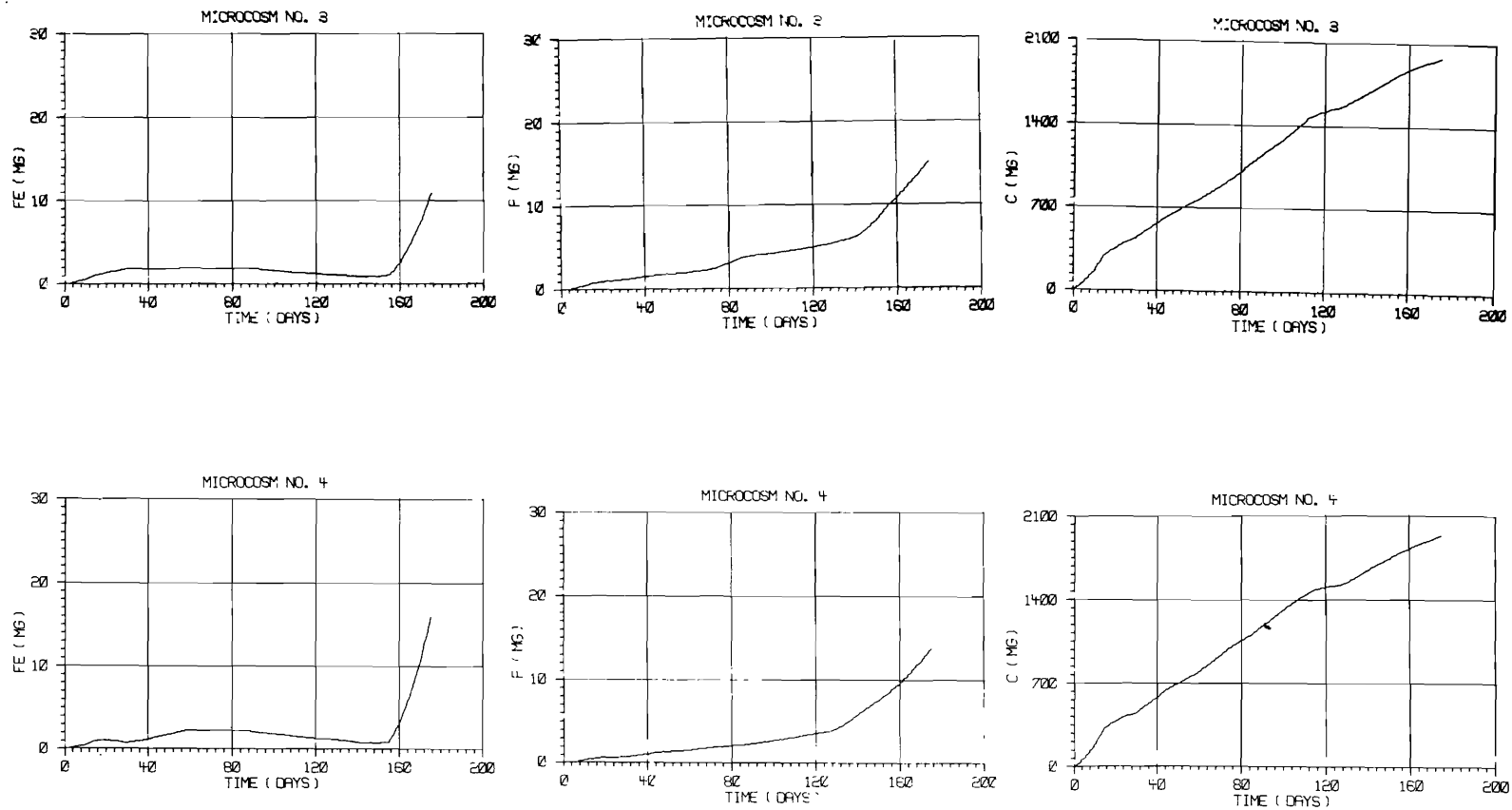
Figure 48. Phase II. Mass balances of nutrients detected in Microcosms 3 and 4 accumulated over Phase II, in mg.

the acid spill and neutralization favored a slightly increased rate of iron release in Microcosm 1 (Figure 47) as compared to the other dark units, but after the nitrogen perturbation was introduced, iron release rates in the other three dark units increased sharply so that by day 175 total iron release was similar in all the dark units.

The increase in the carbon mass balance curve for Microcosm 1 at day 60 is attributed to the $NaHCO_3$ added to the microcosm after neutralization with 1 N NaOH to restore lost buffering capacity (Table 6).

In order to quantify the release of nutrients from the sediments, chemical analyses were performed on the sediments prior to and after incubation within the microcosms (Table 14). The average mass of wet sediment placed in each microcosm was 2821 g and the water content was 70.7 percent (29.3 percent sediment dry weight). Initial masses of elements in the sediments were then calculated as shown below, and listed in Table 14:

$$\underbrace{\frac{(2821 \text{ g wet sediment}) (0.293)}{(826.6 \text{ g dry sediment})}}_{} \left(\frac{\text{mg element}}{\text{g element}}\right) =$$

Initial mass of element, g

Organic carbon analyses of the sediments showed a drop in organic carbon concentration (20 mg/g to 18 mg/g) during incubation of the sediments indicating a release of organic carbon from the sediments of 1653 mg Org C [(826.6) (20-18)]. This carbon loss to the sediments is closely negated by increases in inorganic carbon concentrations of sediments from Nos. 1, 2, and 4. Only No. 3 showed a

decrease in inorganic carbon which amounted to 0.24 mg C/g or a loss of 198 mg C [(0.24) (826.6)]. The total loss of carbon from the sediments of No. 3 was 1851 mg C [1653 + 198] which closely approximated the estimate from the mass balances of 1975 mg C (Table 13).

Phosphorus analyses for the sediments showed a decrease in sediment phosphorus of 8.3, 24.8, 49.6 and 49.6 mg P, which did not agree with mass balance estimates (Table 13).

Iron analyses of the sediments showed an increase in the iron content of the sediments in the dark units after the experiments. This increase was exactly opposite to what was indicated from the mass balance data (output > input; therefore, iron must have been released from the sediments). The initial and final sediment analyses for iron were performed in different laboratories, and although the same procedure was utilized, results differed between analysts. It was concluded (as it was in Phase I sediment analysis) that the sediment analyses were not sufficiently sensitive to identify nutrient fluxes of the magnitude observed in the microcosms.

Light microcosms

Iron input had little if any effect upon the iron and the carbon mass balances; yet, phosphorus mass balances seemed to be inversely related to the iron input into the light units (Table 13, Figures 49 and 50).

The iron mass balances for Microcosms 5, 7, and 8 were nearly identical, and the iron released from the sediments of No. 6 was approximately three times the level attained from the other light units. All four light microcosms had high aqueous phase iron

Table 14. Sediment characteristics, Phase II.

	Initial Conditions (Initial Mass of Element, g)			Microcosms (After Experimental Run 2)							
				1	2	3	4	5	6	7	8
Total P, mg/g	0.94	(777)	Composite	0.93	0.91	0.88	0.88	0.88	0.88	0.83	0.87
			Cores[a] #1	0.95	0.89	0.88	0.86	0.90	0.87	0.83	0.84
			#2	0.90	0.91	0.89	0.90	0.86	0.88	0.83	0.87
			#3	0.93	0.92	0.89	0.90	0.88	0.91	0.85	0.89
Avail P, mg/g	0.017	(14)		0.062	0.061	0.050	0.044	0.046	0.041	0.045	0.047
Total N, mg/g	1.82	(1504)	Composite	1.81	1.94	1.86	1.74	1.89	1.82	1.88	1.93
			Cores #1	2.16	1.72	1.94	1.72	1.91	1.82	2.32	1.86
			#2	1.97	2.04	1.98	1.94	1.85	1.83	1.85	1.97
			#3	1.31	2.01	2.03	1.83	1.84	1.87	1.57	1.98
Org C, mg/g	20.	(16,532)		18.	18.	18.	18.	18.	18.	18.	18.
Inorg C, as $CaCO_3$, mg/g	201.	(166,147)		214.	206.	199.	212.	207.	219.	213.	212.
Total Fe, mg/g	25.	(20,665)	Composite	30.	30.	32.	32.	33.	32.	32.	33.
			Cores #1	29.	30.	31.	31.	33.	33.	32.	33.
			#2	30.	30.	31.	31.	33.	32.	32.	33.
			#3	29.	30.	31.	31.	32.	32.	32.	32.

[a]Cores #1 Top 5 cm
#2 Middle 5 cm
#3 Bottom variable 3.5 - 4.8 cm
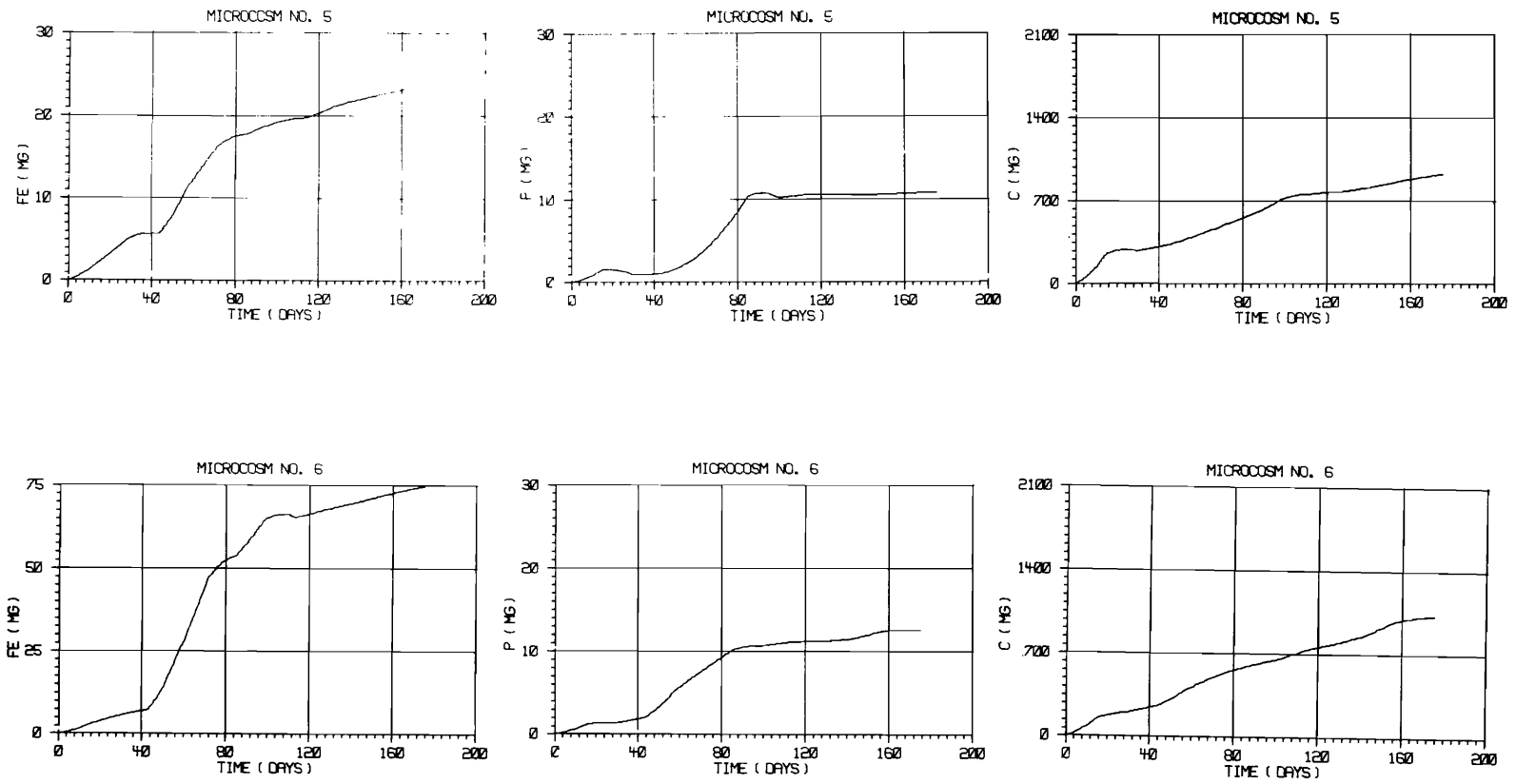
Figure 49. Phase II. Mass balances of nutrients detected in Microcosms 5 and 6 accumulated over Phase II, in mg.
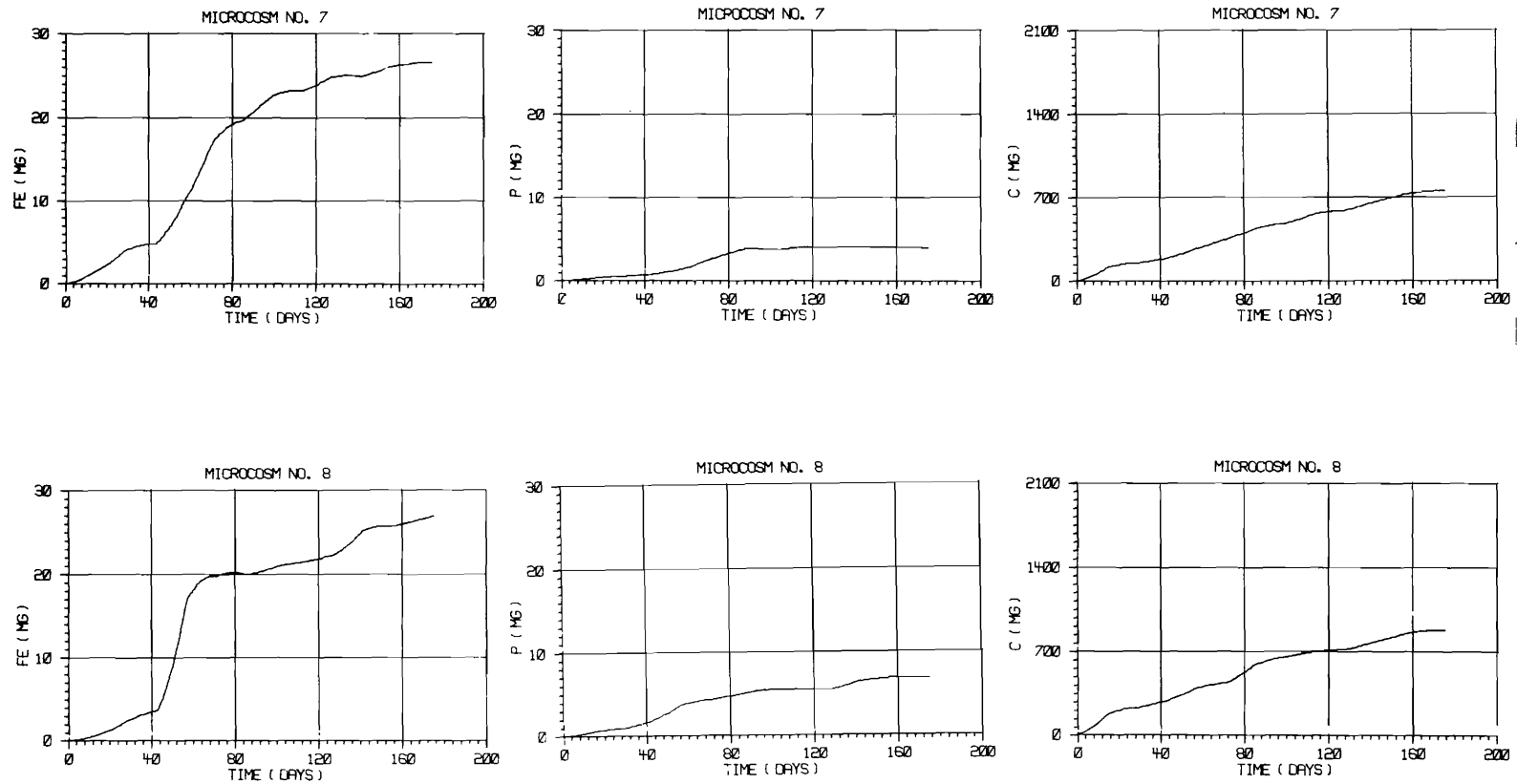
Figure 50. Phase II. Mass balances of nutrients detected in Microcosms 7 and 8 accumulated over Phase II, in mg.

concentrations (also high S.S. levels) during the period of day 40 to day 80 as shown in Figure 45 and by the steep slope portion of the iron mass balance curves (Figures 49 and 50).

To get a total picture of the iron mass balances, the wall scrapings were analyzed for total iron (Table 15). The largest amount of iron released from the sediments became attached to the walls of the unit. The total iron released from sediments (Table 15) showed that input iron variation had no effect upon the iron mass balances.

Iron analyses of the sediments showed an increase in iron content of the sediments (Table 14) in the light units after this experiment, a result which is exactly opposite to the mass balance data (Table 13). The sediment analyses were not sufficiently sensitive to identify the iron fluxes which occurred in the light microcosms; therefore, mass balance and periphyton data were utilized to quantify such iron fluxes (Table 15).

The phosphorus mass balances showed the only effects of input iron variation. Mass balance calculations showed that the phosphorus released from Microcosms 7 and 8 (33 μg Fe/l input) was half that released from No. 5 (0 μg Fe/l input) and No. 6 (9.9 μg Fe/l). The light units released phosphorus until about day 85 (Figures 49 and 50) and then the light microcosms remained at steady state (change in mass balance phosphorus = 0). Table 16 shows that the periphyton offered an adequate sink for the phosphorus released from the sediments. Phosphorus analyses of the sediments quantified the phosphorus released from the sediments, and this amount less the amount of phosphorus released from the microcosm yielded an estimate of phosphorus available to periphyton (Table 16).

Table 15. Periphyton after Phase II.

Microcosm Number	Nutrient Treatment μg Fe/l	Dry Weight (103°C) g	% Fe	X mg Fe	Y Net Fe Flux (Table 13)[a] mg Fe	X + Y mg Total Fe Released from Sediments
5	0.0	11.01	2.3	253	24.0	277.0
6	9.9	9.69	2.6	252	74.7	326.7
7	33.	10.62	2.4	255	26.6	281.6
8	33.	11.31	2.8	317	26.9	343.9

[a]Iron released (OUTPUT > INPUT) from microcosm.

Table 16. Periphyton and phosphorus flux.

Microcosm Number	Nutrient Treatment μg Fe/l	X Net P Flux (Table 13)[a] mg P	Y P Released From Sediment[b] mg P	Y-X Phosphorus Available to Periphyton mg P	Periphyton Phosphorus 1% Total Dry Weight[c] mg P
5	0.0	10.8	49.6	38.8	110.
6	9.9	12.5	49.6	37.1	96.
7	33.	3.99	90.9	86.9	106.
8	33.	6.78	57.9	51.1	113.

[a]Phosphorus released (OUTPUT > INPUT) from microcosms.
[b]Phosphorus released from sediments = [(Initial Total P, mg/g) - (Final Total P, mg/g)]
[826.6 g Dry Wt. Sediment]
[c]Stumm and Leckie (1970).

DISCUSSION: PHASE II

Statistical analysis of the iron data showed that in the second phase of the microcosm experiments, higher total iron concentrations existed in the light microcosm effluent (0.246 mg Fe/l) than in the dark effluent (0.083 mg Fe/l). These results were exactly opposite to those in Phase I and were opposite to those expected from the physical and chemical aspects of iron chemistry. The dark units should favor iron solubility and the light units should favor iron deposition onto the sediments. The higher aqueous phase iron concentrations present in the light units could only be rationalized through biological considerations.

The iron present in the light units was mostly particulate (87 percent particulate), but because biologically bound iron and suspended $Fe(OH)_3$ floc could not be analytically separated, absolute conclusions as to the amount of particulate iron which was biologically bound could not be formulated. Although most of the particulate matter in the light units was non-volatile (72 percent non-volatile), the average V.S.S. value for these microcosms was 5.49 mg/l. This level of volatile solids indicated the presence of biologically bound iron.

Statistical analysis of the phosphorus data in Phase II of the study duplicated the Phase I data with the dark units having higher total phosphorus concentrations (0.134 mg P/l) than the light units (0.086 μg P/l). The phosphorus in the dark units was mostly soluble (86 percent soluble) while the phosphorus in the light units was mostly particulate (73 percent particulate)

Comparing iron and phosphorus concentrations for each microcosm, it was found that iron and phosphorus showed no particular distribution in Phase II. Phosphorus and iron have a molecular weight ratio of P:Fe of 0.56:1. The average influent P:Fe ratio to all microcosms was 1.58:1 (0.030 mg

P/l to 0.019 mg Fe/l). The dark microcosms released slightly less phosphorus relative to iron (0.105 mg P/l to 0.083 mg Fe/l or a P:Fe ratio of 1.27:1) and the light microcosms released considerably less phosphorus relative to iron (0.086 mg P/l to 0.246 mg Fe/l or a P:Fe ratio of 0.35:1) than was present in the input medium.

The iron concentrations in the aqueous phase of the light microcosms (0.246 mg Fe/l) were much greater in Phase II than in Phase I (0.092 mg Fe/l). These differences in light microcosm effluent iron concentrations could only be attributed to the increased nitrogen concentrations in Phase II (10 mg N/l, Phase II; 0 and 0.300 mg N/l, Phase I). Therefore, eutrophic impoundments with excessive inorganic nitrogen aqueous concentrations will contain even greater aqueous phase iron levels than will lower nitrogen eutrophic systems.

Iron could not be a limiting nutrient in a system containing sediments from a eutrophic impoundment (Hyrum Reservoir) with a considerable iron concentration (25 mg Fe/g sediment or 2.5 percent Fe). Phase II was conducted with extremely high nitrogen input (10 mg NO_3-N/l), and the biological activity generated within the microcosms was such that a large quantity of iron was present in the aqueous phase. Organic matter being present, both soluble and particulate, iron limitation in high iron sediment systems was impossible. The nitrogen perturbation imposed upon the microcosm system could simulate an enforcement of proposed zero or low discharge requirements in an agricultural area. This perturbation had immediate effects upon the hypolimnetic phosphorus concentration and a slightly delayed effect upon the hypolimnetic iron concentration. Proposed implementation of such a perturbation on Hyrum Reservoir could have drastic effects upon the hypolimnetic aqueous iron and phosphorus concentration at the reservoir.

SUMMARY AND CONCLUSIONS

Phase I

1. The parallelism of effluent iron and phosphorus concentrations from the biologically active aerobic systems (the light microcosms) indicates that mechanisms of distribution of iron and phosphorus are directly related.
2. The light microcosms effluent iron is associated with organic material, with a portion of the particulate fraction undoubtedly being the suspended inorganic precipitate $Fe(OH)_3$.
3. Phosphorus complexes with Fe(III) iron and precipitates out of solution and onto the sediments ($FePO_4 \cdot 2H_2O$, $Fe(OH)_2H_2PO_4$).
4. Phosphorus is taken up by the periphyton in the light microcosms and is thus removed from the aqueous phase.
5. Phosphorus forms insoluble compounds with other elements and precipitates.
6. The extremely large fluxes indicated by iron analysis of sediments of the microcosms is in error due to lack of analytical sensitivity.

Phase II

1. Iron added to any experimental system must be chelated in order to make it soluble and available to microorganisms.
2. If chromagenic reagents are utilized to quantify iron, extraction procedures must be used in order to concentrate iron and decrease turbidity interferences.
3. The boiling step in the bathophenanthroline extraction analysis of Fe(II) improved recoveries in biologically complex Fe(II) systems.
4. Both iron and phosphorus were present in the soluble form in the dark microcosm and in the particulate form in the light microcosms.
5. Periphyton phosphorus must be considered to balance phosphorus fluxes in the light microcosms.
6. The dominate algal species in the sealed systems containing sediments from the eutrophic Hyrum Reservoir was the blue-green alga, *Microcystis incerta.*

7. Accidental acid spills in two microcosms had a significant effect on gas mass balances in the microcosms.
8. Total gas production, oxygen utilization and carbon dioxide production were not responsive to iron variability in the dark microcosms.
9. Total gas production was greater, by an order of magnitude, in the light units than in the dark units.
10. D.O. and pH were directly related to trends in suspended algal populations in the light microcosms.
11. Oxygen production was inversely related to amounts of periphyton, and iron variability had no apparent relation to oxygen production in the light microcosms.
12. The nitrogen perturbation (input of 10 mg NO_3-N/l stopped) had no effect upon D.O., pH or solids in the dark units.
13. The nitrogen perturbation caused an immediate increase in phosphorus and a delayed increase in iron concentrations in the aqueous phases in the dark units.
14. The nitrogen perturbation directly affected pH values and D.O. concentrations in the light microcosms; recovery of algal growth showed up in pH values and D.O. concentrations.
15. Iron was more important than $CaCO_3$ in regulating phosphorus content of material suspended in light units (approximately day 57).
16. The nitrogen perturbation had no effect upon aqueous iron or phosphorus concentration in the light microcosms.
17. Input iron variation did not affect iron and/or phosphorus concentrations in the dark units.
18. Iron input variation had no effect upon the phosphorus, carbon, and iron mass balances in the dark microcosms.
19. Aqueous concentration of iron and phosphorus (mostly non-volatile particulate matter) increased as algal populations decreased after day 43 in the light microcosms. The iron and phosphorus came either from the sediments or from the bacterial decomposition of algae which formed particulate fractions in the light microcosms.
20. Iron fluxes were not related to input iron concentration in the light microcosms.

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21. Phosphorus fluxes in the light microcosms appeared to be inversely related to the iron inputs.

22. Predictions of iron flux in a three phase microcosm cannot always be made through chemical and physical data. Highly variable biological considerations must be utilized in certain instances to make such predictions.

23. Increases in aqueous phase iron concentrations will occur in the hypolimnetic regions of eutrophic impoundments containing high iron sediments when high inorganic nitrogen inputs are stopped.

24. The shallow littoral regions of eutrophic impoundments containing high iron sediments and exposed to high aqueous phase inorganic nitrogen inputs will have excessively high aqueous phase iron concentrations.

RECOMMENDATIONS FOR FURTHER STUDY

The microcosm approach offers a method of studying the effects of environmental perturbations on natural sediment-water systems in the laboratory and to observe and to quantify nutrient and other elemental fluxes due to those perturbations.

1. Further research is needed to determine how accurately these laboratory microcosms simulate actual aqueous environments. Subsequent studies need to be performed simultaneously in the laboratory and in the field in order to determine how useful a predictive tool microcosms can be in the future.

2. An analysis of the precision of the microcosm technique needs to be performed in order to determine the reproducibility of the methodology. Experimentation might include a minimization of the number of variables thus allowing replicate treatments to be performed.

3. If the gas-water-sediment microcosm is to become a useful tool in predicting biological responses to environmental perturbations, the hydraulics of the system must be evaluated. In addition to varying input media concentration levels, the residence time (detention period) of both semi-continuous and continuous flow systems could be varied and biological responses measured. Results from such studies could determine optimum operating conditions for microcosms.

4. Studies must be performed on iron analytical techniques, specifically, iron in complex biological systems. A technique must be developed which would separate organically bound iron and the inorganic precipitate, $Fe(OH)_3$, in the particulate phase; such a technique would make it possible to quantify particulate phase iron.

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APPENDICES

Appendix A

Analytical Methods

Parameter (PHASE)	Method	Ref.
A. (AQUEOUS)		
I. Unfiltered		
1. Total Phosphorus	Persulfate Digestion	1
2. Organic Nitrogen	Digestion, Distillation	1
3. Total Carbon	Combustion, Infrared	2
4. Inorganic Carbon	Combustion, Infrared	2
5. Total Iron		
Phase I	Bathophenanthroline	3
Phase II	Bathophenanthroline, Extraction	4
6. Total Ferrous Iron		
Phase I	Phenanthroline	1
Phase II	Bathophenanthroline, Extraction	4
7. Total Mercury		5
A. Flameless AA		
B. Gas Chromatograph: Mercury Column		

Column Description: 3 ft. of 1/8"
O.D. stainless steel GLC column
Packing Description: 3% Hi-Eff 4BP
on Gas Chrom Q, 80-100 mesh
Detector: Flame
Oven Temperature: 140°C
Injector Temperature: 180°C
Detector Temperature: 280°C
Carrier Gas: Helium
Sample Size: 1.0 μl
Methane, dimethyl mercury, and monomethyl mercury all had similar retention times

Parameter (PHASE)	Method	Ref.
8. Suspended Solids	Glass Fiber Filter (103°C)	1
9. Volatile Suspended Solids	Glass Fiber Filter (550°C)	1
10. pH	Electrometric	6
11. Dissolved Oxygen	Winkler; Azide Modification	1
12. Chlorophyll	Relative Fluorescence	7
II. Filtered		
1. Ortho-Phosphate	Antimony-Molybdate, Ascorbic Acid	1
2. Total Soluble Phosphorus	Persulfate Digestion	1

Parameter (PHASE)	Method	Ref.
3. Nitrite Nitrogen	Diazotization	3
4. Nitrate Nitrogen	Cadmium Reduction	3
5. Ammonia Nitrogen	Indophenol	8
6. Soluble Organic Nitrogen	Digestion, Distillation	1
7. Soluble Total Carbon	Combustion, Infrared	2
8. Soluble Inorganic Carbon	Combustion, Infrared	2
9. Soluble Total Iron		
Phase I	Bathophenanthroline	
Phase II	Bathophenanthroline, Extraction	4
10. Soluble Total Mercury	A. Flameless AA	4
	B. Gas Chromatograph: Mercury Column	
B. (SEDIMENT)		
1. Total Phosphorus	A. Persulfate Digestion	1
	B. HCLO$_3$ Digestion	1
2. Total Available Phosphorus	A. Dilute Fluoride-Dilute HCl Soluble P	
	B. NaHCO$_3$ Extraction	1
3. Total Nitrogen	Combustion	9
4. Total Organic Carbon	A. Dichromate Oxidation	10
	B. Colorimetric	11
5. Inorganic Carbon	Monometric	11
6. Total Iron	HCLO$_4$ digest, AA	12
7. Total Extractable Iron	Diethylenetriamine pentaacetic acid extraction, AA	12,13
8. Nitrate Nitrogen	Phenol disulfonic acid colorimetric	
C. (GAS)		
1. Nitrogen	Gas Chromatograph molecular sieve 5A	15
2. Oxygen	Gas Chromatograph molecular sieve 5A	15
3. Methane	Gas Chromatograph molecular sieve 5A	15

Parameter   Method   Ref.
(PHASE)

Column Description: 6 ft. of 1/8"
    stainless steel
Packing: Molecular Sieve 5A
Carrier gas: Helium
          Flow rate = 30 ml/min
Detector: Thermal conductivity
Sample size: 1.0 μl
Attenuation: Variable
Column Temperature: 100°C
Detector Temperature: 260°C
Injector Temperature: 100°C

4. Carbon Dioxide Gas Chromatograph Porapak S 10
5. Ethylene   Gas Chromatograph Porapak R 10
Column Description: 6 ft. of 1/8"
    stainless steel
Packing: Porapak R
Carrier gas: Helium
          Flow rate = 30 ml/min
Detector: Flame
Sample size: 0.5 cc
Attenuation: Variable
Column Temperature: 50°C
Detector Temperature: 260°C
Injector Temperature: 100°C

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Appendix B

Analytical Results

Table B-1. Gas mole fraction.

Microcosm 1

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.928	.061	.0023		
29	.948	.042	.0018	.0001	
43	.951	.04	.0013	.0001	
57	.921	.07	.002	.0001	
71	.876	.094	.0005	.015	
85	.942	.041	.001	.017	
99	.931	.045	.009	.007	
113	.938	.043	.0027	.01	
127	.936	.045	.0018	.006	
141	.865	.12	.0017	.002	
155	.886	.098	.0022	.0008	
175	.89	.089	.0007	.012	

Microcosm 3

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.913	.076	.0017		
29	.936	.052	.0019	.0001	
43	.941	.048	.0014	.0001	
57	.921	.068	.0014	.0001	
71	.915	.067	.0013	.004	
85	.871	.102	.011	.008	
99	.906	.075	.0054	.004	
113	.898	.088	.0018	.004	
127	.887	.098	.0012	.004	
141	.923	.061	.0013	.006	
155	.916	.061	.0016	.01	
175	.892	.071	.0018	.024	

Microcosm 2

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.9	.089	.0013		
29	.937	.052	.0015	.0001	
43	.951	.039	.0012	.0001	
57	.931	.058	.0011	.0001	
71	.916	.067	.001	.006	
85	.934	.053	.001	.004	
99	.931	.049	.001	.01	
113	.916	.065	.0008	.01	
127	.929	.058	.0007	.004	
141	.922	.06	.0008	.007	
155	.912	.066	.001	.01	
175	.886	.075	.0013	.028	

Microcosm 4

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.91	.079	.0015		
29	.938	.051	.0018	.0001	
43	.928	.061	.001	.0001	
57	.918	.07	.0014	.0001	
71	.921	.062	.001	.004	
85	.93	.052	.002	.008	
99	.932	.052	.001	.006	
113	.929	.056	.0016	.004	
127	.92	.066	.001	.004	
141	.898	.086	.0009	.004	
155	.925	.061	.0016	.002	
175	.9	.075	.0016	.014	

Table B-1. Continued.

Microcosm 5

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.794	.198	.0004		
29	.582	.409	.0003	.0001	
43	.507	.485	.0002	.0001	
57	.575	.417	.0004	.0001	
71	.515	.453	.002	.0001	
85	.597	.394	.0004	.0001	
99	.612	.377	.0002	.002	
113	.664	.327	.0001	.002	
127	.653	.337	.0002	.002	
141	.676	.312	.0001	.004	
155	.666	.314	.0001	.7096	
175	.675	.298	.0001	.019	

Microcosm 7

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.71	.282	.0002		
29	.607	.384	.0004	.0001	
43	.492	.5	.0002	.0001	
57	.547	.445	.0004	.0001	
71	.495	.493	.0002	.0001	
85	.578	.413	.0004	.0001	
99	.603	.384	.0002	.004	
113	.627	.364	.0001	.002	
127	.618	.37	.0002	.002	
141	.659	.329	.0001	.004	
155	.675	.31	.0001	.008	
175	.697	.289	.0001	.005	

Microcosm 6

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.768	.222	.0004		
29	.585	.406	.0003	.0001	
43	.467	.524	.0002	.0001	
57	.526	.464	.0004	.0001	
71	.468	.521	.0002	.0001	
85	.532	.459	.0004	.0001	
99	.58	.408	.0002	.002	
113	.646	.343	.0001	.002	
127	.637	.352	.0002	.002	
141	.673	.314	.0001	.003	
155	.689	.295	.0001	.006	
175	.706	.281	.0001	.004	

Microcosm 8

Day	N_2	O_2	CO_2	CH_4	C_2H_4
0	.781	.209	.0003	.0000	.0000
15	.754	.236	.0003		
29	.547	.447	.0004	.0001	
43	.444	.547	.0002	.0001	
57	.532	.458	.0004	.0001	
71	.475	.514	.0002	.0001	
85	.601	.391	.0004	.0001	
99	.584	.402	.0002	.005	
113	.631	.358	.0001	.002	
127	.635	.355	.0002	.002	
141	.657	.331	.0001	.003	
155	.661	.325	.0001	.004	
175	.653	.336	.0001	.001	

Table B-2. Nutrients, mg/l.

Unfiltered

Microcosm 1

Day	TP	TC	TFe	SS	VSS	Fe^{++}	I.C.	T.O.C.
0	.035	3.1	.0005	.05	.05	.0005	1.	2.1
15	.375	21.	.132	20.	4.2	.031	19.5	1.5
29	.081	17.	.104	11.	1.6	.070	16.	1.
43	.085	16.	.073	8.5	1.	.047	13.5	2.5
57	.070	14.	.063	5.5	1.5	.021	10.	4.
71	.448	158.	.075	5.	2.2	.044	130.	28.
85	.279	40.	.057	4.4	2.7	.035	24.	16.
99	.265	23.	.116	9.6	3.1	.114	13.5	9.5
113	.239	25.	.155	6.	.8	.186	10.	15.
127	.191	13.	.055	5.	.8	.027	9.	4.
141	.149	13.	.013	2.1	1.2	.011	8.	5.
155	.190	12.	.023	1.5	.9	.005	5.	7.
175	.261	12.	.036	1.1	.9	.036	12.	.01

Microcosm 3

Day	TP	TC	TFe	SS	VSS	Fe^{++}	I.C.	T.O.C.
0	.035	3.1	.033	.05	.05	.0005	1.	2.1
15	.088	22.	.107	18.	3.6	.033	20.	2.
29	.069	15.	.090	6.8	1.4	.048	15.	.01
43	.071	16.	.041	5.9	1.2	.006	14.	2.
57	.059	14.	.042	4.4	.9	.014	12.	2.
71	.067	14.5	.030	3.	1.6	.01	12.5	2.
85	.130	16.	.038	1.6	1.6	.013	9.5	6.5
99	.078	15.	.011	4.3	2.1	.008	9.5	5.5
113	.077	18.	.011	2.2	1.6	.007	12.	6.
127	.080	10.5	.018	3.1	1.1	.003	10.5	.01
141	.108	13.	.012	4.4	1.7	.006	9.	4.
155	.270	14.	.049	2.9	.8	.049	5.5	8.5
175	.342	10.	.585	2.	.7	.291	10.	.01

Microcosm 2

Day	TP	TC	TFe	SS	VSS	Fe^{++}	I.C.	T.O.C.
0	.035	3.1	.0099	.05	.05	.0005	1.	2.1
15	.087	18.	.171	11.	3.	.041	18.	.01
29	.071	15.	.069	8.	.9	.038	15.	.01
43	.075	17.	.041	6.2	1.8	.006	14.5	2.5
57	.070	14.	.052	7.4	.05	.023	12.	2.
71	.070	17.5	.042	5.2	.7	.014	12.	5.5
85	.068	.01	.021	2.8	1.4	.003	11.	6.
99	.071	17.5	.012	3.1	1.9	.006	10.5	7.
113	.086	17.	.011	2.6	1.8	.010	11.	6.
127	.086	11.	.009	2.8	.5	.004	10.5	.5
141	.167	12.5	.032	2.2	1.8	.029	9.	3.5
155	.353	15.	.131	1.3	1.1	.113	5.5	9.5
175	.211	9.	.401	2.6	1.8	.273	9.	.01

Microcosm 4

Day	TP	TC	TFe	SS	VSS	Fe^{++}	I.C.	T.O.C.
0	.035	3.1	.033	.05	.05	.0005	1.	2.1
15	.071	24.	.098	12.	7.3	.095	20.	4.
29	.052	14.	.026	4.2	.1	.019	16.	.01
43	.071	17.	.077	8.3	1.6	.010	14.	3.
57	.060	14.	.091	8.6	1.4	.029	12.	2.
71	.065	17.	.045	7.	1.5	.009	14.	3.
85	.059	16.	.029	5.4	1.5	.020	11.	5.
99	.070	17.5	.011	3.7	1.8	.011	11.	6.5
113	.083	16.	.003	1.8	1.6	.004	12.	4.
127	.078	8.	.012	2.3	1.1	.004	8.	.01
141	.190	13.	.003	2.8	.8	.003	9.	4.
155	.223	13.	.034	2.8	1.2	.019	6.	7.
175	.333	11.	.885	3.6	.8	.409	10.	1.

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Table B-2. Continued.

Microcosm 5

Day	TP	TC	TFe	SS	VSS	Fe++	I.C.	T.O.C.
0	.035	3.1	.0005	.05	.05	.0005	1.	2.1
15	.134	20.	.156	34.	7.4	.148	15.	5.
29	.012	7.	.213	27.	6.1	.185	2.	5.
43	.041	7.	.059	16.	3.2	.004	3.5	3.5
57	.131	9.	.383	63.	8.8	.052	3.	6.
71	.262	10.	.395	41.	6.8	.056	3.5	6.5
85	.382	10.	.165	18.	4.7	.047	4.	6.
99	.073	13.	.119	21.	6.3	.063	2.5	10.5
113	.070	7.	.055	12.	2.3	.041	3.	4.
127	.035	5.	.102	14.	3.8	.030	2.	3.
141	.033	6.	.077	13.	2.2	.059	2.5	3.5
155	.045	7.	.067	11.	.5	.041	2.	5.
175	.046	6.	.065	8.5	.2	.028	5.	1.

Microcosm 7

Day	TP	TC	TFe	SS	VSS	Fe++	I.C.	T.O.C.
0	.035	3.1	.033	.05	.05	.0005	1.	2.1
15	.059	11.	.140	20.	7.8	.045	6.5	4.5
29	.053	6.5	.216	25.	5.4	.212	2.	4.5
43	.052	6.5	.105	20.	3.6	.011	2.5	4.
57	.079	9.	.392	47.	8.	.025	3.	6.
71	.123	9.	.573	38.	7.4	.054	2.	7.
85	.122	10.	.274	30.	6.7	.147	2.	8.
99	.047	7.	.268	15.	4.1	.120	2.5	4.5
113	.056	9.	.105	17.	4.4	.047	3.	6.
127	.042	6.	.158	25.1	5.1	.043	2.5	3.5
141	.035	8.	.057	15.	3.3	.057	2.5	3.5
155	.035	8.	.113	13.	1.8	.056	2.	6.
175	.032	5.	.062	9.	2.2	.029	3.	2.

Microcosm 6

Day	TP	TC	TFe	SS	VSS	Fe++	I.C.	T.O.C.
0	.035	3.1	.0099	.05	.05	.0005	1.	2.1
15	.112	13.	.201	15.	6.5	.053	11.	2.
29	.059	8.	.210	31.	8.6	.209	2.	6.
43	.083	7.5	.161	24.	6.	.017	3.	4.5
57	.252	13.	1.140	177.	27.	.252	4.	9.
71	.24	12.5	1.705	113.	17.1	.258	3.	9.5
85	.229	10.	.765	49.	7.8	.167	3.	7.
99	.100	8.	.868	51.	9.8	.363	2.5	5.5
113	.077	9.5	.164	34.	7.2	.062	3.	6.5
127	.047	8.	.188	24.	5.1	.038	3.5	4.5
141	.055	8.5	.175	29.	5.5	.086	3.	5.5
155	.096	11.5	.189	24.	2.6	.100	3.	8.5
175	.041	6.	.166	13.	1.1	.054	5.	1.

Microcosm 8

Day	TP	TC	TFe	SS	VSS	Fe++	I.C.	T.O.C.
0	.035	3.1	.033	.05	.05	.0005	1.	2.1
15	.077	15.	.086	28.	2.7	.045	10.5	4.5
29	.064	8.	.159	22.	5.8	.137	2.	6.
43	.104	8.	.128	30.	8.7	.015	2.	6.
57	.168	11.	.937	135.	18.	.067	3.	8.
71	.094	7.	.355	72.	10.3	.056	2.	5.
85	.090	14.	.092	10.	2.3	.054	5.	9.
99	.076	9.	.109	31.	6.4	.046	2.5	6.5
113	.038	7.	.086	11.	3.4	.033	3.	4.
127	.035	5.	.092	18.	4.1	.024	2.	3.
141	.099	7.5	.238	30.	5.7	.120	3.	4.5
155	.069	8.5	.098	21.	1.1	.098	3.	5.5
175	.034	5.	.100	10.	.05	.046	2.	2.

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Table B-2. Continued.

Filtered

Microcosm 1

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.0005
15	.329	.329	7.215	.038	7.177	.296	21.	18.	3.	.016
29	.063	.077	6.407	.011	6.396	.120	17.	16.	1.	.035
43	.070	.066	7.093	.002	7.091	.070	15.	13.5	1.5	.046
57	.052	.053	7.896	.002	7.894	.082	14.	10.	4.	.015
71	.258	.343	7.347	2.010	5.337	.636	158.	130.	28.	.044
85	.202	.245	5.930	.400	5.530	.315	31.	24.	7.	.057
99	.197	.196	7.161	.008	7.153	.121	23.	13.5	9.5	.063
113	.221	.239	9.168	.034	9.134	.238	20.	10.	10.	.081
127	.159	.159	1.420	.002	1.418	.176	13.	9.	4.	.055
141	.141	.141	.149	.002	.147	.266	11.	8.	3.	.013
155	.140	.148	.022	.0005	.022	.316	11.	5.	6.	.010
175	.152	.261	.005	.0005	.005	.254	12.	12.	.01	.0005

Microcosm 2

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.0099
15	.047	.054	7.085	.098	6.987	.399	18.	17.	1.	.037
29	.054	.050	5.828	.131	5.697	.141	15.	15.	.01	.029
43	.063	.066	7.263	.055	7.208	.054	15.5	14.5	1.	.035
57	.056	.056	7.400	.027	7.373	.048	14.	12.	2.	.019
71	.064	.064	7.191	.085	7.106	.046	16.	12.	4.	.028
85	.058	.058	6.021	.066	5.955	.075	16.	11.	5.	.004
99	.055	.055	6.892	.014	6.878	.049	13.	10.5	2.5	.012
113	.054	.086	7.205	.051	7.154	.093	17.	11.	6.	.007
127	.068	.072	.820	.016	.804	.019	10.	10.	.01	.007
141	.155	.159	.012	.0005	.012	.192	12.5	9.	3.5	.012
155	.303	.303	.0005	.0005	.0005	.224	12.5	5.5	7.	.047
175	.202	.202	.0005	.0005	.0005	.202	9.	9.	.01	.091

Microcosm 3

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.033
15	.042	.049	7.378	.145	7.233	.079	22.	19.	3.	.028
29	.047	.054	6.299	.183	6.116	.032	15.	15.	.01	.023
43	.056	.060	7.604	.129	7.475	.018	15.	14.	1.	.016
57	.048	.050	7.247	.188	7.058	.050	14.	12.	2.	.008
71	.059	.059	7.155	.111	7.044	.052	14.5	12.5	2.	.015
85	.111	.111	6.460	.059	6.401	.063	9.5	9.5	.01	.038
99	.061	.069	7.354	.013	7.341	.049	14.	9.5	4.5	.006
113	.053	.069	8.175	.135	8.040	.086	18.	12.	6.	.007
127	.057	.061	1.200	.036	1.164	.005	9.5	9.5	.01	.004
141	.095	.101	.011	.0005	.011	.104	13.	9.	4.	.012
155	.230	.236	.0005	.0005	.0005	.230	13.	5.5	7.5	.008
175	.305	.305	.002	.002	.0005	.145	10.	10.	.01	.356

Table B-2. Continued.

Microcosm 4

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.033
15	.042	.046	7.768	.034	7.734	.206	24.	19.	5.	.018
29	.042	.053	6.226	.100	6.126	.048	14.	14.	.01	.009
43	.048	.052	7.263	.061	7.202	.020	15.5	14.	1.5	.027
57	.041	.044	7.961	.190	7.771	.033	14.	12.	2.	.011
71	.052	.052	7.413	.199	7.214	.046	15.	14.	1.	.021
85	.049	.049	9.002	.108	8.894	.053	15.	11.	4.	.004
99	.054	.054	7.007	.023	6.984	.049	15.5	11.	4.5	.004
113	.058	.083	7.238	.023	7.215	.120	16.	12.	4.	.001
127	.062	.066	1.180	.003	1.177	.010	8.	8.	.01	.009
141	.093	.097	.033	.001	.032	.077	13.	9.	4.	.003
155	.213	.223	.0005	.0005	.0005	.168	13.	6.	7.	.007
175	.263	.294	.0005	.0005	.0005	.169	10.	10.	.01	.390

Microcosm 5

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.0005
15	.040	.046	6.272	.055	6.217	.041	20.	14.	6.	.003
29	.012	.012	4.634	.055	4.579	.022	5.5	2.	3.5	.030
43	.012	.015	5.183	.185	4.998	.046	5.5	3.5	2.	.018
57	.019	.029	5.820	.100	5.720	.037	6.	3.	3.	.141
71	.016	.048	6.123	.187	5.936	.268	8.5	3.5	5.	.043
85	.013	.017	5.198	.148	5.050	.310	6.	4.	2.	.005
99	.016	.029	6.699	.025	6.674	.162	6.5	2.5	4.	.005
113	.009	.016	5.997	.108	5.889	.171	7.	3.	4.	.0005
127	.007	.013	.481	.021	.460	.024	5.	2.	3.	.005
141	.012	.024	.004	.0005	.104	.051	5.5	2.5	3.	.010
155	.006	.029	.0005	.0005	.0005	.079	7.	2.	5.	.001
175	.012	.020	.0005	.0005	.0005	.031	6.	5.	1.	.008

Microcosm 6

Day	PO$_4$-P	T.P.	(NO$_3$+NO$_2$)-N	NO$_2$-N	NO$_3$-N	NH$_3$-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.0009
15	.010	.008	7.605	.093	7.512	.045	13.	11.	2.	.005
29	.008	.008	4.344	.042	4.302	.024	6.5	2.	4.5	.020
43	.011	.018	4.876	.120	4.756	.077	6.	3.	3.	.010
57	.038	.050	4.921	.139	4.782	.219	8.	4.	4.	.295
71	.034	.045	4.993	.286	4.707	.427	6.5	3.	3.5	.102
85	.021	.027	5.013	.290	4.723	.546	10.	3.	7.	.043
99	.012	.023	6.160	.083	6.077	.409	8.	2.5	5.5	.145
113	.012	.013	5.856	.759	5.097	.295	7.	3.	4.	.006
127	.006	.013	.347	.176	.171	.236	6.	3.5	2.5	.003
141	.016	.017	.004	.001	.003	.079	8.5	3.	5.5	.031
155	.013	.042	.002	.002	.0005	.309	11.	3.	8.	.036
175	.018	.070	.001	.001	.0005	.019	5.5	5.	.5	.008

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Table B-2. Continued.

Microcosm 7

Day	PO₄-P	T.P.	(NO₃+NO₂)-N	NO₂-N	NO₃-N	NH₃-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.033
15	.001	.007	6.078	.066	6.012	.040	11.	6.5	4.5	.007
29	.010	.021	4.272	.040	4.232	.018	6.5	2.	4.5	.036
43	.007	.014	5.217	.189	5.028	.016	5.5	2.5	3.	.004
57	.009	.014	5.890	.146	5.744	.225	5.	3.	2.	.022
71	.014	.023	5.937	.187	5.750	.156	7.	2.	5.	.019
85	.009	.009	4.871	.184	4.687	.222	10.	2.	8.	.018
99	.008	.021	6.660	.066	6.594	.645	7.	2.5	4.5	.038
113	.006	.009	6.379	.257	6.122	.231	6.	3.	3.	.007
127	.007	.014	.410	.049	.351	.024	4.	2.5	1.5	.0005
141	.010	.027	.003	.0005	.003	.032	7.	2.5	4.5	.003
155	.004	.024	.0001	.0005	.0005	.069	6.5	2.	4.5	.032
175	.011	.023	.003	.0005	.003	.030	5.	3.	2.	.006

Microcosm 8

Day	PO₄-P	T.P.	(NO₃+NO₂)-N	NO₂-N	NO₃-N	NH₃-N	T.C.	I.C.	T.O.C.	T.Fe
0	.035	.035	10.4	.0005	10.4	.052	3.1	1.	2.1	.033
15	.004	.009	6.500	.069	6.431	.045	15.	11.	4.	.0005
29	.005	.005	4.851	.034	4.817	.013	5.	2.	3.	.0005
43	.003	.009	6.479	.152	6.327	.177	4.	2.	2.	.006
57	.012	.029	6.248	.128	6.120	.274	6.	3.	3.	.245
71	.015	.027	5.589	.36	5.229	.668	7.	2.	5.	.023
85	.009	.015	5.034	.438	4.596	.766	7.	5.	2.	.002
99	.017	.025	6.391	.042	6.349	.255	6.	2.5	3.5	.002
113	.004	.015	5.823	.380	5.443	.343	6.	3.	3.	.0005
127	.005	.009	.530	.114	.416	.236	5.	2.	3.	.001
141	.014	.023	.004	.002	.002	.162	5.5	3.	2.5	.039
155	.010	.045	.002	.002	.0005	.072	8.5	3.	5.5	.087
175	.011	.015	.0005	.0005	.0005	.031	5.	3.	2.	.030

Table B-3. Atmospheric pressure (mm Hg), room temperature and effluent temperature (°C) for specific micro-cosms 1-8.

Day	Atm P	R.T.	M 1	2	3	4	5	6	7	8
1	643.8	24.	21.4	21.6	22.2	22.7	22.2	22.3	22.3	22.4
2	644.	21.3	22.8	20.6	21.	21.5	20.8	21.	21.1	21.
3	642.	22.	20.3	20.6	21.1	21.6	20.9	21.1	21.	21.
4	640.	21.3	20.1	20.4	20.9	21.3	20.6	20.9	20.6	20.6
5	643.3	20.3	19.8	20.1	20.6	21.	20.2	20.4	20.3	20.2
6	647.	22.	20.8	21.1	21.7	21.2	21.7	21.8	21.8	21.7
7	648.3	20.7	20.2	20.7	21.	21.4	20.7	21.1	20.9	20.8
8	649.8	20.6	19.1	19.9	20.5	20.9	20.4	20.4	20.4	20.4
9	641.4	22.1	22.	20.6	21.3	21.6	21.	21.2	21.4	21.1
10	638.8	21.1	20.1	20.4	20.1	21.4	20.8	20.9	20.8	20.8
11	644.3	20.	18.9	19.3	19.8	20.6	19.2	19.5	19.3	19.2
12	643.2	19.2	18.4	18.7	19.3	19.8	18.6	18.5	18.6	18.5
13	639.5	20.6	18.7	18.9	19.5	19.9	18.7	18.7	18.2	18.8
14	640.4	20.	18.7	19.1	19.6	20.	19.	18.8	18.9	19.1
15	639.9	20.	18.8	18.9	19.4	19.9	18.9	18.9	18.8	18.9
16	637.9	20.	18.6	18.9	19.5	20.	19.	18.9	18.9	19.1
17	648.2	22.5	20.4	21.1	21.7	22.1	21.6	21.6	21.4	21.4
18	649.3	18.9	18.7	18.9	19.4	19.7	19.2	19.1	19.1	19.
19	642.8	18.3	17.4	17.5	18.	18.3	17.9	17.8	17.9	17.8
20	641.0	23.1	20.	19.9	20.4	21.	20.4	20.4	20.6	20.7
21	648.6	23.5	21.4	21.4	21.9	22.3	21.5	21.6	21.6	21.7
22	649.2	25.5	21.5	21.6	22.3	22.2	21.8	21.8	21.8	22.
23	644.3	23.	21.5	21.8	22.5	22.9	22.3	22.3	22.2	22.4
24	653.9	22.8	21.6	21.8	22.5	23.	22.1	22.1	22.1	22.2
25	651.3	23.5	21.7	22.	22.1	23.2	22.2	22.4	22.4	22.6
26	647.6	22.5	22.4	21.8	22.7	23.1	22.3	22.5	22.4	22.4
27	642.	22.5	20.8	21.3	21.8	22.4	21.3	21.4	21.4	21.5
28	641.7	23.4	21.2	21.4	22.2	22.6	21.9	21.7	21.3	21.9
29	645.1	22.6	21.4	21.7	22.3	22.7	21.9	22.	22.	22.1
30	647.5	22.9	21.6	22.2	22.5	23.2	22.1	22.3	22.3	22.3
31	638	23.2	21.6	21.9	22.6	23.1	22.1	22.2	22.3	22.3
32	635.6	23.8	21.7	22.	22.6	23.1	22.2	22.2	22.3	22.4
33	634.6	23.4	21.6	21.9	22.4	23.	22.1	22.1	22.2	22.3
34	633.5	22.9	21.4	21.8	22.4	22.8	22.	22.2	22.1	22.1
35	641.3	22.5	20.7	21.1	21.4	21.9	21.5	21.6	21.7	21.5
36	649.5	22.1	20.9	21.1	21.6	22.1	21.7	21.7	21.7	21.9
37	642.5	23.	20.9	21.3	21.8	22.2	21.7	21.6	22.6	21.7
38	651.6	22.3	20.8	21.1	21.7	22.2	21.1	21.8	22.	21.9
39	655.8	22.9	21.	21.3	21.7	22.3	21.8	21.9	22.	22.
40	651.	22.1	20.9	21.3	21.5	21.3	21.7	21.9	22.	21.9
41	642.2	21.3	20.7	20.9	20.5	20.9	21.3	21.4	21.5	21.5
42	642.9	21.9	20.5	20.8	21.3	21.8	21.3	21.4	21.5	21.3
43	636.2	22.	20.9	21.2	21.8	22.3	21.7	21.8	21.8	22.
44	639.1	22.9	21.4	21.6	22.2	22.6	22.2	22.3	22.3	22.4
45	630.4	23.	21.7	22.	22.6	22.9	22.4	22.6	22.7	22.7

Table B-3. Continued.

Day	Atm P	R.T.	M 1	2	3	4	5	6	7	8
46	635.1	22.7	21.4	21.6	22.2	22.7	22.	22.2	22.3	22.4
47	643.6	23.	21.4	21.7	22.1	22.6	22.	22.3	22.4	22.6
48	642.1	23.3	21.3	22.	22.7	23.9	22.6	22.8	22.9	22.9
49	634.5	23.4	22.	22.2	22.3	23.3	22.3	22.9	23.	23.1
50	632.1	24.1	22.3	22.6	23.1	23.6	23.	23.3	23.4	23.6
51	636.	24.1	22.3	22.3	23.3	23.7	23.3	23.4	23.5	22.3
52	645.8	23.8	22.4	22.6	23.2	23.6	23.2	23.3	23.3	23.5
53	642.7	24.	22.6	22.8	23.4	23.9	23.3	23.4	23.5	23.7
54	644.3	24.1	22.1	21.1	23.5	23.7	23.4	23.6	22.5	23.6
55	643.4	24.	22.7	22.8	23.4	23.8	23.2	23.3	23.4	23.5
56	643.7	24.	22.3	22.9	23.5	23.8	23.4	23.5	23.9	23.6
57	643.3	23.5	22.4	22.4	22.9	23.4	22.9	23.	23.1	23.2
58	646.1	23.7	22.	22.5	23.	23.5	22.8	23.2	23.2	23.3
59	645.	23.7	22.4	22.7	23.3	23.6	23.1	23.3	23.3	23.5
60	644.5	23.8	22.8	22.9	23.4	23.2	23.5	23.4	23.1	23.7
61	643.7	24.	22.3	22.8	23.2	23.9	23.4	23.6	23.4	23.5
62	641.5	23.6	22.4	22.5	23.1	23.4	22.6	22.9	23.1	23.3
63	646.7	23.5	22.8	23.1	23.5	24.2	23.2	23.2	23.4	23.7
64	645.6	23.1	23.2	22.5	23.	23.4	22.8	22.9	23.3	23.4
65	638.5	23.5	22.1	22.3	22.8	23.3	22.5	22.8	23.1	23.4
66	640.	23.5	22.1	22.5	22.9	23.3	22.8	23.	23.2	23.5
67	642.	23.5	22.4	22.6	23.2	23.6	22.9	23.2	23.4	23.7
68	641.8	24.2	22.5	22.8	23.2	23.5	23.2	23.2	23.4	23.6
69	642.2	24.5	23.3	23.4	23.9	24.3	23.7	23.9	24.1	24.4
70	641.4	24.	22.9	23.1	23.7	24.1	23.6	23.5	23.7	24.1
71	640.	24.5	22.8	23.1	23.6	24.	23.4	23.4	23.6	24.
72	645.6	23.3	22.3	22.7	23.2	23.6	22.7	22.7	22.9	23.1
73	635.	23.5	22.4	22.5	22.3	22.4	22.8	22.2	23.4	23.
74	638.2	22.3	21.5	21.6	22.	22.5	21.3	21.2	21.8	21.7
75	636.2	23.	22.1	22.2	22.8	23.3	22.5	22.8	22.7	23.
76	632.8	23.1	22.3	22.4	23.	23.5	22.9	23.1	23.1	23.4
77	640.9	23.	22.4	22.6	23.1	23.7	22.9	23.	23.1	23.4
78	649.8	23.5	22.7	22.8	23.3	23.8	23.	23.2	23.3	23.5
79	649.3	23.6	22.7	22.9	23.4	23.8	23.	23.3	23.4	23.5
80	640.	23.5	22.8	23.	23.4	23.9	23.	23.2	23.3	23.6
81	648.2	24.	22.8	23.	23.4	23.9	23.2	23.3	23.3	23.5
82	646.4	23.5	22.8	23.1	23.5	23.9	23.4	23.4	23.4	23.6
83	632.9	24.5	23.3	23.5	24.	24.5	23.8	23.8	23.9	24.2
84	632.2	23.8	23.3	23.4	23.9	24.5	23.9	23.8	23.9	24.1
85	641.3	23.5	22.6	22.7	23.3	23.7	23.1	23.	23.1	23.4
86	639.2	23.5	22.4	22.8	23.3	23.7	23.2	23.2	23.4	23.7
87	645.	23.5	22.2	22.9	23.3	23.7	23.	23.	23.1	23.5
88	644.7	23.5	22.2	22.7	23.2	23.6	22.9	23.	23.1	23.3
89	647.9	23.8	22.8	23.3	23.8	24.3	23.4	23.4	23.6	23.8
90	645.8	24.2	23.7	24.2	24.9	25.4	24.1	24.1	24.2	24.3

Table B-3. Continued.

Day	Atm P	R.T.	M	1	2	3	4	5	6	7	8
91	645.	23.5	23.5	24.	24.6	25.	24.2	24.2	24.3	24.4
92	638.5	21.	21.4	22.	22.4	22.6	21.9	22.	22.3	22.3
93	635.2	25.	24.	24.4	24.6	24.9	24.7	24.8	24.7	25.
94	637.9	25.	24.1	24.5	25.	25.5	24.7	24.8	25.	25.1
95	645.2	23.5	23.	23.5	24.	23.9	23.6	23.7	23.5	23.8
96	646.2	23.3	22.2	22.5	23.	23.5	22.4	22.4	22.5	22.7
97	639.4	25.4	23.8	24.2	24.9	25.4	24.7	25.	25.	25.1
98	640.	25.5	24.7	25.	25.6	26.1	25.4	25.5	25.6	25.8
99	642.2	26.4	25.3	25.4	26.3	26.7	26.1	26.2	26.3	26.4
100	641.2	27.5	26.	26.4	27.	27.4	26.8	26.9	27.	27.2
101	643.8	25.5	25.	25.5	25.9	26.4	25.6	25.5	25.7	25.8
102	645.8	25.6	24.9	25.4	25.9	26.3	25.4	25.5	25.6	25.7
103	649.7	25.5	24.6	25.1	25.6	26.	25.	25.1	25.2	25.4
104	648.5	25.5	24.2	24.5	25.2	25.6	25.1	25.4	25.4	25.6
105	642.2	25.2	24.4	25.	25.3	25.8	25.3	25.6	25.5	25.7
106	642.4	27.	25.	25.6	25.9	26.4	26.2	26.5	26.5	26.6
107	643.2	26.	24.7	25.3	25.6	25.9	25.6	25.8	25.9	26.
108	644.5	26.	24.3	25.4	25.7	26.1	25.2	25.9	26.	26.2
109	644.5	26.5	25.4	25.9	26.3	26.7	26.3	26.5	26.5	26.7
110	642.4	26.8	25.7	26.4	26.6	27.	26.5	26.4	26.8	27.
111	642.6	25.2	26.6	27.3	27.4	27.9	27.8	27.8	27.8	27.7
112	641.4	24.5	24.1	24.6	24.7	25.	24.7	24.9	24.8	24.9
113	638.6	23.8	23.4	23.9	24.	24.3	24.1	24.2	24.3	24.3
114	639.9	24.2	23.	23.7	24.	24.1	23.5	24.	24.	24.
115	641.2	22.5	22.	22.5	22.7	23.	22.7	22.9	22.9	22.7
116	635.5	22.5	22.1	22.6	22.8	23.1	22.7	22.9	22.9	22.9
117	641.	22.5	21.8	22.3	22.5	22.7	22.3	22.4	22.3	22.3
118	639.2	22.5	22.2	22.7	22.9	23.1	22.4	22.7	22.4	22.6
119	636.7	23.5	22.4	27.8	23.	23.3	22.5	22.8	22.7	22.8
120	638.5	22.5	22.2	22.6	22.8	23.	22.6	22.7	22.7	22.7
121	634.	23.	22.8	23.4	23.6	23.8	23.2	23.3	23.3	23.2
122	637.2	23.5	23.	23.4	23.6	23.9	23.4	23.5	23.5	23.5
123	634.5	23.7	23.1	23.5	23.8	23.9	23.5	23.6	23.6	23.6
124	640.	22.5	22.1	22.5	22.8	22.9	22.4	22.5	22.4	22.5
125	645.4	20.8	20.8	21.4	21.4	21.5	20.9	21.	21.	20.8
126	644.4	21.4	21.2	21.4	21.6	21.9	21.3	21.4	21.4	21.3
127	643.2	22.5	22.2	22.4	22.5	22.7	22.3	22.4	22.4	22.4
128	644.9	23.6	23.2	23.4	23.5	23.4	23.	23.4	23.3	23.2
129	646.8	24.5	24.1	24.1	24.4	24.1	24.2	24.2	24.4	24.3
130	646.2	25.4	23.9	23.8	24.	24.	24.4	24.5	24.7	24.6
131	639.2	26.	24.8	24.8	25.	25.	25.4	25.1	25.1	25.6
132	641.3	26.	25.3	25.9	25.2	25.3	25.7	25.7	25.8	25.7
133	637.8	26.	25.4	25.1	25.4	25.5	25.7	25.8	26.	26.
134	642.6	24.5	24.2	24.2	24.5	24.4	24.7	24.9	24.7	24.6
135	644.2	25.	24.1	24.2	24.6	24.8	24.6	24.7	24.7	24.7

Table B-3. Continued.

Day	Atm P	R.T.	M	1	2	3	4	5	6	7	8
136	644.3	24.5	24.3	24.5	25.	25.	24.5	24.6	24.6	24.6
137	645.	25.2	24.6	25.	24.6	24.9	25.1	24.6	24.6	25.
138	642.7	25.2	25.	25.3	25.4	25.6	25.1	25.1	25.1	25.2
139	641.9	25.5	24.9	25.	25.4	25.7	25.4	25.4	25.4	25.5
140	641.1	25.5	24.8	25.	25.5	25.5	25.3	25.2	25.3	25.2
141	642.	24.	23.7	23.7	24.	24.3	24.	24.	24.	24.1
142	635.9	24.	23.1	23.3	23.6	23.9	23.5	23.4	23.7	23.7
143	642.3	23.5	22.4	22.6	22.9	23.	22.6	22.6	22.6	23.
144	644.4	22.5	22.3	22.4	22.8	22.4	22.6	22.6	22.7	22.1
145	645.	23.8	22.7	22.9	22.9	23.4	23.3	23.4	23.5	23.5
146	645.8	24.6	22.9	22.9	23.3	23.4	23.	23.1	23.2	23.2
147	643.8	25.	23.	23.	23.5	24.	23.	23.5	23.5	23.5
148	641.8	26.	23.8	23.9	24.2	24.6	24.2	23.9	24.3	24.4
149	643.7	27.8	24.9	25.	25.2	25.5	25.7	25.8	25.7	25.8
150	643.6	27.3	24.9	25.	25.4	25.4	25.4	25.8	25.8	25.9
151	640.9	27.8	25.5	25.7	26.0	26.	26.	26.	26.	26.1
152	641.5	28.	25.5	25.5	25.8	26.1	26.3	26.3	26.6	26.3
153	640.6	28.5	25.8	25.5	25.5	25.5	27.	26.8	26.8	26.7
154	640.5	28.	26.	26.	26.	26.	26.	26.	26.	27.
155	641.	28.8	26.1	26.1	26.5	27.	27.	27.1	27.1	27.
156	645.6	27.6	27.1	26.1	26.6	26.6	26.7	26.8	27.	26.8
157	641.6	27.5	26.	26.	26.5	26.9	26.5	26.5	26.	26.
158	642.2	28.5	26.3	26.6	26.6	26.8	26.8	26.8	26.8	26.1
159	644.	28.8	26.8	27.	27.2	27.3	27.2	27.3	27.2	27.3
160	642.8	29.	26.8	26.9	27.1	26.8	27.1	26.8	27.1	27.5
161	645.1	29.	26.8	26.4	26.4	27.8	26.4	27.1	26.8	27.8
162	645.5	28.7	27.	27.	27.5	27.5	27.5	28.	27.5	27.5
163	646.3	28.	26.3	26.5	26.8	26.5	26.5	26.8	26.9	26.6
164	647.8	27.5	25.5	25.8	25.8	25.8	26.3	27.	26.5	26.5
165	644.7	28.5	26.5	26.8	27.	27.	27.	27.2	27.3	27.
166	639.5	29.	27.1	27.3	27.3	27.3	27.9	27.5	27.5	27.5
167	638.9	29.	27.	27.	27.2	27.2	27.5	27.7	27.5	27.5
168	648.4	27.5	26.	26.1	26.9	26.8	26.8	27.	27.	26.5
169	647.1	27.	26.	26.1	26.1	26.1	26.5	26.1	26.1	26.1
170	641.7	27.5	25.4	26.	26.1	26.3	25.2	26.1	26.4	26.
171	639.5	28.5	26.2	26.2	27.	27.	27.	26.9	27.1	27.
172	640.9	28.5	26.3	27.1	27.5	27.1	27.5	27.1	27.1	27.1
173	641.5	28.5	29.4	27.1	27.1	28.	27.6	27.5	27.8	27.3
174	641.9	28.5	26.4	26.8	27.1	27.1	27.	27.	27.1	27.1
175	642.2	28.8	26.9	27.	27.5	27.2	27.6	27.5	27.5	27.5

Table B-4. Nutrient/gas (mg/l) accumulation values (mg).

Microcosm 1

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	5.2027	2.0187	277.7700	-2.1352	35.3657	-169.0794	37.7272	0.0000	0.00
29	5.1201	3.2661	450.8941	35.0823	74.9867	-272.9969	56.7599	0.0489	0.00
43	5.7694	4.1329	618.8433	74.0368	118.4408	-370.9491	69.5085	0.0751	0.00
57	6.1806	4.9107	758.2100	114.9261	146.7505	-435.5171	97.1996	0.1008	0.00
71	12.2432	5.8849	3044.2930	117.6398	115.4487	-495.6820	92.7523	7.4797	0.00
85	14.9293	6.5608	3245.6024	54.4416	43.8361	-624.6580	101.2241	10.6078	0.00
99	17.8666	8.1823	3482.2691	58.9855	-9.6390	-724.0029	226.2904	9.5817	0.00
113	20.3850	10.2272	3783.1076	74.7211	-14.6504	-819.1389	241.1118	12.2561	0.00
127	22.2459	10.6981	3888.2620	78.9886	-38.7686	-913.3221	256.4052	12.5575	0.00
141	23.5878	10.7674	4017.6073	74.1511	-106.4841	-945.9823	271.4000	12.0261	0.30
155	25.6330	11.0797	4132.7093	87.7252	-121.5305	-1029.2094	294.3555	11.9243	0.00
175	29.6977	11.7262	4301.5552	122.8342	-130.8488	-1135.8362	296.8249	17.3640	0.00

Microcosm 2

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	0.7071	2.4693	229.8569	1.4606	6.4671	-135.0509	16.8099	0.0000	0.00
29	1.2147	2.9845	376.9165	30.9587	32.9556	-241.1394	32.3199	0.0436	0.00
43	1.7277	3.3134	560.3162	65.6198	72.0924	-341.8539	43.3951	0.0684	0.00
57	2.1574	3.8686	693.9649	116.0580	120.2245	-416.8203	53.5918	0.0946	0.00
71	2.5984	4.2505	837.3435	156.8255	155.2082	-492.2707	62.2003	2.9187	0.00
85	3.0097	4.3431	812.2336	191.8495	204.4707	-584.0123	71.6945	3.5080	0.00
99	3.4749	4.3503	1038.3625	227.7797	236.2805	-675.9676	79.7538	7.3182	0.00
113	4.1512	4.3619	1216.0719	262.1026	267.3303	-747.8273	84.8548	9.8257	0.00
127	4.7959	4.3461	1304.3075	269.3941	280.7630	-835.9972	88.9690	9.3973	0.00
141	6.6413	4.6763	1428.8294	278.5735	287.8875	-916.5779	94.9094	11.6761	0.00
155	11.0666	6.4249	1588.0806	289.7333	278.7718	-997.0365	102.8432	14.4904	0.00
175	14.3159	13.3537	1708.3623	314.6573	281.0799	-1098.3321	120.1741	26.2920	0.00

Table B-4. Continued.

Microcosm 3

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	0.8124	1.1343	292.2851	-7.3570	32.3744	-166.7674	25.7485	0.0000	0.00
29	1.2001	1.8174	432.8979	25.2167	62.5915	-271.0512	47.3375	0.0520	0.00
43	1.6582	1.8080	602.6755	59.2254	95.2031	-364.5585	60.6233	0.0766	0.00
57	1.9336	1.9236	739.5036	96.2121	118.5230	-432.7595	75.2921	0.1013	0.00
71	2.3566	1.8594	890.0114	119.9130	131.5864	-515.8042	88.1571	2.2400	0.00
85	3.6953	1.9404	1082.4605	128.6332	101.6059	-561.7744	287.9666	5.2859	0.00
99	4.1201	1.6025	1262.9890	137.2396	114.1806	-658.0294	340.6379	4.9372	0.00
113	4.6513	1.3231	1472.0523	139.8539	104.9406	-722.5882	350.6462	5.8447	0.00
127	5.2268	1.1491	1554.0839	144.4214	93.6511	-784.4037	360.9190	6.7650	0.00
141	6.2170	0.8687	1690.0384	133.5986	85.6396	-891.9228	373.0315	8.5690	0.00
155	9.5571	1.1535	1836.3228	142.9089	66.0343	-978.7538	388.4354	11.7262	0.00
175	15.0651	10.8692	1975.0037	137.7250	16.8012	-1086.3136	412.8168	21.5696	0.00

Microcosm 4

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	0.5518	0.9983	322.2802	7.0758	19.7404	-138.4268	20.6914	0.0000	0.00
29	0.7233	0.7461	442.1711	37.9846	53.2323	-239.1376	41.9526	0.0437	0.00
43	1.2196	1.4153	628.1091	56.6630	62.9596	-321.4099	48.2014	0.0676	0.00
57	1.5158	2.1880	764.3800	83.9737	83.3970	-398.5638	64.6496	0.0924	0.00
71	1.9059	2.2362	950.8262	107.2742	105.3326	-485.4951	72.0808	1.8796	0.00
85	2.1948	2.1498	1117.3175	122.4016	126.9097	-576.7251	100.7843	4.6641	0.00
99	2.6606	1.8321	1307.7211	145.7124	147.5663	-664.5264	105.1404	5.6326	0.00
113	3.2946	1.4361	1470.9421	149.2989	152.1762	-747.5892	125.3886	6.1349	0.00
127	3.8266	1.1909	1519.3741	149.8829	139.2951	-826.2607	131.8677	7.0079	0.00
141	6.0346	0.7919	1658.0134	128.4505	75.5843	-897.3372	136.1366	7.6814	0.00
155	8.4818	0.8737	1785.7321	127.2226	39.8915	-995.7248	150.0129	7.6580	0.00
175	13.7963	15.6268	1936.9263	143.3150	19.1881	-1099.5463	170.4229	13.1327	0.00

Table B-4. Continued.

Microcosm 5

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	1.5147	2.3859	258.2017	112.3159	90.2108	0.7003	0.3492	0.0000	0.00
29	0.9504	5.1980	277.8248	475.3340	214.4916	373.9172	-2.2343	0.0715	0.00
43	1.0903	5.6034	326.3158	765.7002	288.2026	718.0277	-6.5171	0.1164	0.00
57	2.5024	11.1582	404.6032	954.7881	385.5720	869.1065	-6.2741	0.1535	0.00
71	5.6574	16.1622	497.4131	1001.6956	303.6010	1011.8933	25.8082	0.1804	0.00
85	10.3195	17.7436	588.3952	1131.8943	376.8149	1117.8280	19.8652	0.2137	0.00
99	10.1106	19.1474	728.4169	1202.1904	373.8159	1196.5217	14.9482	1.3552	0.00
113	10.5517	19.7104	736.2022	1248.8904	398.0525	1221.4379	9.0743	1.8918	0.00
127	10.4736	21.1044	774.9636	1348.9532	419.9624	1320.8869	5.7495	2.5270	0.00
141	10.4439	22.0184	813.6050	1414.2922	441.9030	1360.9821	0.0506	4.2603	0.00
155	10.5969	22.8401	866.1893	1493.3012	450.4166	1421.4507	-5.5088	8.6980	0.00
175	10.7945	24.0110	923.0468	1593.6650	478.0089	1487.0554	-12.7206	18.4065	0.00

Microcosm 6

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	1.1828	2.9356	151.5830	113.2913	55.3854	16.3713	-0.3758	0.0000	0.00
29	1.3710	5.4966	202.5052	437.0442	193.9269	317.7018	-2.8576	0.0590	0.00
43	2.0298	7.2902	256.0455	741.5409	262.4419	700.4439	-6.9924	0.1050	0.00
57	5.1443	23.7322	392.6402	999.2667	370.5219	949.4794	-6.6401	0.1469	0.00
71	7.7003	46.3618	509.4236	1207.7185	389.7296	1245.9111	-11.1353	0.1854	0.00
85	10.1199	53.7610	590.2088	1404.3558	464.0483	1451.5928	-10.7424	0.2234	0.00
99	10.6579	64.8429	647.7235	1531.5748	501.5103	1571.5294	-15.4969	1.2268	0.00
113	11.1375	65.2108	731.2488	1645.7637	562.2114	1644.6854	-21.1916	1.8541	0.00
127	11.2252	67.5358	789.6916	1725.3748	573.8243	1741.9935	-24.4963	2.4635	0.00
141	11.4964	69.6026	858.7835	1791.0627	596.5499	1783.9567	-30.1198	3.5061	0.00
155	12.3572	71.8908	971.2283	1844.8795	601.5959	1814.7681	-35.6050	5.7194	0.00
175	12.4900	74.7109	1025.0989	1927.6729	628.6024	1865.4740	-42.6941	6.9084	0.00

Table B-4. Continued.

Microcosm 7

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	0.3672	1.6373	119.9744	175.6235	71.6021	115.1008	-4.2720	0.0000	0.00
29	0.5811	4.1189	152.2998	458.5361	188.8443	370.5970	-4.0489	0.0649	0.00
43	0.7931	4.7764	194.5915	789.9432	274.2325	765.4272	-8.5338	0.1125	0.00
57	1.4082	9.9455	274.0237	992.3089	362.3615	953.8981	-8.2368	0.1504	0.00
71	2.6219	17.1953	348.2648	1176.9255	371.7712	1216.3793	-12.8553	0.1871	0.00
85	3.7273	19.6104	437.7458	1321.6218	446.9242	1336.5369	-12.4914	0.2215	0.00
99	3.7110	22.5650	488.9552	1430.0573	475.7331	1434.7281	-17.2612	2.5267	0.00
113	3.9963	23.1107	558.9536	1532.7597	512.2035	1528.5559	-22.9388	2.5939	0.00
127	4.0530	24.8050	587.9893	1637.1968	537.4042	1648.8797	-26.1239	3.2335	0.00
141	4.0381	24.8987	655.2332	1686.1742	550.5151	1683.7543	-31.7939	4.9075	0.00
155	4.0381	26.0366	718.1967	1751.0354	563.9090	1724.8904	-37.2696	8.2465	0.00
175	3.9854	26.5834	753.2212	1845.2867	602.8113	1781.5583	-44.3052	9.4736	0.00

Microcosm 8

Day	P	Fe	C	V_{NET}	N_2	O_2	CO_2	CH_4	C_2H_4
15	0.6438	0.8124	181.7645	28.7978	-24.7013	10.0438	-2.0676	0.0000	0.00
29	0.9898	2.5870	229.8148	187.5194	-47.8224	269.5311	-2.3819	0.0523	0.00
43	1.9522	3.7193	291.1981	318.6493	-101.2015	543.2768	-7.0139	0.0855	0.00
57	3.7720	16.9300	396.8975	408.8098	-87.8801	655.9583	-6.9157	0.1152	0.00
71	4.3511	19.6911	436.5971	512.6492	-133.2111	871.2451	-11.5506	0.1457	0.00
85	5.0369	19.8496	589.3217	585.1093	-95.1060	925.2089	-11.2532	0.1747	0.00
99	5.5234	20.8475	653.3754	674.6247	-103.0700	1044.0694	-15.9595	2.7075	0.00
113	5.4780	21.4716	697.6496	748.1148	-82.2042	1111.5296	-21.6172	2.6164	0.00
127	5.4714	22.2324	716.5067	810.9225	-78.4188	1197.6367	-24.8089	3.1990	0.00
141	6.4244	25.1534	777.2231	827.0323	-103.3925	1230.1135	-30.3737	4.1614	0.00
155	6.7853	25.6574	846.8647	851.0044	-136.4544	1264.0357	-35.7943	5.3212	0.00
175	6.7838	26.8644	880.9895	868.2359	-202.5220	1316.1740	-42.9554	5.0782	0.00

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