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David W. Hendricks

Utah State University

Logan, Utah 84321

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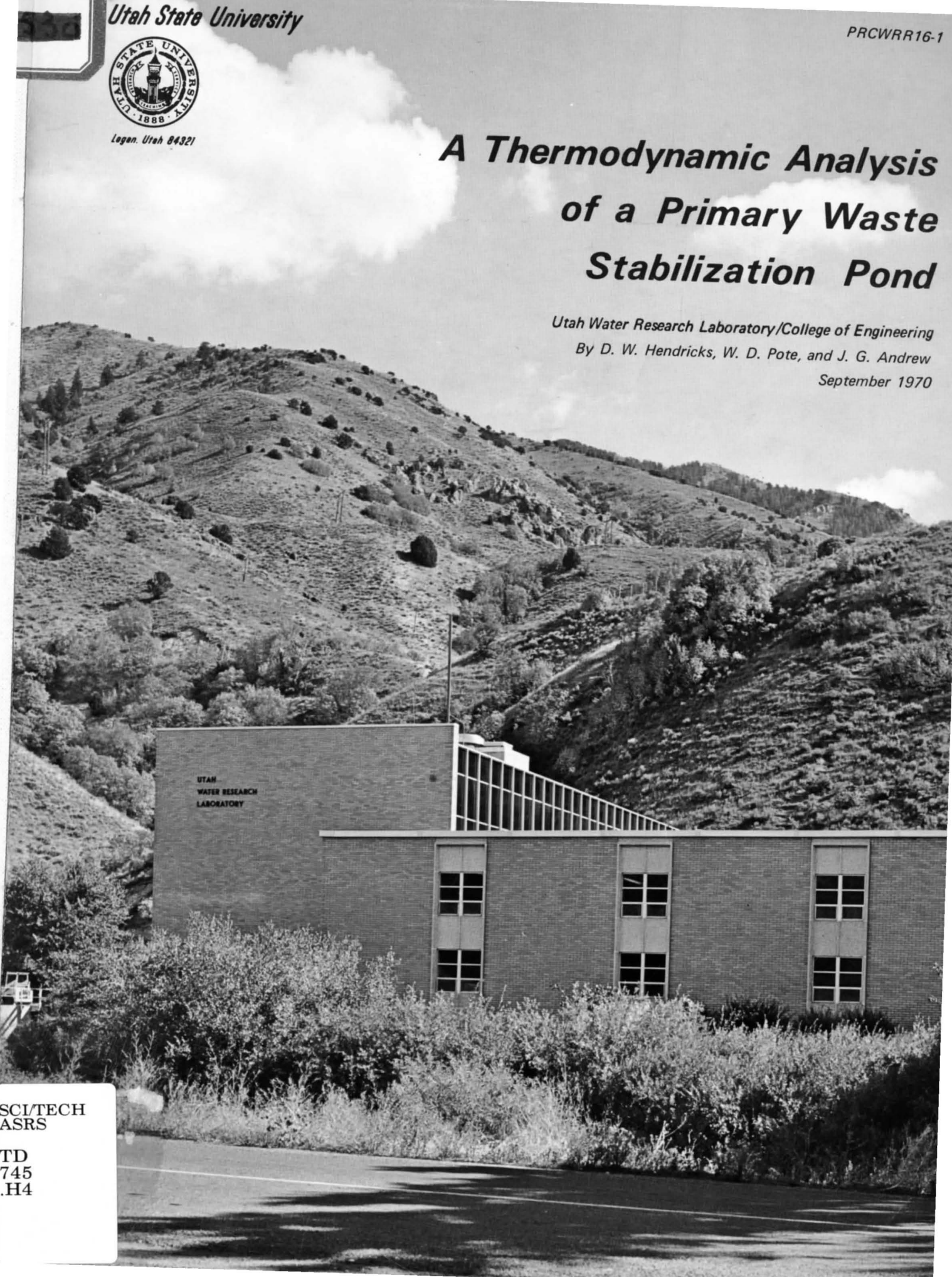

A THERMODYNAMIC ANALYSIS OF A PRIMARY WASTE STABILIZATION POND

by

D. W. Hendricks,
W. D. Pote,
and J. G. Andrew

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Utah Water Research Laboratory
College of Engineering
Utah State University
Logan, Utah 84321

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ABSTRACT

Traditional design practice for waste stabilization ponds is based upon the premise that sufficient photosynthetic oxygen must be produced within the pond to satisfy oxygen requirements of the incoming waste flow. Thus, because algae production is proportional to pond surface area, surface organic loading rate is a principal design criterion (hydraulic detention time is the other). That a possible adverse energy trade exists in the sequence of coupled reactions (aerobic waste degradation–photosynthesis) has been largely ignored. This work is focused on quantitatively articulating this energy trade, in terms of algae produced vis a vis waste degraded. This is done by: (1) defining the chemical reactions involved–both stoichiometrically and thermodynamically (the latter in terms of equilibrium conditions), (2) measuring terms in a daily mass balance model of an operating primary pond, and (3) evaluating the "algae production potential" for the pond studied, based upon available solar insolation. These results define respectively, (1) the *calculated* absolute lower limit of daily algae synthesis necessary for production of the stoichiometric oxygen to satisfy the daily influent BOD requirement, (2) a *measured* daily synthesis rate of algae to compare with the daily influent TOC (total organic carbon), under conditions of maximum sunshine in the annual cycle, and (3) the calculated absolute upper limit of daily algae synthesis, through the annual cycle, if all usable solar energy were utilized. Results, for a daily waste inflow of 415 kg TOC, showed: (1) 167 kg per day of algae TOC must be synthesized to provide the stoichiometric oxygen for 415 kg TOC waste, as glucose, (2) *measured* algae synthesis rate, in early July, was +12,600 kg TOC per day, and (3) algae production potential in early July was 44,000 kg algae TOC per day. The effluent flux was 110 kg TOC per day.

These results establish: (1) that even in the lower limit, algae production is significant in relation to the waste degraded, (2) that the actual production of algae is almost 100 times the stoichiometric requirement–for the maximum day in the annual cycle, and (3) the upper limit of algae production is about three times the actual production. A vast energy overturn exists for relatively little net effect. In fact effluent TOC concentrations exceeded influent TOC concentrations.

PREFACE

This project is exploratory in nature. Consequently project objectives are limited to two tasks. The first task is to ascertain the lower and the upper limits of algae production for a primary waste stabilization pond. From this we wish to assess, in a preliminary sense, the relative importance of the aerobic and anaerobic zones in pond design. The second task is to imply a theoretical direction for further more comprehensive work, which might involve all three ponds—primary, secondary, tertiary—modeled over the annual cycle. These tasks are handled by our formulation of two models: (1) an equilibrium model which depicts the stoichiometry and thermodynamics of the coupled reactions energetically significant in pond functioning (we are concerned here about the total amount of standard state free energy of formation contained in organic molecules either synthesized or degraded), and (2) a TOC (or COD or ΔF_f°) mass balance model of the primary pond, using a daily time increment.

While we believe that our models were formulated with a degree of structural rigor sufficient for our purposes, limitations should be mentioned. First, the value of our equilibrium model is in the delineation of reaction equations, and in showing the disposition of products and reactants *in the limit sense.* Also any kinetic equation proposed *must* be tied back to a corresponding reaction equation. Second, the system we have modeled is an open system, while our reaction thermodynamics is structured for an equilibrium condition for each equation. This is not an uncommon manner of handling problems in water chemistry. However, we should caution that more explicit delineation of the thermodynamics is in order. Ours is a first step and should be considered as such. Furthermore some of our empirical formulas for cellular materials and values obtained for free energies of formation represent only the best data and calculations we could find; some are questionable and we have endeavored to call attention to this, as appropriate, and to document by showing calculations so that any questionable premise can be traced. These points in question are, in fact, possible fruitful directions for continued work. Finally, our mass balance model of the primary pond was simulated numerically only for a single day—and this was the most extreme day, in terms of daily sunshine, in the annual cycle. Again this result implies direction for further work; a suitable simulation model over the annual cycle could provide a better understanding of pond behavior relating to time delays in reactions and give rational guidance in pond design.

RECOMMENDATIONS

1. The other cells in the pond system should be studied to determine the final disposal of the algae contained in the primary pond effluent.
2. The study should be continued over an annual cycle where Equation (9) is formulated or summed over a longer time period.
3. Our results imply, as suggested by Meenaghan (1963) and Parker et al. (1950), that pond design should be deeper with less surface area, in order to promote more activity in the anaerobic zone. A primary anaerobic pond as suggested by Parker et al. (1950) is another rational approach.
4. Design criteria should be volume oriented rather than area oriented.
5. The loading rates to an aerobic pond need to be determined by kinetic studies, in situ.

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Background

The beneficial effects of impounding wastewater were first observed in the United States when, in 1901, a sewage holding pond was built at San Antonio, Texas, to use the reclaimed wastewater as irrigation water (Smallhorst, 1961). Many observations of the improvement in quality of impounded wastewater were made during the 1920's and 30's (Smallhorst, 1961; Svore, 1961). In Texas, for example, such ponds were used subsequent to primary treatment. However, the first *purposeful* design of waste stabilization ponds was not introduced until 1948 (Wright, 1966). As a result of continued investigations and favorable operating experiences with ponds based upon empirically developed design and operating criteria, confidence has increased in the lagoon system as a means for the economical and effective treatment of domestic and industrial raw wastes.

Pond systems are classified according to their mode of operation—aerobic, anaerobic, or facultative (Wright, 1966). The two most common types are "facultative" and "anaerobic." The facultative pond, by definition contains both an aerobic zone and an anaerobic zone. The other ponds operate as implied by their respective names.

The biological and biochemical processes which occur in waste stabilization ponds are not well understood. Originally it was thought that algae were the causative organisms through which stabilization of waste occurred in ponds (Stone and Abbot, 1950). More recently, however, the role of algae has been redefined. Algae are now considered to function as the suppliers of oxygen necessary to meet the metabolic requirements of the aerobic and facultative bacteria present in the pond system. The bacteria are currently considered to be the principal stabilizers of organic matter in the pond environment (Pipes, 1961; Bartsch, 1961; Oswald et al., 1957; King, 1966), and now the algae are considered to have a secondary role in the functioning of waste stabilization ponds.

The design of ponds is based on (1) organic loading rate, and (2) detention time. It is generally agreed that algae are the principal source of oxygen in the pond system (Bartsch, 1961). Thus, present day ponds are designed to produce algae in order to supply the necessary oxygen for the aerobic bacteria. In the early history of ponds, empirical experience indicated a loading rate of about 45 lbsBOD/acre/day (Caldwell, 1946). Today, the range in loading rate is 20 to 50 lbsBOD/acre/day (Bartsch, 1961; Meenaghan and Alley, 1963; Utah State Department of Health, 1965). In general, the presence of algae is encouraged, based upon the assumption that the role of the aerobic zone is of primary significance; and satisfactory pond performance is believed associated with good algal populations. In fact the existence of area loading rates is a tacit recognition of the importance of algae presumed by most authorities. Little attention is given in the literature to the detention time criteria; long detention times appear to be associated with satisfactory pond performance. However, most investigators appear to neglect the consideration that excessive detention times may nullify any rationale used to determine loading rates.

The fact that algae *per se* may pose a significant problem in pond effluent is generally ignored in most of the literature on the subject. An ambivalent and rationalizing attitude is often the response in those instances (Oswald et al., 1957; Bartsch, 1961; Parker et al., 1950) where the problem is fully acknowledged. Results of several observations on pond functioning are given in Table 1. Those observers advocating a benefit attributed to the presence of algae often fail to state whether the pond effluent was filtered before BOD analysis (this is a common practice but such results are meaningless without this information).

That algae can be produced in abundance is well documented. Fitzgerald and Rohlich (1958) report laboratory yields of 16 gm/m^2/day and mention other reported values of 7-10 gm/m^2/day and 14-21 gm/m^2/day. Oswald et al. (1957) report a pilot pond production rate of 30 tons dry algae per acre annually (18.5 gm/m^2/day) and compare this to 6-9 tons per acre for alfalfa yields in California. They advocate separation of the algae for use as animal feed.

While algae currently have formal status as a pollutant, this has not always been the case. In years past, investigators have tended to stress the positive role of algae as an oxygen source in streams, largely ignoring the fact that this can be more than canceled by oxygen deficits resulting from nocturnal algal respiration. Other major nuisance effects of algae (e.g., aesthetic depreciation of streams and lakes) have also been dismissed as not significant in years past.

Table 1. Observations comparing pond effluent and influent.

Observer	Pond influent	Pond effluent
Oswald (1957)	210 mg/l BOD	195 mg/l BOD (largely in S.S. form)
Meenaghan (1963)	720 mg/l V.S.	421 mg/l V.S.
Parker (1950)	94 S.S.	194 S.S.

S.S. = suspended solids
V.S. = volatile solids

Statements questioning conventional hypotheses regarding the net beneficial role of algae have come from Meenaghan and Alley (1963) and McKinney (1965). The former has conducted extended measurements on an operating pond, pointing out that algae volatile solids concentration in the pond effluent were 25 percent of the volatile solids concentration of the pond influent. In fact, they suggest that the anaerobic process is the real workhorse of the lagoon and that algae have a secondary role of polishing and odor inhibition (also suggested by Oswald et al., 1964). Foree and McCarty (1968) and Parker et al. (1950) also suggest that the anaerobic zone has a primary role in waste reduction and stabilization. In fact, Parker et al. (1950) have reported on studies of Melbourne's ponds and described BOD reductions of 530 mg/l raw sewage to 160 mg/l anaerobic lagoon effluent and then 51.3 mg/l aerobic lagoon effluent (6.6 mg/l filtered).

A comprehensive investigation of the role of algae and the importance of the anaerobic process in waste stabilization ponds seems to have never been given any appreciable momentum. Designs are still based upon the criteria of area loading. This is a surrogate measure of algae synthesis potential, and hence oxygen production rate.

Objectives

Our prime objective is to quantitatively delineate the thermodynamic role of algae in the operation of a primary waste stabilization pond in Logan, Utah, for July conditions of temperature and light. Specifically we wish to: (1) calculate the daily amount of algae synthesis needed to aerobically degrade the daily influent waste, (2) calculate the algae production potential for the primary pond studied, based upon usable light, (3) measure the daily rate of algae synthesis and compare this with other kinetic terms on a daily basis, considering the pond as a reactor, and (4) relate our results to pond design.

Significance

Our research challenges the basic assumption of conventional design—that photosynthesis, on the scale specified in design, is a key mechanism for the successful operation of aerobic waste stabilization ponds. This hypothesis is completely fallacious thermodynamically, since the photosynthetic oxygen produced to decompose COD *creates* COD in the form of algal protoplasm (McKinney, 1965). This implies that the pond either discharges algae in its effluent with a consequent oxygen demand, or that the algae sinks to the bottom of the pond and is decomposed anaerobically. If the latter situation occurs, it would be better to cause anaerobic decomposition of the waste initially. This is thermodynamically more rational anyway since less cell material is produced in anaerobic treatment than in aerobic treatment. This does not say the algae have no role, even though they may have a negative role thermodynamically. The algae may provide a "capping" action to keep the ponds less objectionable in odor, as stated previously.

Our work is a thermodynamic analysis of pond functioning. Through this analysis, we can better understand the relative importance of the aerobic zones, anaerobic zones, and photosynthesis, in pond design.

Scope

Our project is intended as neither a definitive nor a comprehensive study. It is a pilot study designed to quantitatively delimit the production rate of algae vis a vis waste degradation in the primary waste stabilization pond. The results and direction developed may serve as a point of departure for further research.

CHAPTER II

WASTE STABILIZATION POND REACTIONS

Functions of waste treatment

The basic functions of a waste stabilization pond are identical to those of any waste treatment system. First, it is necessary to cause a predictable *separation* between the water and its undesirable constituents—both dissolved and suspended. Second it is necessary to reduce the energy level of the separated waste constituents to some *stabilized* products (that is the activation energy of the stabilized products must be high enough that nuisance reactions are no longer a problem). Waste stabilization ponds must be designed and operated to achieve these ends. However, the unit processes common to conventional waste treatment plants are not separable and explicitly identifiable in this type of system, though they are operative (but not subject to control as is a waste treatment plant).

Reactions

Several facets of the waste stabilization pond ecology are outlined in Figure 1, and include: (1) sewage influent, (2) sunlight, (3) algae, (4) aerobic and facultative bacteria, (5) anaerobic and facultative bacteria, and (6) gaseous end products. All of these components are participants in successive reactions[1] which begin with the sewage substrate as an initial reactant and end, ultimately, with gaseous end products. Our quantitative analysis of pond reactions and thermodynamics is based upon the assumptions that all reactions are coupled and that they proceed to equilibrium. These assumptions are true in the limit sense only and form the basis for deriving Figures 2 and 4.

[1] This is true only in the equilibrium sense, that the reactions are successive "coupled" reactions.

Figure 2 represents the fate of the COD that is removed in the pond system (assuming coupled reactions proceeding to equilibrium). As the wastewater enters the pond system, 28 percent of the COD (from Table 2) settles directly to the anaerobic zone. The 44 percent suspended and 28 percent dissolved COD (Table 2) are metabolized in the aerobic zone (actually some of this will go the anaerobic zone but this amount is unique to the pond hydraulics) where the aerobic heterotrophes oxidize 45 percent of the 72 percent (or 32 percent of the original COD) of the organic matter they receive for energy (endogenous respiration). They incorporate 55 percent of the nonsettleable COD organic matter (which is 40 percent of the original) into cell mass[2] which eventually settles to the anaerobic zone. The algae use the oxidized end products of the aerobic bacteria and produce algal cell mass which also will settle to the anaerobic zone. In the anaerobic zone about 40 percent of the algae is inert to decomposition and deposits on the bottom. The anaerobic bacteria use the remaining COD for energy and cell mass. Table 2 gives the percentage breakdown for these various depositions of incoming COD. The numerical data given in Figure 2 were obtained from the stoichiometry of the respective reactions, given in Appendix A. It should be noted also that an outside source of carbon (i.e. from HCO_3^-) must be available in order to produce sufficient algae in order to provide the 44.5 gm O_2 shown. Thus it is clear that for aerobic decomposition of 68 gm (.38 moles) glucose, 27.9 gm (.16 moles) of algae must be produced in order to satisfy the stoichiometry of the reactions involved. This is not, however, the upper limit of algae production, since additional outside carbon sources may be available. Figure 2 should not be interpreted literally,

[2] The actual distribution of substrate utilization depends upon the C:N:P ratio; the figures given should be considered nominal.

Table 2. Average composition of domestic sewage (Fair, Geyer, and Okun, 1968).

State of Solids	Mineral	Organic	Total	BOD	COD	%COD
			(mg/l)			
Suspended	65	170	235	110	108	72
Settleable	40	100	140	50	42	28
Nonsettleable	25	70	95	60	66	44
Dissolved	210	210	420	30	42	28
Total	275	380	655	140	150	100

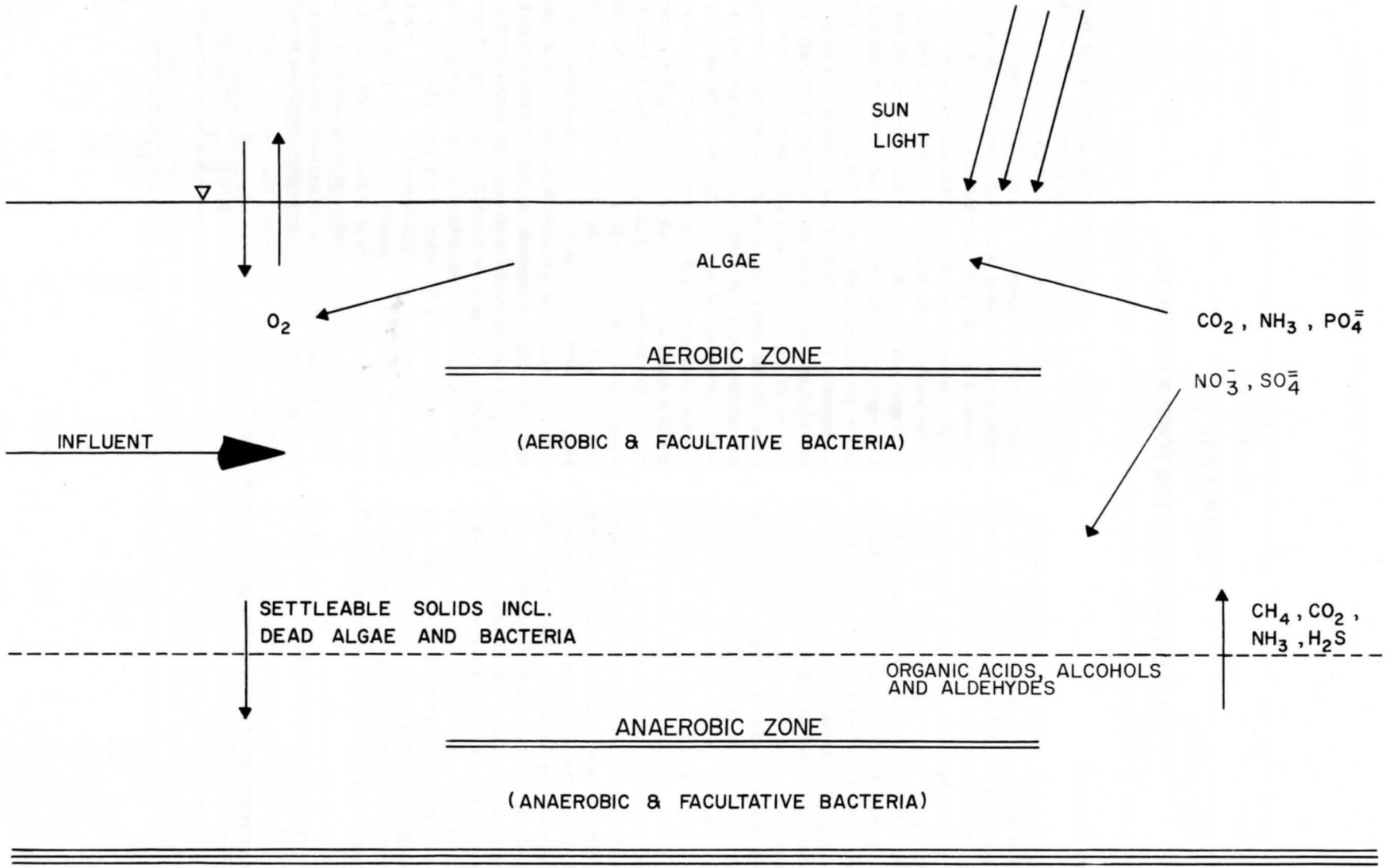


Figure 1. Representation of the aerobic and anaerobic zones in a facultative pond.

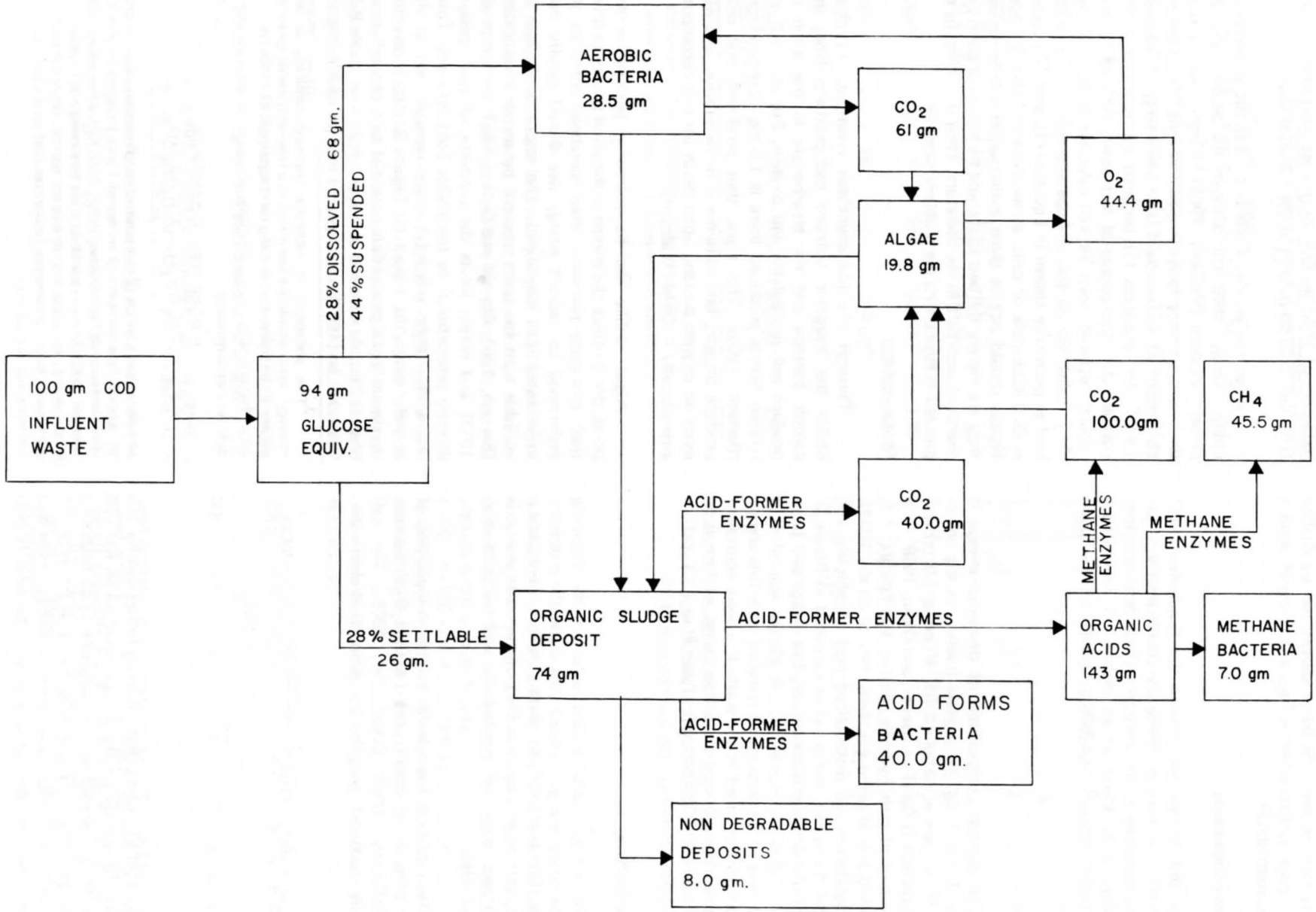


Figure 2. Disposition of influent COD through pond processes.

of course, as the sources of reactants and depositions of products may or may not be as shown; we are dealing with an open system whereas Figure 2 is based upon a closed system idealization.

Reaction components

In this section we outline the assumptions and calculations necessary to define the stoichiometry of the reactions occurring within the pond. These determinations then allow a synthesis of an unreal but nevertheless conceptually useful, equilibrium model of the pond reactions.

Influent

The average composition of domestic sewage is given in Table 2. Of the organic fraction in the wastewater; 50 percent is carbohydrate, 40 percent is protein and 10 percent is fat (Fair, Geyer, and Okun, 1968). The heat content of carbohydrates, protein and fats are: 4.1, 4.1, and 9.3 kcal/gm respectively (White et al., 1959). These substances are metabolized both aerobically and anaerobically within the pond as indicated in Figure 2. Subsequent calculations concerning free energy will be in terms of "glucose equivalents." A glucose equivalent is defined here as the amount of standard state free energy of formation contained in the waste if it were equivalent to glucose. The standard state free energy of formation for glucose is -217.56 kcal/mole (Table 3) or 1.13 kcal/gm COD (Appendix A).

Aerobic bacteria

The aerobic bacteria oxidize part of the incoming waste to obtain energy to drive their metabolic processes. The resultant end products of this oxidation are carbon dioxide, water and ammonia. Part of the waste matter is incorporated into cell protoplasm and becomes new bacterial cells.

The empirical formula for cellular composition of aerobic bacteria has been reported as $C_5H_7O_2N$ (Symons and McKinney, 1958; Eckenfelder, 1965). The cell synthesis reaction is given by Equation (1) (Eckenfelder, 1965).

$$6C_6H_{12}O_6 + 16O_2 + 4NH_3 \xrightarrow{\text{enzymes}} 4C_5H_7O_2N + 16CO_2 + 28H_2O$$

$$\Delta F_R^o = -3760 \text{ kcal}^1 \quad \ldots\ldots\ldots\ldots \quad (1)$$

McCarty (1965) reports that substrate utilization for cell synthesis, for a wide variety of substrates, varies from 26 percent to 65 percent of the substrate COD becoming bacterial protoplasm. The stoichiometry of Equation (1) shows, using the COD equivalents of 192 gm/mole and 160 gm/mole for glucose and aerobic bacteria respectively, that 640 gm COD of cells results from the oxidation of 1158 gm COD of glucose. This former figure turns out to be 55 percent of the substrate COD.

When the aerobic bacteria oxidize the substrate to obtain energy, they are between 40 percent and 70 percent efficient (McCarty, 1965) in capturing and using the released energy to synthesize new cells.[3] If we assume 55 percent of the standard state free energy of reaction, ΔF_R^o,[2] for Equation (1) becomes incorporated as the standard state free energy of formation, ΔF_f^o, of the new cellular material, then we can calculate both ΔF_R^o and ΔF_f^o (cells). We do this in Appendix A-2; ΔF_R^o is -3760 kcal as previously shown in Equation (1), and ΔF_f^o (cells) is -520 kcal/mole of cells, as we show in Table 3. These figures should not be given authoritative interpretation; they are merely the best that could be arrived at given the dearth of such data in the literature. Their use allows us to proceed in structuring the problem, however.

Photosynthesis

Through the photosynthesis reactions, the algae utilize the inorganic nutrient end products from the aerobic bacteria and the bicarbonate in the water to produce cell protoplasm and oxygen. For each 1.6 mg oxygen that is produced there is 1.0 mg algae produced (Bartsch, 1961). The algae, thus produced, not only produce oxygen, but consume it in respiration, and will exert an oxygen demand upon death (or will decompose anaerobically if oxygen is absent).

Algae, unlike the heterotrophic bacteria in the pond, don't utilize the energy in the pond system to drive their biological reactions; their metabolic activities are maintained by radiant energy (we should qualify that some exceptions may exist). The response of algae to available light has been reported by several investigators (Bartsch, 1961; Oswald and Gotaas, 1957; Oswald et al., 1957) and within limits the synthesis of algal mass is directly proportional to the visible light intensity. Total and visible light intensity varies annually within the bounds shown in Figure 3. Figure 3 also shows the maximum algae production potential as a proportion of observed visible radiation at Logan, Utah, from Table B-3. Oswald et al. (1957) have reported that for each milligram

[3] This statement is nebulous thermodynamically as any "energy" term should be identified in a thermodynamic sense; we assume an interpretation ensuing for the purpose of this work.

[4] ΔF_R^o is the standard state *free energy of reaction* and is defined mathematically

$$\Delta F_R^o = \sum_i^{\text{n products}} N_i \Delta F_f^o(i) - \sum_j^{\text{n reactants}} N_j \Delta F_f^o(j)$$

in which i and j are particular products and reactants, respectively; N_i and N_j are numbers of moles of i and j respectively in the stoichiometric equation. Also, $\Delta F_{ox} \equiv \Delta F_R^o$, when oxygen is a reactant. $\Delta F_f^o(i)$ is the standard state free energy of formation of compound i, it is the free energy of reaction in synthesizing a compound from its elements (using standard state values of both elements and compounds).

Table 3. Compounds and their properties.

Compound	Molecular Weight (gms)	COD per mole or Equivalent (gms O_2)	ΔF_f kcal/mole	ΔF_f kcal/gm COD
Glucose	180	192[c]	-217.56[a]	-1.13
Carbon dioxide	44	32	- 92.31[a]	-2.88
Ammonia	17		- 19.00[a]	
Bacteria				
1) Aerobic	113	160[c]	-520.00[b]	-3.24
2) Anaerobic	131	160[c]	-260.00[b]	-1.62
Algae	172	256	-900.00[b]	-1.69
Water	18		- 56.69[a]	
Oxygen (O_2)	32		0.00[a]	
Organic sludge[b] composite	166[b]	168[b]	-438.00[b]	-2.60

[a] Values taken from Williams and Williams (1967).
[b] Determined analytically in this paper in Appendix A.
[c] McCarty (1965).

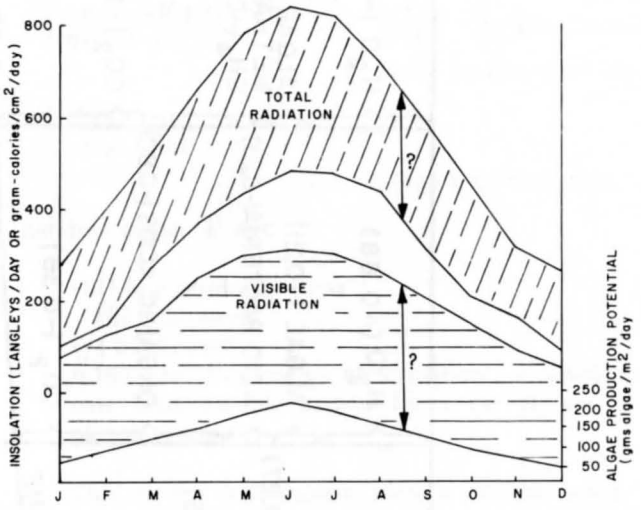


Figure 3. Insolation and maximum algae production at Logan, Utah, latitude 41°44'N, Elevation 4430' MSL, plotted from data in Table B-3.

of oxygen produced in photosynthesis, 3.68 calories[5] of solar energy are needed. Thus, as the amount of insolation increases, the energy available for photosynthesis also increases.

Algae do not utilize all of the incident radiation. Some of the solar energy is reflected and some is lost as heat. Oswald and Gotaas (1957) report that energy conversion efficiency seldom exceeds 10 percent; Oswald et al. (1957) report 3 percent to 4 percent for pond environments.

Table B-2, Appendix B, gives additional detail showing the effect of latitude and month on solar insolation.

The empirical formula for algae is calculated in Appendix B as $C_8H_{14}O_3N$. With this formula it is assumed that the algae have ΔF_f^o of -900 kcal/mole. This assumption is based on the premise that algae, with 7 carbon to carbon bonds have 7/4 times the ΔF_f^o of aerobic bacteria with 4 carbon to carbon bonds; or (7/4) (-520) = -900 kcal/mole. This is, of course, a hazardous assumption; nevertheless we feel it important to call attention to it and continue.

The stoichiometry of algae production in photosynthesis is:

$$NH_3 + 8CO_2 + 4.5H_2O \xrightarrow{\text{light}} C_8H_{14}O_3N + 8.75\,O_2$$

$$\Delta F_R^o = +112 \text{ kcal/mole algae} \quad \ldots\ldots \quad (2)$$

and is based on the premise that 1.6 grams of oxygen are produced per gram of algae produced (Bartsch, 1961) and the empirical formula for algae as determined in Appendix B. The ΔF_R^o for the photosynthetic reaction is +112 kcal/mole algae and this determination is outlined in Appendix A.

[5] In Appendix A, we calculate ΔF_R^o = + 112 kcal/mole algae for the photosynthesis reaction; since the molecular weight of algae is taken as 172, this figure is + 0.65 kcal/gm algae; since 1.6 mg oxygen are produced per mg of algae synthesized, as have + 0.40 kcal of solar energy are needed per gm of oxygen or, + 400 cal/mg oxygen, which is in close agreement with Oswald and Gotaas.

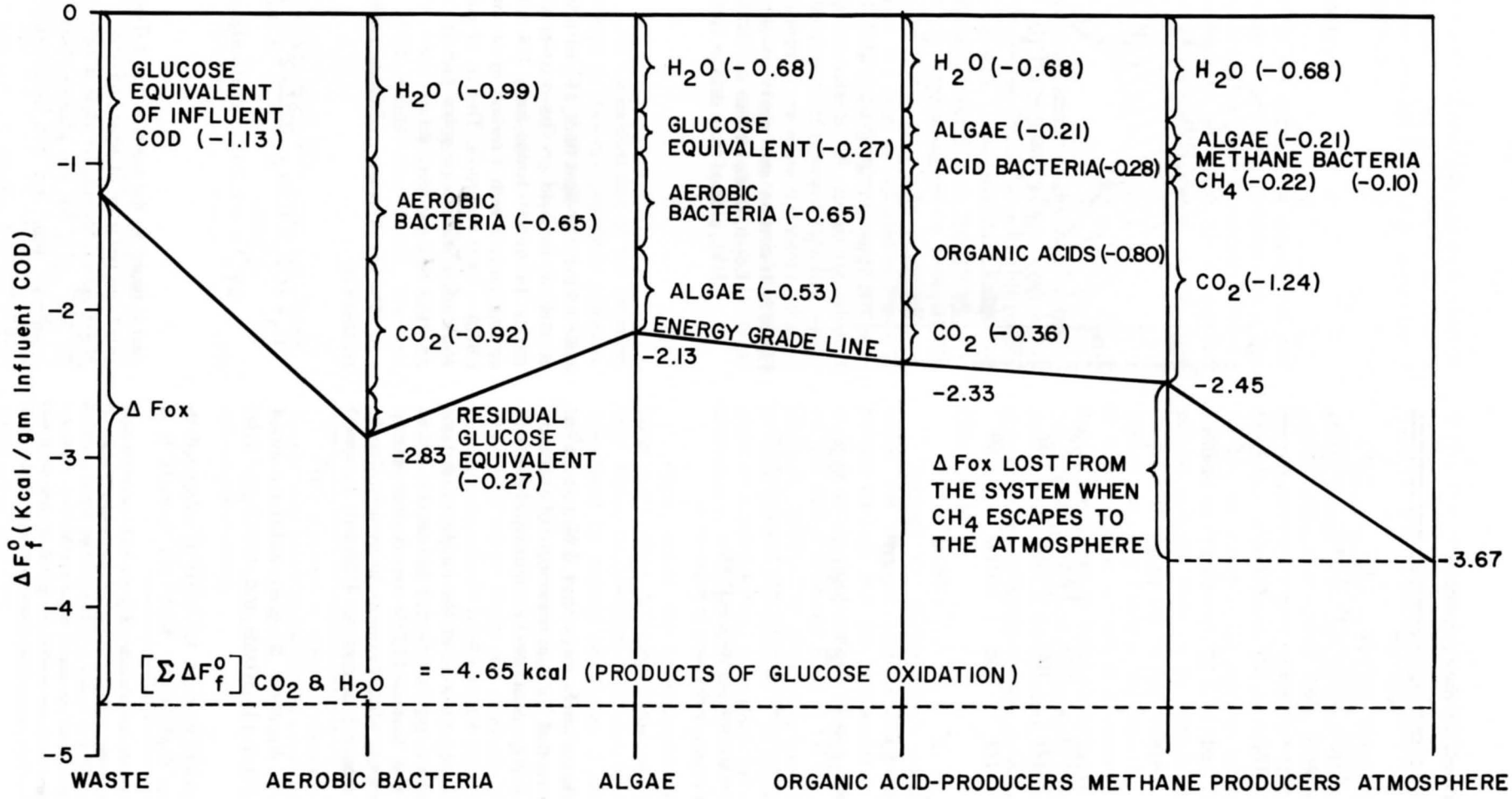


Figure 4. Energy gradient in the pond system.

We calculate the potential algal production caused by glucose degradation as follows:

(1) From the stoichiometry of Equation (1), 6 moles of glucose will produce 16 moles of CO_2.

(2) From the stoichiometry of Equation (2), 16 moles of CO_2 will produce 2 moles of algae (we assume here that the molecular formula and the empirical formula for algae are identical).

(3) Thus 6 moles of glucose can produce 2 moles of algae—for algae the production potential is 1/3 mole algae per mole of glucose.

(4) To express this in terms of COD (Table 2) 1 mole of glucose contains 192 gm COD; also 1 mole of algae contains 256 gm COD; thus we calculate

$$\frac{1/3 \text{ mole algae}}{\text{mole glucose}} \times \frac{256 \text{ gm algae COD/mole algae}}{192 \text{ gm glucose COD/mole glucose}} \times \frac{.445 \text{ gm algae COD}}{\text{gm glucose COD}}$$

(5) To express again, but in terms of standard state free energies of formation, we find in Table 3 that
ΔF_f° (glucose) = -217.56 kcal/mole
ΔF_f° (algae) = -900 kcal/mole;
thus we calculate

$$\frac{1/3 \text{ mole algae}}{\text{mole glucose}} \times \frac{-900 \text{ kcal algae/mole algae}}{-217.56 \text{ kcal glucose/mole glucose}} \times \frac{1.38 \text{ kcal algae}}{\text{kcal glucose}}$$

Algae as an oxygen source. In conventional design of aerobic ponds it is assumed that oxygen required for the aerobic degradation of glucose, as represented by Equation (1), is produced entirely by photosynthesis, Equation (2). Assuming we have 100 gm COD, which is 94 gm of glucose (of which 68 gm is subject to aerobic degradation), the calculation of algae required to provide the necessary oxygen follows directly from the stoichiometry of the two reactions. Thus from Equation (1):

$$\left.\begin{array}{l}\text{gms oxygen needed to degrade 68 gm glucose, containing -82 kcal, } \Delta F_f\end{array}\right\} = \frac{16\times32}{6\times180} \times 68$$
$$= 32.2 \text{ gm oxygen}$$

Using this value in Equation (2), we have

$$\left.\begin{array}{l}\text{gms algae needed to result in 32.2 gm oxygen}\end{array}\right\} = \frac{172}{280} \times 32.2$$
$$= 19.8 \text{ gm}$$

$$\therefore [\Delta F_f]_{\substack{19.8 \text{ gm} \\ \text{algae}}} = \frac{19.8}{172} \times -900 \frac{\text{kcal}}{\text{mole}}$$
$$= -103 \text{ kcal}$$

Thus we have these conclusions:

(1) that 19.8 gm algae are needed to provide oxygen for 72 gm COD (as glucose equivalents) (with 26 gm out of the original settleable)

(2) that -103 kcal of ΔF_f° in new cell material (algae) result from disappearance of -68 kcal of dissolved glucose

However, the energy trade results also in

$$\left.\begin{array}{l}\text{gms aerobic bacteria from 68 gm glucose}\end{array}\right\} = \frac{4\times113}{6\times180} \times 68 \text{ gm glucose}$$
$$= 28.5, \text{ by Equation (1)}$$

or

$$[\Delta F_f]_{\substack{28.5 \text{ gm} \\ \text{aerobic} \\ \text{bacteria}}} = \frac{28.5}{113} \times (-520)$$
$$= -131 \text{ kcal, again by Equation (1)}$$

Therefore we gain in net:

-131 kcal aerobic bacteria
-103 kcal algae
- (-82 glucose)
=-152 kcal free energy of formation contained in new biodegradable carbon forms per 68 gm of glucose reacted.

This is not a complete picture of the energy relationships, however, since aerobic respiration is ignored.

Algae production potential from alkalinity. The algae also utilize bicarbonate, which is present as alkalinity in the water, as a source of carbon for synthesis. As algae use the dissolved CO_2 in the water they shift the equilibrium that exists in natural buffered systems. The buffering equation is represented by the following sequence:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad \ldots\ldots (3)$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^= \quad \ldots\ldots\ldots\ldots (4)$$

$$CO_3^= + H_2O \rightleftharpoons HCO_3^- + OH^- \quad \ldots\ldots\ldots (5)$$

As the CO_2 is used, the equilibrium in Equations (3) and (4) is shifted to the left and in Equation (5) is shifted to the right. With this shifting there is an increase in the OH^- concentration with a resultant increase in the pH. Since most aerobic organisms have an *optimum* pH range from 7 to 8, it appears likely then that the elevated pH caused by algal synthesis may inhibit the reduction of organic matter by aerobes. We do not pursue this point further, however; many aerobes will operate well over a wide pH range and even up to a pH of 10.

The algae can utilize also the carbon in the pond alkalinity; this is supplemental to organic carbon sources in the pond influent.

We calculate this increase as follows:

(1) For each liter of pond water containing 100 mg/l of HCO_3^- there is 72 mg/l CO_2 equivalent.

(2) We assume the molecular formula and empirical formula for algae are identical.

(3) Based upon the stoichiometry of Equation (2), we calculate the corresponding algae production (potential):

mg algae per liter of water containing initially 100 mg/l HCO_3

$$= \frac{172}{8\times44} \times 72 = 35.2$$

Thus 35.2 mg of algae can be produced by 100 mg of HCO_3^-.

Anaerobic reactions

In the anaerobic zone essentially two reactions take place: (1) Facultative and anaerobic bacteria metabolize the organic matter; the end products are: volatile acids, carbon dioxide and alcohols. (2) Anaerobic bacteria metabolize the volatile acids; the end products are methane and carbon dioxide. These reactions are perhaps the most important in the pond system, as it is in the anaerobic zone that final stabilization of organic solids must occur.

If sulfates and nitrates are present in the anaerobic zone, they will be reduced by the bacteria, the end products being: sulfide and nitrogen gas. At a low pH the sulfides may be given off in the form of hydrogen sulfide gas, which may be oxidized again if an aerobic zone is present.

The anaerobic zone contains a sludge deposit made up of material from three sources: 1) settleable portion of the influent wastewater, 2) dead bacteria from the aerobic and anaerobic zones, and 3) algae that settles into the anaerobic zone. Without the anaerobic reactions, these materials will build up continuously.

The empirical formula for anaerobic bacteria is $C_5H_9O_3N$ (Speece and McCarty, 1964). If we assume that the empirical formula is identical to the molecular formula, then the molecular weight is 131 gm/mole. Table 2 shows the COD equilvalent and the free energy of formation.

Acid production. The acid formation stage for anaerobic decomposition is given by Equation (6). The ΔF_R^o value for this reaction is calculated in Appendix A.

$$4.2\ \text{Organic sludge} \xrightarrow[\text{acid formers}]{\text{[anaerobic bacteria]}} C_5H_9O_3N + 8\ \text{Org. Acids} + 4CO_2$$

$$\Delta F_R^o = -182.7\ \text{kcal} \quad \ldots\ldots\ldots\ldots \quad (6)$$

The "organic sludge" reactant is a conglomerate of the material from the dead algae, dead bacteria and influent organics as shown in Figure 2. The psuedo molecular weight of this conglomerate is 142 gm/mole–which is a weighted average of these components.

Methane bacteria. The reaction representing the methane production process is approximated by Equation (7).

$$100\,[\text{Organic Acids}] \longrightarrow 3C_5H_9O_3N + 93CH_4 + 92CO_2$$

$$\Delta F_R^o = -863.5\ \text{kcal} \quad \ldots\ldots\ldots\ldots \quad (7)$$

This ΔF_R^o value is calculated in Appendix A. Acetic acid is assumed to be the organic acid in all calculations involving free energy of formation and molecular weight.

Combined reactions

One objective of the treatment process is to stabilize the organic matter by reducing the energy of the system or, at least, making the energy unavailable to biological systems by separating or letting it escape from the system. Figure 4 shows the energy disposition of various reactants and products in pond treatment. The "energy gradeline" is computed by solving for the change of free energy between two states; $\Delta F_R^o = \Delta F^o_{f(\text{products})} - \Sigma \Delta F^o_{f(\text{reactants})}$. When ΔF_R^o is negative, there is a decrease in the energy of the system in going from the state of the reactants to the state of the products. When ΔF_R^o is positive, as in the case of algae, there is an increase in the energy of the system in going from reactants to products. In Figure 4, the reference line at -4.65 kcal/gm COD represents the summation of the ΔF_R^o of the end products if the glucose waste was directly oxidized, in one step, to the stable end products of carbon dioxide and water. The closer the "energy gradeline" approaches this reference line, the more stable will be the waste effluent.

The net reaction, after combining Equations (1) and (2) is:

$$6C_6H_{12}O_6 + 6NH_3 \longrightarrow 4C_5H_7O_2N + 2C_8H_{14}O_3N + 19H_2O + 7.5O_2$$

$$\Delta F_R^o = -1.0\ \text{kcal/gm removed COD} \quad \ldots \quad (8)$$

The gases produced (as reaction end products) either react further or immediately leave the system. The measure of pond effectiveness in handling waste thus involves an evaluation of the system's capacity for the stabilization of organic solids. This characteristic, as standard state free energy of formation of each organic solid, ΔF_f^o, is observable in Figure 5. It is readily apparent in Figure 5 (from an energy standpoint) that the aerobic zone, which is dependent upon the production of oxygen by algae, is superfluous; the anaerobic zone, utilizing methane releasing fermentation reactions appears as the "workhorse" reaction zone. By implication then, the algae

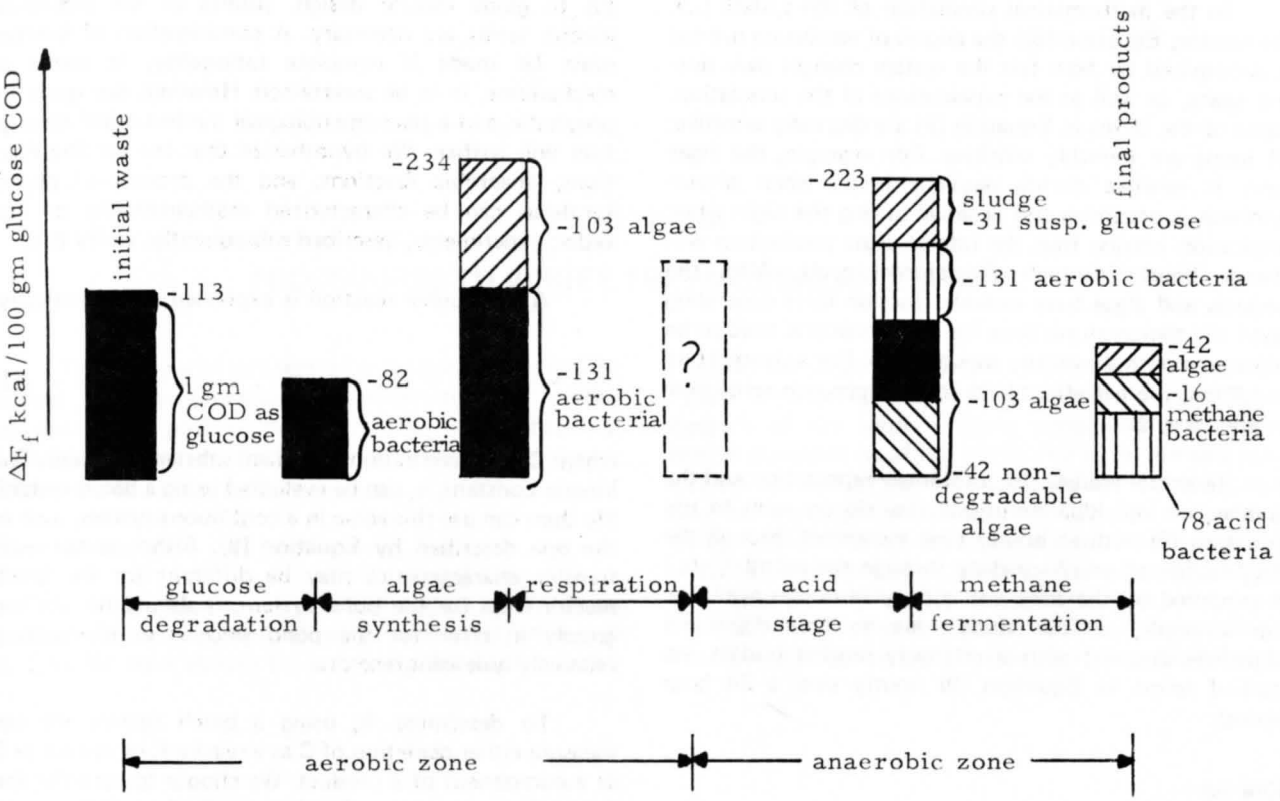


Figure 5. Degradable solid products from Figure 4.

are superfluous to efficient pond function and the enhancement of their reproduction (i.e. expansive ponds of shallow depth) may, in fact, reduce pond efficiency through the inhibition of fermentation by oxygen.

The waste stabilization pond as a continuous flow reactor

The coupled sequence of reactions outlined in foregoing sections, and summarized quantitatively in Figure 2, represents the upper limit distribution of various reactants and products. In a real system, however, we realize, of course, that the *rates* of each of these reactions will determine the *actual* distribution of reactants and products at any given time. For this analysis, we consider the waste stabilization pond as a continuous flow reactor and combine each of the kinetic equations, representing the foregoing reaction equations, into a single mass or energy balance equation.

The mass balance reactor equation states that the time rate of change of a given substance equals its rate of delivery to the reactor minus its rate of outflow plus its rate of change due to the various reactions occurring within the system. Equation (9) is the mathematical representation of this statement. We can state Equation (9) in terms of either mass or energy content of the various reactants and products involved. The important point is that we use a common unit of measurement for the various reactants and products involved in this heterogeneous reactor. It matters not whether we choose units such as biochemical oxygen demand, BOD, chemical oxygen demand, COD, total organic carbon, TOC, standard state free energy of compound formation, ΔF_f^o, or standard state free energy of oxidation ΔF_{ox}^o; these are all acceptable measures of degradable organic matter. In this work we have obtained measurements in terms of TOC; we also use ΔF_f^o and ΔF_{ox}^o interchangeably with TOC. Our general term for degradable organic matter we call F, which is not specific to any of these measures. Using the symbol F then for degradable organic matter we state Equation (9), which represents mass fluxes entering and leaving the system, and sources and sinks of degradable energy.

$$\left[\frac{dF}{dt}\right]_{\substack{\text{net}\\ \text{effect}}} = [Q\cdot\Delta F]_{\text{influent}} - [Q\cdot\Delta F]_{\text{effluent}} + \left[\frac{dF}{dt}\cdot A\right]_{\text{algae}} + \left[\frac{dF}{dt}\cdot V\right]_{\substack{\text{aerobic}\\ \text{zone}}} + \left[\frac{dF}{dt}\right]_{\substack{\text{anaerobic}\\ \text{zone}}} + \left[\frac{dF}{dt}\cdot F\right]_{\substack{\text{volume}\\ \text{storage}\\ \text{change}}} \quad \ldots\ldots\ldots\ldots (9)$$

- Q = rate of flow of water into or out of the pond (liters/sec)
- F = degradable organic matter (kcal/liter or mg/liter)
- t = time (seconds, hours, days, months or years)
- A = pond area (square meters)
- V_V = portion of reactor being considered (liters)

In the mathematical simulation of the system (i.e. the reactor, Equation (9)) the degree of resolution needed is determined by how fast the system changes over time and space, as well as the expectations of the simulation. Some of the terms in Equation (9) are diurnally sensitive; all terms are monthly sensitive. For example, the algae term is positive during daylight hours when photosynthesis is occurring and negative during the night when respiration occurs; thus the rate of algae production will change hourly through the diurnal cycle. Also, the bacteria and algae have variable reaction rates depending upon the temperature; thus the rate constants need to be adjusted monthly over the annual cycle. For a steady state condition, the net effect term should approach zero—over an annual cycle.

In actual studies the resolution expectation and the number of individual solutions (i.e. do we wish to use Equation (9) with an hourly time increment through the day, month, or year?—or daily through the month?, etc.) is tempered by the time and money resources available. For example, in our study, due to limited project objectives coupled with a relatively modest budget, we studied terms in Equation (9) hourly over a 24 hour period.

Kinetics

Kinetics is all important in the study or design of reactors, as is evident in Equation (9). In using Equation (9) to guide reactor design, studies of the individual kinetic terms are necessary. A consideration of kinetics must be made if complete rationality, in terms of mechanisms, is to be ascertained. However, our goals are pragmatic and a phenomenological mathematical description will suffice. We hypothesize that the aerobic reactions, anaerobic reactions, and the process of photosynthesis can be characterized mathematically as first order; experiments, described subsequently, verify this.

A first order reaction is expressed mathematically:

$$\frac{dC}{dt} = -kC \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

where C = concentration of a given substance in mg/l. The kinetic constant, k, can be evaluated using a batch system. We then can use this value in a continuous system, such as the one described by Equation (9). Although the mass transfer characteristics may be different for the batch reactor than for the pond system, it should be not too grossly in error for the pond since it is inherently a relatively quiescent reactor.

To determine, k, using a batch system we can measure either depletion of C as a reactant, or uptake of C as a component of a product. We choose to consider the latter, but we process the data received so that we can calculate reactant *depletion* also. This allows us to utilize Equation (10).

CHAPTER III

PROCEDURES TO VERIFY THE MASS BALANCE MODEL

The purpose of this section is to describe the reactor system, sampling procedures, measurements, and analysis of data. Our goal is to experimentally ascertain the individual terms in the mass balance reactor model, Equation (9).

The reactor

To ascertain the individual terms in the mass balance model, our studies were conducted on a real system. We have chosen for our study the south primary pond in the Logan City waste stabilization pond system. Figure 6 shows this system as a whole; Figure 7 is a diagram of the south primary pond; and, Figure 8 is several photographs of this pond. The south primary pond receives one half of the Logan City sewage effluent. Logan City has a population of about 25,000; its discharge of sewage varies through the year, as influenced by groundwater infiltration from irrigation, ranging from 3.37 MGD in winter to 14.07 MGD in summer. BOD levels range from 30 mg/l in the summer to 300 mg/l in the winter. The operation of this pond system started in 1967.

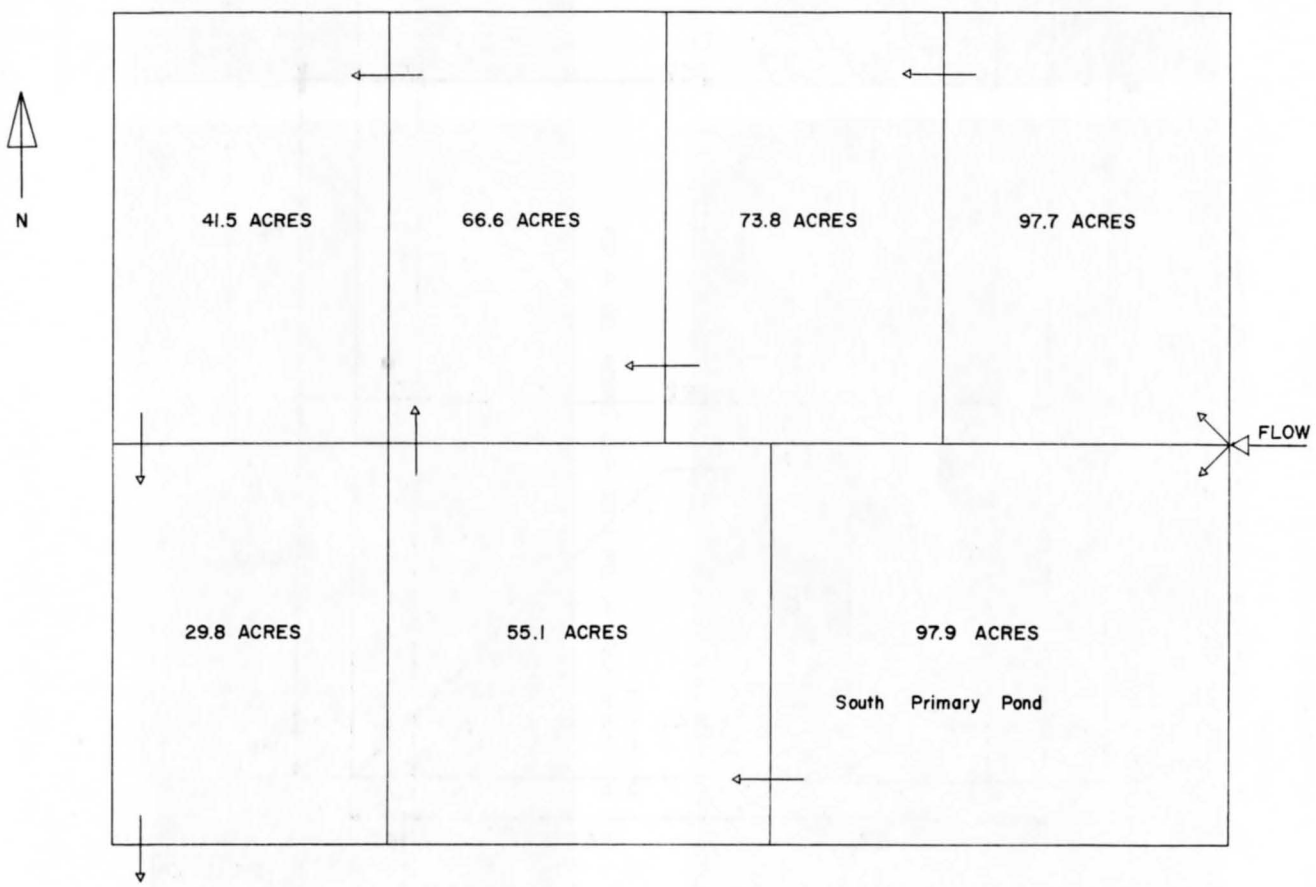


Figure 6. Schematic drawing of Logan waste stabilization ponds (after Keith Hanson, Logan City Office, Logan, Utah).

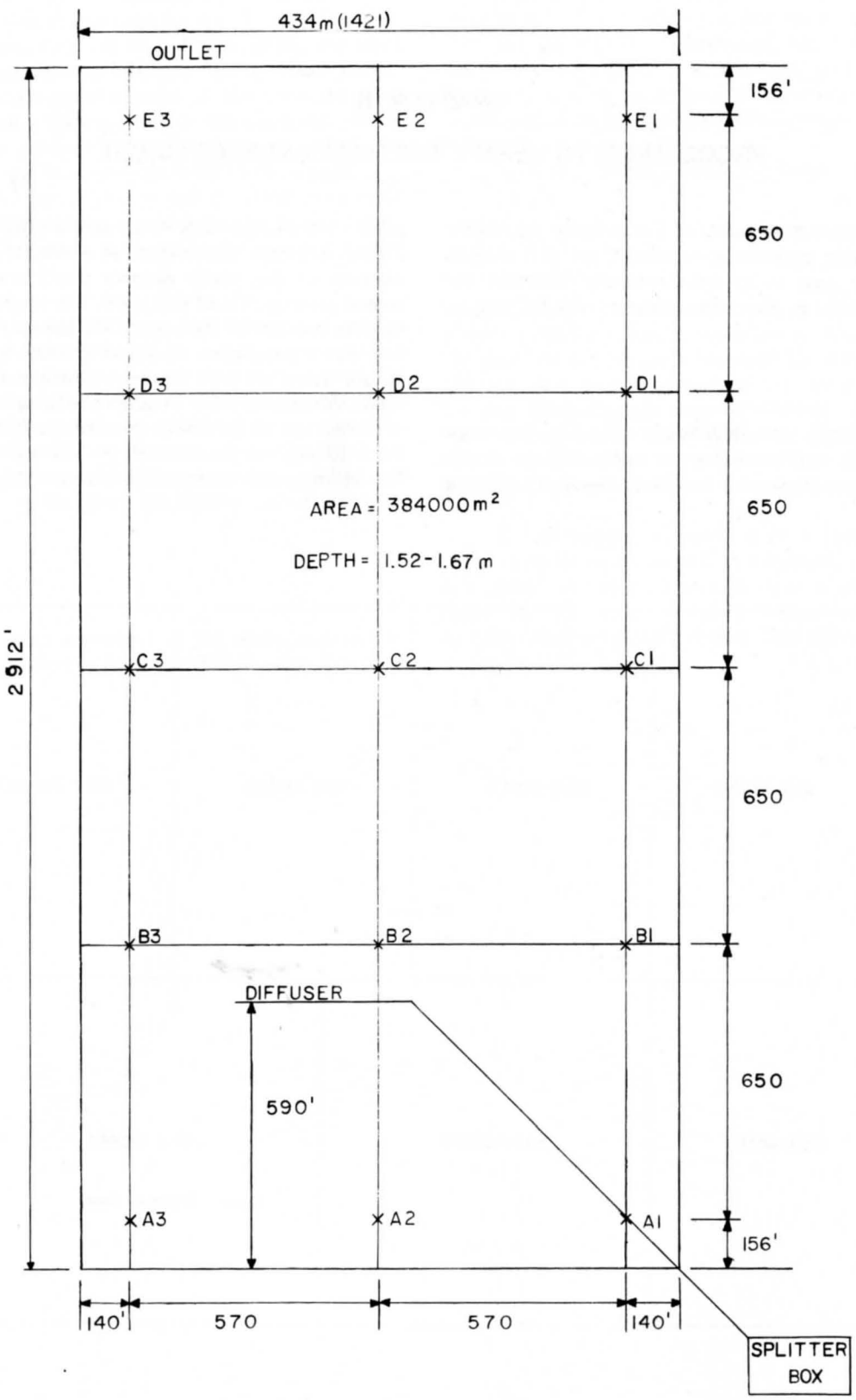
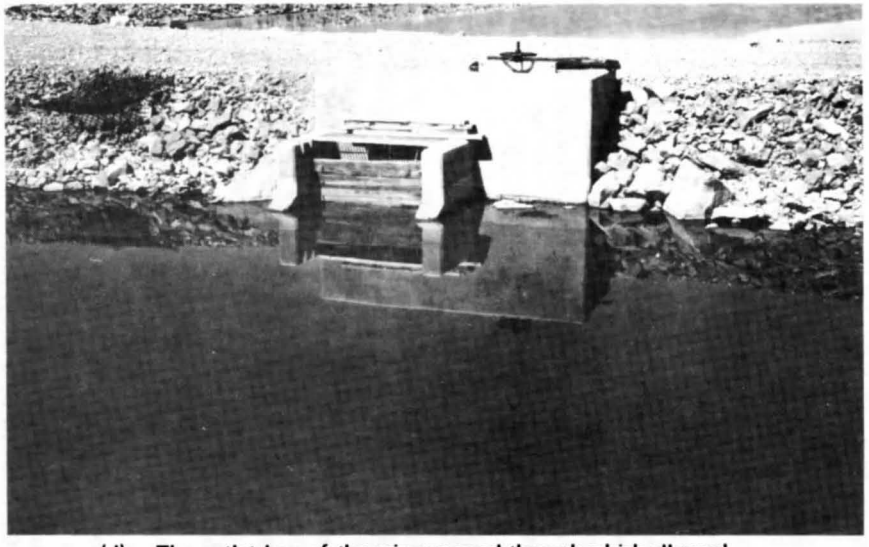


Figure 7. South primary waste stabilization pond.

(a) An overall view of the pond with Logan City in the background.

(b) The algae mats and associated film which covers the surface of the pond.

(c) A close up view of the surface in an undisturbed corner of the pond.



(d) The outlet box of the primary pond through which all pond effluent water must pass.

Figure 8. Various views of the Logan City sewage pond.

Sampling procedures

Pilot sampling

Pilot data were collected on the south primary pond (hereafter called the "pond") in September 1969 and June 1970. These data were collected: (1) to ascertain the requirements for data and to guide in the selection of appropriate procedures, and (2) to obtain a "feel" for the behavior of the pond. These pilot data and other supporting information regarding procedures are found in Appendix B.

Diurnal sampling and measurement

Because of the marked diurnal influence on many of the operative factors in pond behavior, we concentrated our efforts on a single 24-hour period to obtain a comprehensive set of measurements (0500 July 8, 1970, to 0500 July 9, 1970). This sampling period, combined with prior point sampling in time and space (described above and in Appendix B) provides an adequate picture of pond behavior.

Pertinent physical, chemical, and biological parameters of the south primary pond were measured, as described herein, every two hours at: inlet; outlet; and depths of: surface to 1', 2', 3', 4', 5', bottom, over the 24-hour sampling period. The depth sampling was done at point C2 (marked by a buoy) located in the middle of the pond as shown in Figure 7. In addition to in situ oxygen-temperature measurements (using the oxygen-temperature probe), water samples were obtained at these depths every two hours using a Kemmerer water bottle (Kemmerer Water Bottle, Foerst Mechanical Specialties Company, Chicago, Ill.). The sample was then transferred to a 250 milliliter plastic bottle and placed on ice.

Temperature and dissolved oxygen. Both temperature and dissolved oxygen were measured directly, in situ, with the EIL D.O.–Temperature Meter. (EIL Dissolved Oxygen Temperature Meter, Electronic Instruments LTD, Richmond-Surrey, England.) The instrument reads directly in degrees centigrade and percent saturation (either 0 - 100 percent or 0 - 200 percent) for temperature and dissolved oxygen respectively. We converted dissolved oxygen percent saturation to mg/l concentration using standard conversion tables.

pH measurements. pH was determined colorimetrically on all samples collected using a Lamotte field pH kit. (Lamotte Chemical Products, Inc., Chestertown, Maryland.)

Carbon dioxide, carbonate ion, and bicarbonate ion determinations. These determinations were carried out on all samples collected by titration analysis, using the procedures outlined in *Standard Methods of Water and Wastewater Analysis.* While carbon dioxide concentrations were determined directly in terms of mg/l CO_2, the bicarbonate and carbonate ion concentrations were determined first in terms of mg/l as $CaCO_3$. These data were then subsequently converted to mg/l $CO_3^{=}$ and mg/l HCO_3^{-} by the following formulae:

$CO_3^{=}$ in mg/l = 1.2 (phenolphalein alkalinity)

HCO_3^{-} in mg/l = (61/50) (Total alkalinity) - (2) (phenolphalein alkalinity)

Light penetration measurements. Light penetration measurements were made every two hours at point C2. These measurements were obtained using the Submarine Photometer shown in Figure 9. Light transmission was measured in terms of milliamper response on an ammeter. These measurements were converted to percent transmission for each depth using the surface reading in milliamperes as 100 percent (e.g. if the surface reading equals 6.8 ma and the reading at three feet equals 0.61 ma then the percent light transmission equals 0.61 ma/6.8 ma X 100 = 8.97 percent).

Flow measurements. Inflow measurements were read directly from Logan City's Stevens Total Flow Meter (Leupold & Stevens Instruments, Inc., Portland, Oregon).

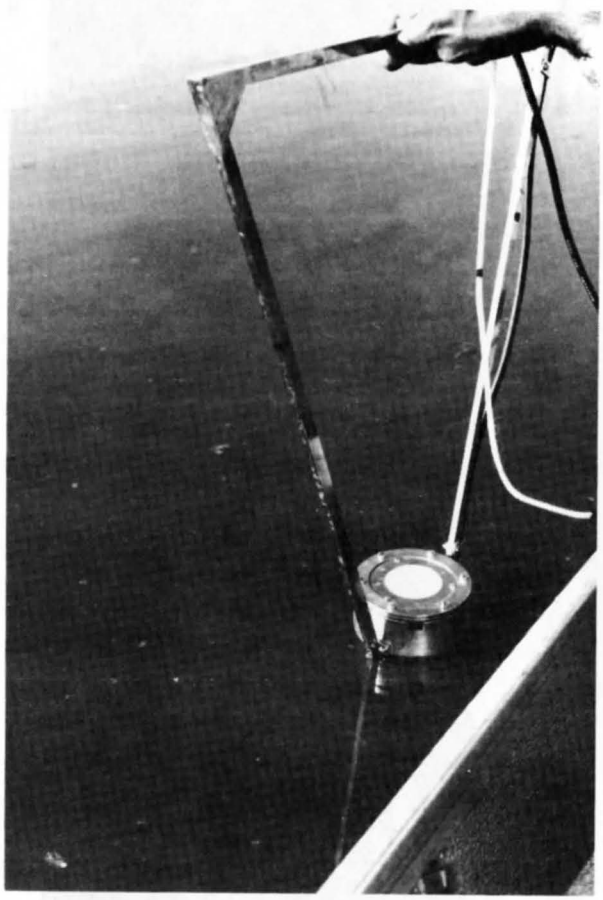


Figure 9. Submarine photometer used to measure light intensities at various depths of water.

This recorder gives continuous readings in both million gallons per day (mgd) as well as the total gallons of water passing through the Parshall measuring flume over any time interval chosen. Both of these readings were recorded at each two hour sampling interval. These readings were then converted to cubic feet per second, and then to metric units for use in Equation (9).

All water effluent from the south primary pond was through the outlet box shown in Figure 8(d). This flow was measured, at four hour intervals, at the entrance to the box, by a Gurley Current Meter. (Gurley Current Meter, Model 622D, Gurley Engineering Instruments, Troy, New York). Table B-8, Appendix B, shows the points of measurement within the flow cross section and also illustrates the computational procedure. Accurate measurements were difficult to acquire because of eddy currents induced by the configuration of the box, however, the measurements were probably within the normal current meter accuracy of $\pm$ 5 percent.[1]

Total organic carbon. Total organic carbon was used as the measure of carbon containing compounds. Samples were taken, for TOC analysis, from all stations at two hour intervals as described previously. These samples were placed immediately in an ice-filled styrofoam chest and transported, within two hours, to the laboratory where they were frozen.

On July 23 and 24 these samples were thawed and two 100 ml aliquots were removed from the bottles. One of the aliquots was passed through a millipore filter (which had been previously acid-washed as described in Appendix B-4). The other 100 ml aliquot was blended for 1 minute in a Waring Blender ("liquify" setting). Twenty milliliter aliquots of the resulting solution were removed from both blended and filtered samples, placed in a test tube, and frozen.

On July 27 and 29, 1970, TOC analyses were carried out using the Beckman Total Carbon Analyzer, according to procedures outlined in Appendix C.

Data processing. All data obtained above for the 24-hour sampling cycle were reduced and are displayed in tabular form in Appendix D.

Kinetic measurements

The purpose of kinetic measurements was to evaluate first order rate constants for the individual kinetic terms–$[dF/dt]_{algae}$, $[dF/dt]_{aerobic\ zone}$ and $[dF/dt]_{anaerobic\ zone}$ in Equation (9), and to ascertain that the reactions can be described as first order. The technique used to study kinetics involved measurement of the uptake of radioactive substrates over time and were developed during this study. Samples used in kinetic studies were collected at 1100 hrs July 20, 1970. These data were applied with the chemical and physical measurements of July 8-9, 1970, in Equation (9). It was assumed that little difference in rate constants had occurred in the interim. Weather had not changed during the period.

Materials

Glucose C^{14}(U), New England Nuclear Corporation, Boston, Massachusetts, 02118, lot number 379-226.

Sodium Bicarbonate ($Na_2C^{14}O_3$), New England Nuclear Corporation, Boston, Massachusetts, 02118, lot number 386-54.

Swinnex Adapters and 0.45 μ (25 mm diameter) filters, Millipore Filter Corporation, Bedford, Massachusetts, 01730.

Nuclear Chicago Gas Flow Alpha-Beta Detector and Scaler Model 8703, Nuclear Chicago Corporation, Boston, Massachusetts, 02118.

Carrier Gas: 1.3% Butane, 98.7 % Helium.

Eckman Dredge, Wildlife Specialties Company, Inc., Saginaw, Michigan.

Constant Temperature Bath (Warburg), Precision Scientific Co., Chicago, Illinois.

Submarine Photometer.

Methods –aerobic bacteria and algae

Experimental design. To evaluate the rate of substrate uptake for aerobic bacteria, glucose C^{14} was mixed in samples of pond water taken from zero and 5½ feet depths. We assume, as a *working hypothesis,* that the unit rate of glucose C^{14} uptake (the rate constant) is representative of the unit rate of uptake of other substrates present in the reaction vessel. We realize that metabolism rates differ for each substrate. Equation (1) is the reaction assumed predominant.

To evaluate the rate of algae synthesis, bicarbonate C^{14} ($Na_2HC^{14}O_3$) was mixed with samples of pond water taken from zero and 5½ feet depths. It must be true that the unit rate of uptake of radioactive bicarbonate is identical to the unit rate of uptake of natural bicarbonate. The reaction equation describing algae synthesis is Equation (2) (and Equations (3), (4), and (5) sequence).

Preparation of reaction vessels. Figure 10 identifies the position, color (dark or light), and radioactive substrate for each reactor bottle used in the aerobic bacteria and algal kinetic experiments. The experiments were done in closed reactor bottles immersed in the pond at the depth corresponding to the simulation, and containing pond water obtained from those respective depths.

The rationale for addition of substrates to their respective reactor bottles is given below:

A. Glucose C^{14} (U)
   1. Light bottle: gives rate of uptake of substrate by bacteria

[1] Personal communication with Mr. Lloyd Austin, research engineer, Utah Water Research Laboratory.

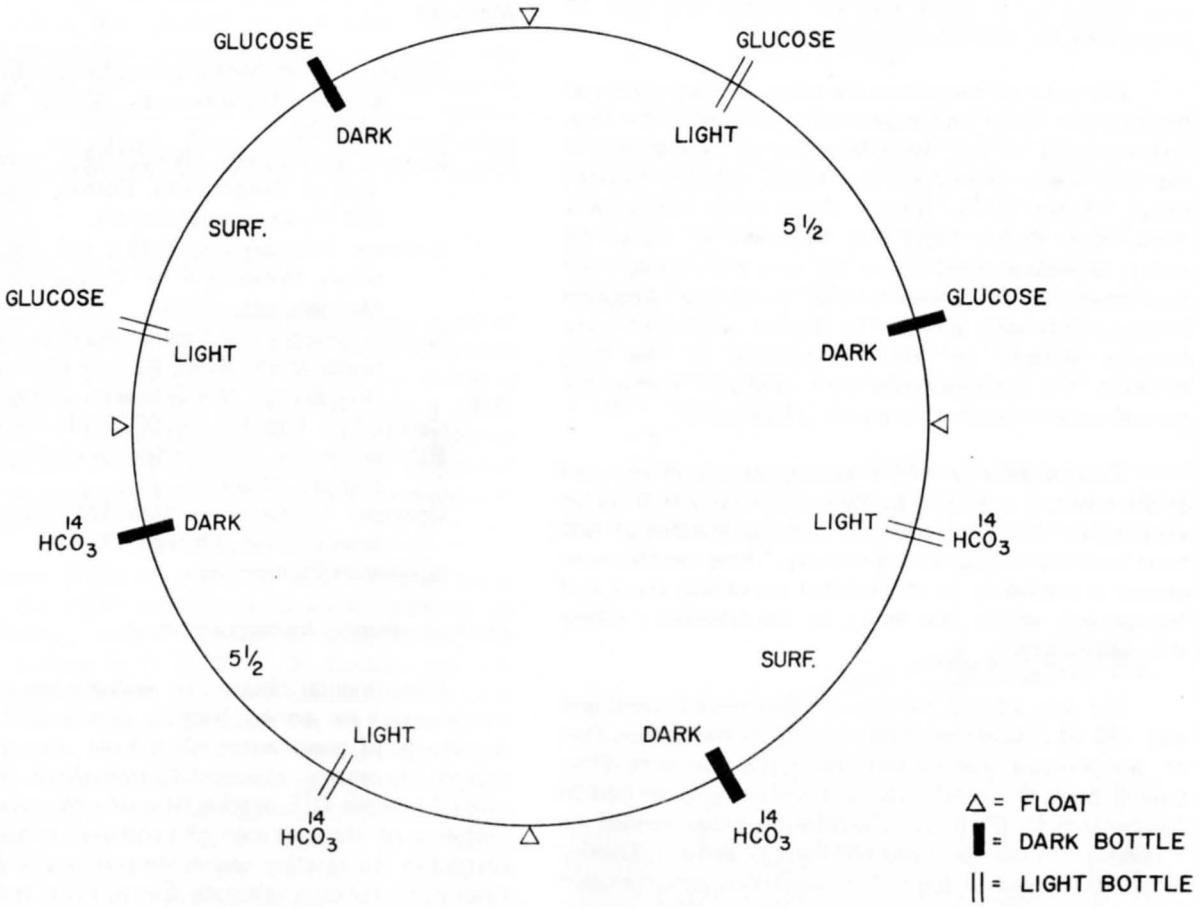


Figure 10. Float ring holding reactor bottles—showing bottle array.

2. Dark bottle: gives rate of uptake of substrate during respiration of both algae and bacteria

B. Bicarbonate C^{14}
   1. Light bottle: gives rate of bicarbonate uptake by algae during photosynthesis
   2. Dark bottle: (control) gives the rate of bicarbonate uptake in the absence of light (non-photosynthetic environment)

Figure 10 shows the positions of the bottles during the period of the kinetic experiments. The 4 inch and 5 foot 6 inch depth positions, shown in Figure 10, were chosen to determine: (1) the difference in synthesis rates between surface (at 4" depth) and the bottom bacterial organisms (at 5'6" and the bottom muds), and (2) to show the difference in synthesis rates between the surface algae (at 4" depth) and the bottom algae (at 5'6" and the bottom muds), in different sunlight environments.

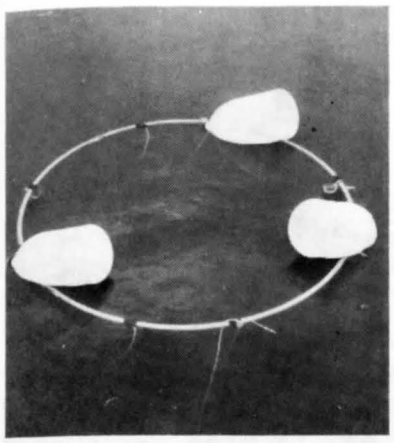
Reaction vessels were 300 ml BOD bottles. Dark bottles were prepared by wrapping the 300 ml BOD bottles, first with black polyethylene sheeting, and then with a double wrapping of black tape. Light bottles were unwrapped bottles.

Water samples used to fill the bottles were obtained at depths of 4" and 5'6" for the reaction vessels located at those respective depths. Some bottom muds were intentionally included with the samples obtained at the 5'6" depth.

Steps in sampling and measurement. Figure 11 describes the preparation of reaction vessels described previously—in Figure 11(a) through 11(i). Figure 11 also describes the sample procurement protocol for the kinetic experiments; Figures 11(j) through 11(q) describe this protocol in exact detail. Figure 12, (a) through (e), describes the laboratory measurement of the radioactive samples thus procured.

Methods—anaerobic bacteria

Experimental design. Again, as for aerobic rate experiments, we assume that glucose C^{14} is identical in its uptake rate to substrates in the pond benthos which are



(a) Overall view of the float ring holding reaction bottles suspended at depths of 4 inches and 5 ft. 4 inches—4 bottles at each depth.

(b) Preparing to retrieve a water sample from a depth of 5'3" with the Kemmer water bottle.

(c) Measuring a 250 ml volume of the collected water.

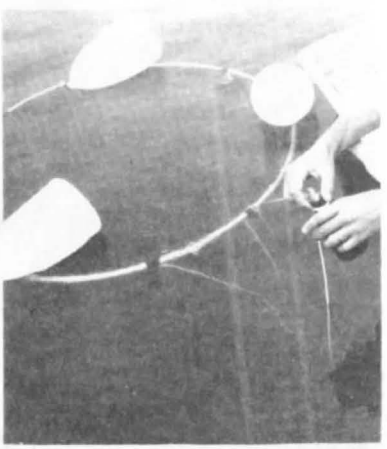
(d) Transferring the 250 ml measured volume to the reaction vessel.

(e) Replacing the reaction vessel to its respective incubation depth for an equibration period.

(f) Measuring 5 ml portions of the radioactive glucose or radioactive sodium bicarbonate solutions prior to transfer to the reaction vessels.

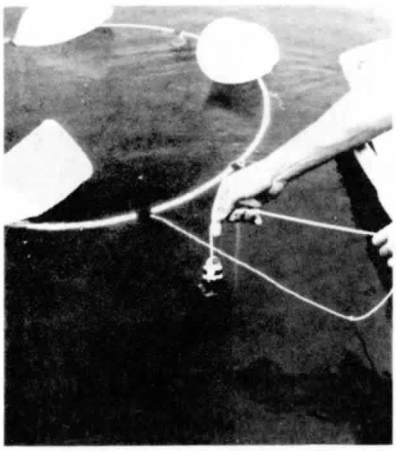
(g) Retrieving the reaction vessel after the equibration period (15 mm) had lapsed.

(h) Transfer the 5 ml radioactive glucose (1 μ c/ml) or bicarbonate (0.5 μ c/ml) solution to the reaction vessel.



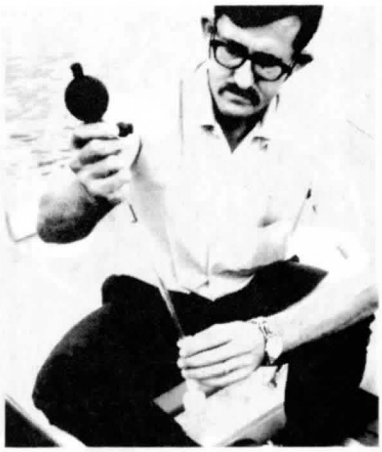
(i) Replacing the reaction vessel to its appropriate incubation depth.

Figure 11. Aerobic bacteria and algae kinetic experiments at the south primary pond July 20, 1970—field data collection procedures.



(j) Retrieving the vessel after the specified reaction interval (30 min.) in order to extract an analysis aliquot.

(k) Removing a 10 ml sample aliquot for radioactivity analysis.

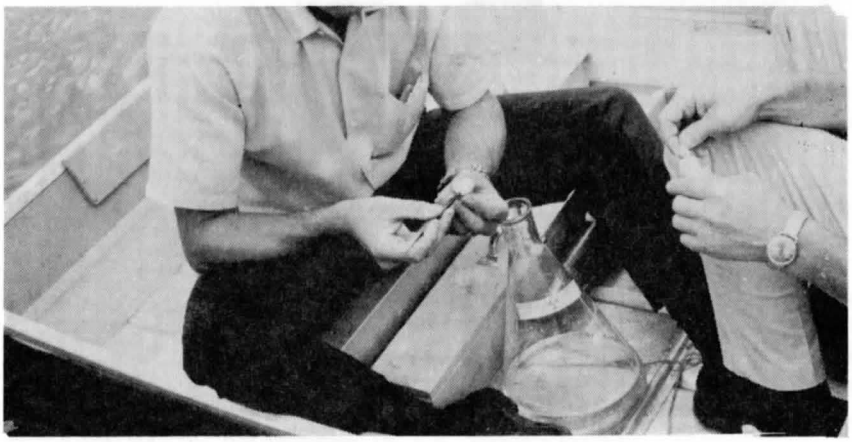


(l) Adding the sample to a 10 ml syringe and Swinnex adapter apparatus.

(m) Filtering the radioactive sample into a waste reservoir.

(n) Washing the filter disc with 50 ml of distilled water (removes unbound radioactive solution).

(o) Removing the radioactive filter disc from the Swinnex adapter with forceps.

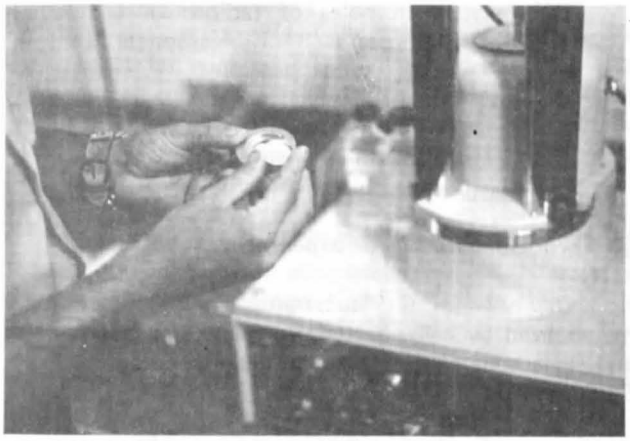


(p) Gluing the filter disc onto an aluminum planchet.

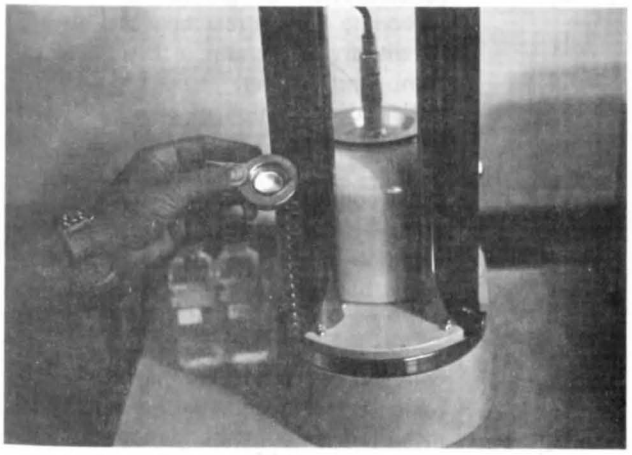
(q) Placing the radioactive samples into a container for transport to the counting laboratory.

Figure 11. Continued.

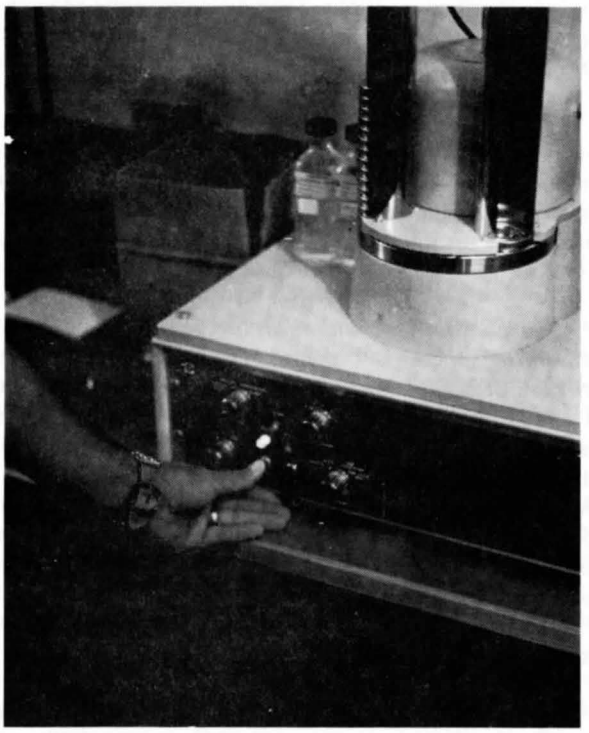
(a) Nuclear Chicago Beta Radiation Counter used for counting the radioactive emissions from the filter discs collected in the kinetic experiments.



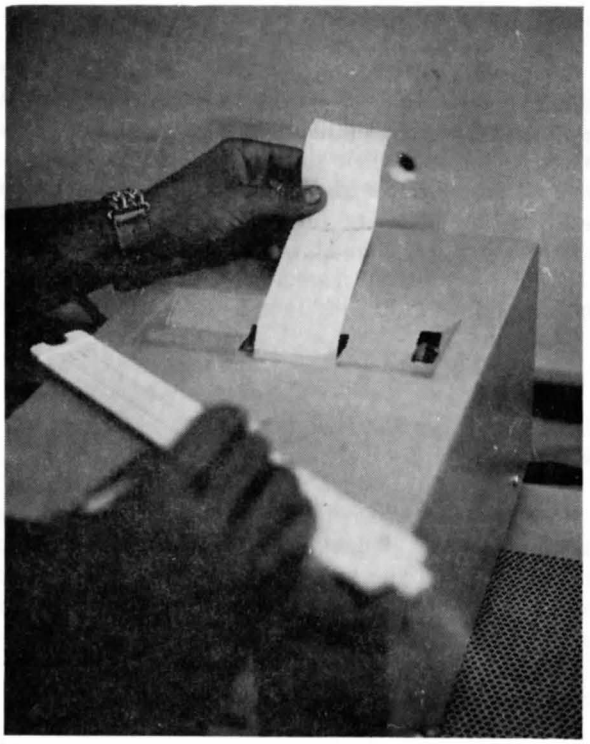
(b) Placing planchet into planchet holder for counting.



(c) Placing planchet holder into counting chamber.



(d) Energizing the machine to begin counting planchets containing radioactive filter discs.



(e) Determination of radioactivity (counts per minute) derived from machine printout.

Figure 12. Measurement of accumulated radioactivity from bacteria and algae kinetic experiments.

utilized by anaerobic bacteria; Equations (6) and (7) are the hypothesized reactions. Thus we pattern the anaerobic kinetic experiments after those previously described, with modifications described subsequently.

Retrieval of reaction medium. A bottom sample was retrieved from the pond bottom by the use of an Eckman Dredge. Approximately 1 liter of bottom mud and water was transferred from the dredge to a one liter beaker. This sample was returned immediately to the laboratory where it was refrigerated until the experiment commenced.

Preparation of the reaction vessel. Prior to the addition of glucose C^{14} the sample was removed from the refrigerator and 250 ml was placed (after thorough mixing of the suspension) into a 500 ml Erlenmeyer flask, which had been previously covered with 2 layers of Scotchlite Black Sealing Tape. This flask was then placed into a Warburg Constant Temperature Bath (24°C) and allowed to equilibrate for 30 minutes. During this equilibration period, and throughout the experiment period, nitrogen gas was continuously bubbled through the apparatus in order to maintain anaerobic conditions within the reaction vessel.

Steps in sampling and measurement. Following establishment of temperature equilibrium ten milliliters of a 1 μ c/ml solution of glucose C^{14}(U) were added to the reaction vessel at time zero. At thirty minute intervals, 5 ml aliquots were removed from the reaction vessels and processed for radioactivity determinations. The experiment was continued for 4 hours.

Due to the difficulty of Millipore filtration with the considerable amount of suspended material present, the sample aliquots were diluted. The 5 ml aliquot was placed in a 250 ml volumetric flask and diluted with distilled water. Five milliliters of this diluted solution were then removed and Millipore filtered through a syringe and Swinnex Adapter apparatus, as previously described in Figure 11. Subsequent measurement steps are identical to those described in Figures 11 and 12 for aerobic bacteria.

Kinetic data processing

Conversion of radiation counter output to C^{14} substrate uptake

The counts per minute output from the Beta radiation counter is directly proportional to the number of molecules of glucose C^{14} or bicarbonate C^{14} (as the case may be) in the sample counted. The sample counted, in all cases, was the residue retained on a Millipore filter. Thus the conversion of the machine reading to micromoles of substrate taken up is given as:

$$\overline{X}_s = CPM/SA \quad \ldots\ldots\ldots\ldots\ldots\ldots (11)$$

where

$\overline{X}_s$ = amount of radioactive compound in sample aliquot taken from reactor vessel in micromoles, taken up by cells

CPM = counts per minute output reported by the Beta counter (which is the number of disintegrations per minute times the efficiency of the counter) from filter residue

SA = CPM of given sample per micromole of C^{14} in standard sample

We determined SA for both glucose C^{14} and bicarbonate C^{14} as outlined by Chase and Rabinowitz (1967).

The solution concentration of the radioactive compound in the sample aliquot is given as:

$$[\overline{X}] = \overline{X}_s / V_s \quad \ldots\ldots\ldots\ldots\ldots\ldots (12)$$

where

$[\overline{X}]$ = micromoles of radioactive compound (glucose C^{14} or bicarbonate C^{14}) in cells retained per liter

V_s = quantity of aliquot taken from reactor vessel in liters (0.010 L in aerobic and algae kinetic studies and 0.005 L in anaerobic kinetic studies)

Data processing for kinetic experiments

Depletion of C^{14} substrates. The amount of C^{14} metabolized by cells—bacterial or algae (for glucose C^{14} and bicarbonate C^{14} respectively)—is determined by Equation (13).

$$C_i = (C_o V_o - \overline{X}_i - \sum^{i} \overline{X}_{s_i} - \sum^{i} C_i V_s)/V_i \quad \ldots\ldots\ldots (13)$$

in which

C_i = concentration of glucose C^{14} or bicarbonate C^{14} in reactor vessel at time of withdrawal of sample i (μ moles of compound per liter)

C_o = initial concentration of glucose C^{14} or bicarbonate C^{14} in reactor vessel (μ moles/L)

V_o = initial volume of reactor vessel (L)

$\overline{X}_i$ = calculated amount of glucose C^{14} or bicarbonate C^{14} (in micromoles) taken up by bacteria or algae in suspension in the reactor vessel, after removal of sample i

i = consecutive sample number of sample removed from reactor vessel

$\overline{X}_{s_i}$ = measured amount of glucose C^{14} or bicarbonate C^{14}, in cell protoplasm, retained by Millipore filter from sample aliquot removed from reactor vessel (μ moles)

V_s = measured volume of sample taken from reactor vessel (L)

V_i = volume of liquid remaining in reactor vessel after removal of sample i (L)

Equation (13) is a mass balance statement, accounting for the number (moles) of tagged particles. Thus $C_o \times V_o$ is the initial number of tagged particles (in moles), $\overline{X}_i$ is the number of tagged particles in cell protoplasm in suspension in the reactor at any time subsequent to removal of sample i, $\sum^{i} \overline{X}_{s_i}$ is the number of tagged particles in cell protoplasm removed by sample aliquots up to and including sample i, $\sum^{i} C_i V_s$ is the number of tagged particles unreacted in reactor solution removed by sampling to and including sample i, and $C_i V_i$ is the number of unreacted tagged particles in the reactor vessel after removal of sample i. Tables E-1, E-2, and E-3 show data reduction for kinetic experiments for aerobic glucose C^{14} degradation, bicarbonate C^{14} photosynthetic uptake, and anaerobic glucose degradation, respectively. These calculations are based upon Equation (13).

Determination of kinetic rate constants. Equation (9) applies for each of the three categories of reaction experiments conducted in the batch reactors. The experimental reactors are designed to include the left hand term and only one of the terms on the right, as dictated by the rate constant in question.

It is hypothesized that first order kinetics with respect to substrate will adequately describe the observations of substrate depletion. Thus integration of Equation (10) yields:

$$\ln C/C_o = kt \quad \ldots\ldots\ldots\ldots\ldots\ldots (11)$$

or in terms of base 10 logarithms:

$$\log C/C_o = [k/2.3]t \quad \ldots\ldots\ldots\ldots\ldots\ldots (12)$$

The slope of such an experimental plot, Equation (12), will yield the reaction rate constant. Figures E-1, E-2, and E-3 are examples of how this is done using data from Tables E-1, E-2, and E-3 respectively. These data appear to fit a first order reaction formulation. It should be emphasized, however, that we are purposely working in terms of a *psuedo* first order reaction, as both algae and bacteria concentrations are ignored in their respective experiments. This omission does not matter for our purposes, however, as long as these and other operative variables do not vary between our experimental reactor which is a sample of the pond, and the pond. We assume they do not. This assumption appears reasonable based upon our observations, and it is sufficient for our purposes, which is not to study kinetics per se, but to *use* kinetics in a way that will result in a reasonable quantitative depiction of Equation (9).

Algae synthesis. Our interest in aerobic and anaerobic glucose C^{14} degradation is in terms of degradation, per se, so the kinetic results can be used directly. For the bicarbonate C^{14} depletion, however, we are interested in this only as it relates to algae synthesis. This relationship can be obtained by the stoichiometry of the synthesis reaction, which shows 8 carbon dioxide molecules (from bicarbonate) are necessary to create one algae molecule. Thus we assume that the rate of creation of algae (in moles) is one eighth the rate of depletion of bicarbonate (in moles). This is a critical assumption.

Mass balance kinetic terms. We are interested in evaluating each individual term in Equation (9). The kinetic terms for aerobic uptake of glucose, and algae synthesis are done using the assumption of first order kinetics with respect to TOC and bicarbonate, respectively. Therefore, both TOC and bicarbonate measurements are needed for the pond water; these are the values used for reactant concentration in the respective kinetic terms in Equation (9).

CHAPTER IV

EXPERIMENTAL RESULTS

Pilot experiments

Miscellaneous measurements

Gross oxygen production insolation and COD. Results of COD measurements of influent to and effluent from the south primary pond, and gross oxygen production from pond water, contained in light bottles, are reported in Tables B-1 and B-2 of Appendix B. Table B-3 shows the comparison between calculated and observed insolation for each month of the year with corresponding algae production potentials using a 10 percent energy conversion efficiency; Figure 3 is a graphical display of this table.

Dissolved oxygen and temperature profiles. Perspectives in space and time of dissolved oxygen and temperature profiles are seen in Tables B-5 and B-6 respectively. Observations outlining the behavior of these data are included also in these tables. It should be noted that depth profiles of these parameters are remarkably consistent over the surface of the pond (Table B-5) and over time—in days (Table B-6).

Dissolved and suspended TOC. Appendix B-5 describes results of pond water TOC, inorganic carbon, and organic carbon measurements before and after successive Buchner and Millipore filtrations. The Buchner filter retained all suspended solids except free bacteria; the Millipore filter retained the free bacteria. Thus a comparison of measurement before and after each of these successive filtrations gives a measure of TOC, etc., for particulate organics and algae (Buchner filter), bacteria (Millipore filter), and dissolved carbon (Millipore filtrate). The results of organic carbon measurements for each of these successive determinations are shown in Table 4 in terms of (a) algae and suspended organics, (b) bacteria, and (c) dissolved organics. Table 4 shows that bacteria are very insignificant in their contribution to the quantity of organics in the pond water and influent sewage. Also dissolved organics and suspended organics and algae are present in comparable concentrations in each sample analyzed (with some expected statistical variation). The total organic carbon concentrations do not vary appreciably with depth; it should further be observed that concentrations for inflow, outflow, and pond water are approximately equal. As expected, the filtration sequence had little effect on inorganic carbon concentrations.

Benthic muds. A sample of bottom mud was obtained for volatile solid determination; Appendix Table B-6 describes the procedure and result. The volatile solids portion of our sample is 31.8 percent. Although merely indicative this figures implies that reactions in the benthic muds must play an important role in pond functioning; the thermodynamic implications, regarding energy dissipation, are profound. We attempted to further verify this aspect of pond functioning by collecting evolved gas from physically disturbed bottom muds. Mass spectrograph analysis was attempted, but was not successful due to an instrument malfunction. It was suspected, however, that the evolved gas was composed of methane and carbon dioxide as well as other gases. This analysis was motivated merely to corroborate the certainty of anaerobic reactions

Table 4. Results of organic carbon analyses showing composition of pond water.

Source of sample	Organic carbon (mg/l)			
	Algae and suspended organics	Bacteria	Dissolved organics	Total organic carbon (mg/l)
inlet	20	2	12	34
outlet	10	4	17	31
1′ depth	19	-	20	39 ±
2′ depth	10	3	15	28
3′ depth	17	1	14	32
4′ depth	11	-	-	-
5½′ depth	12	-	22	34±

[a] This table was derived from Appendix B-5, Part C.

in the benthic muds. Gas bubbles breaking the surface of the pond were profuse at all times as slight stirring of the bottom muds would result in release of large quantities of gas.

Diurnal measurements

Sewage inflow

Table D-1, Appendix D, contains the measurements of sewage inflow obtained during the 24 hour study. The flow rate varied from 20.0 cfs (during the period 0140-0337 hours) to 27.8 cfs (during the period 1345-1525). Total volume inflow during the 23.5 hour period was 7,100,000 gallons (26,900,000 liters).

Pond outflow

Table D-2 contains measurements of pond outflow obtained during the 24 hour study. The flow rate varied from 8.12 cfs to 8.77 cfs (actually we are uncertain whether the differences were due to instrument error).

Pond volume changes

Table D-2 also shows measurements of pond water surface elevation taken during the 24 hour study. Even though the difference between inflow and outflow rates was significant, there was little appreciable difference noted in the elevation measurements.

Measurement of physical and chemical parameters

Table D-3, Appendix D, shows the results of measurements for the chemical and physical parameters measured through the diurnal study. Corresponding graphical displays with time are also shown in this appendix.

Temperature. The surface of the pond shows considerable diurnal response (23°C to 29°C) with atmospheric temperature. The diurnal response range is only 21.7C to 23.4C at 5 ft. depth.

Dissolved oxygen. The pond surface, and to a 3 ft. depth, was supersaturated (greater than 200 percent) during daylight hours, diminishing to about saturation (8 mg/l) at 0600 hours. Surprisingly, the bottom, (at 5 ft. depth), was never devoid of oxygen—varying from 0.5 at 0700 hours to 2.5 mg/l at 1700 hours.

pH. The surface pH varied within the range of 8.4 to 9.1. At the pond bottom the pH varied from 8.2 to 9.0 with no discernible diurnal trend in either case.

Free carbon dioxide. This measurement was zero in virtually every measurement except at the inlet, which measured up to 48 mg/l.

Carbonate. Carbonate varied from 8 to 100 mg/l at the surface and from 0 to 88 mg/l at the bottom with diurnal trend in each case.

Bicarbonate. Bicarbonate varied from 166 to 260 mg/l at the surface and from 192 to 400 at the bottom. At the surface there appears to be a steady depletion throughout the day, while at the bottom it increases steadily at night These observations are consistent with the photosynthesis and respiration phenomena occurring within the pond.

Light transmission. At midday 1 percent light transmission was observed at the bottom. Within the first two feet of depth the light transmission was reduced by 50 percent.

Carbon measurements. Samples were collected and analyses were made for carbon according to the procedures outlined in Chapter III. Analyses before and after Millipore filtration are reported.

Inflow. Results for all samples are given in Table D-4. Total carbon exhibited a definite trend over the 24 hour period, peaking at 79 mg/l at 0925 hours and diminishing to 55 at 0515 hours. Inorganic carbon concentration showed no such trend ranging from 43 to 57.5 mg/l. Organic carbon varied from 6 to 24 mg/l; about 30 to 50 percent of this was suspended organics.

Outflow. Results for all samples are given in Table D-5. The outflow from the pond is representative of the pond itself. Total organic carbon varied from 62 to 78 mg/l over the 24 hour period, with no discernible trend. Inorganic carbon varied randomly from 41 to 51 mg/l. Organic carbon varied randomly also from 16 to 25 mg/l; about 50 percent of this was suspended organics (probably algae).

Depth measurements. Table D-6 shows results of sample analyses for depth measurements at station C2; samples from only four collection periods were analyzed (samples from the other collection periods were discarded because of the labor involved in analyses and because of the consistency in results of those samples analyzed). All measurements were nominally consistent with depth, and the time span of collection (further investigation might reveal diurnal trends in these measurements as there is an indication of such a trend for the organic carbon measurements). Organic carbon concentration was nominally about 25 mg/l. Suspended organics ranged from 6 to 25 percent of the organic carbon but the inflow and outflow figures were 30 to 50 percent.

The most revealing aspect of these data was the measurements of bottom samples. These measurements correlate with the magnitude of the volatile solids content found for the bottom muds sample and the gas evolution observed.

Results of kinetic studies

Substrate uptake

The rate of substrate(s) uptake was determined for: (1) aerobic bacterial synthesis and respiration, (2) algae

synthesis and respiration, and (3) anaerobic bacterial synthesis and respiration. The manner of conducting these experiments has been described previously. Results of all substrate uptake tests over time are given in Appendix E in both tabular and graphical form. The arithmetic plots are useful in displaying the trends. The consistency of the data is best seen, however, in the semi-log plots which have linear form. The greatest scatter occurred in the measurements for the anaerobic system; it is not readily apparent to us whether the uptake shown in Figure E-1i represents a rapid rate of cell synthesis or an initial biosorption, with essentially little discernible cell synthesis.

Rate constants

The kinetic rate constants, derived from the slopes of the semi-log plots in Appendix E, are summarized in Table 5.

Mass balance treatment of the reactor

Units and time increments

We now have sufficient data to ascertain the relative importance of most of the terms in Equation (9). To use Equation (9), however, we must have consistency in all units of measurement. We use total organic carbon to express concentrations of organic matter of whatever category (aerobic bacteria, anaerobic bacteria, and algae), as well as a measure of substrate concentration. To simplify nomenclature, let us designate C as total organic carbon; we assume that dissolved organic carbon in the pond is used as the substrate for aerobic bacteria; we assume that the suspended organic matter in the pond consists of algae.

We conduct our mass balance determination over one hour periods—first at the time of probable maximum photosynthesis, and then for a non-photosynthetic period. We can then apply Equation (9) to an entire 24 hour cycle. Light was available from 0600 hours to 2130 hours.

Values of individual terms

Net effect. This term is expressed mathematically as

$$\left[\frac{d\bar{C}_p}{dt} \cdot V\right]_{\text{net effect}} = 0 \times 6.06 \times 10^8 = 0$$

in which

$\bar{C}_p$ = average total organic carbon (dissolved and suspended) of the pond water (mg/l)
= 22.8 mg/l (Table D-6 average)

V = volume of pond (liters)
= 6.06×10^8 liters (data from Figure 5)

This term must be zero over the annual cycle for a long term equilibrium operational condition; it may not be zero, however, for some days or months during the season.

The $[dC_p/dt]_{\text{net effect}}$ term is the *measured* rate of TOC change of the pond water; this term was zero for our 24 hour cycle, as the TOC values were essentially constant in depth and time, as noted for Table D-6.

Table 5. Kinetic rate constants for aerobic and anaerobic bacteria glucose utilization, and algae synthesis.

Depth of reaction water (ft)	Bottle color	Radioactive substrate	Organism category	Rate constant, k ($min.^{-1}$)	(hr^{-1})
4"	light	glucose C^{14}	aerobic bacteria	9.55×10^{-4}	.057
4"	dark	glucose C^{14}	aerobic bacteria	9.55×10^{-4}	.057
5'3"	light	glucose C^{14}	aerobic bacteria	32.9×10^{-4}	.20
5'3"	dark	glucose C^{14}	aerobic bacteria	32.9×10^{-4}	.20
4"	light	bicarbonate C^{14}	algae	12.8×10^{-4}	.078
4"	dark	bicarbonate C^{14}	algae	0	0
5'3"	light	bicarbonate C^{14} algae	4.25×10^{-4}	.025	
5'3"	dark	bicarbonate C^{14}	algae	0	0
bottom (Eckman dredge)	dark	glucose C^{14}	anaerobic bacteria	11.0×10^{-4}	.066

Degradable mass inflow. This term is expressed mathematically as

$$[Q_I \cdot C_I]_{inflow} = 26.9 \times 10^6 \text{ L/day} \cdot 15.4 \text{ mg TOC/l}$$
$$= 4.15 \times 10^8 \text{ mg TOC/day}$$
$$= 415 \text{ kg TOC/day}$$

Conversion to free energy of formation in glucose equivalents is

$$[Q_I \cdot \Delta F_{f_I}]_{inflow} = 415 \text{ kg TOC/day} \times \frac{\text{mole glucose}}{.072 \text{ kg TOC}} \times \frac{217.5 \text{ kcal}}{\text{mole}}$$
$$= 1.25 \times 10^6 \text{ kcal/day}$$

in which

- Q_I = average daily inflow (liters/day)
  = 26.9×10^6 liters/day
- C_I = average organic carbon concentration (dissolved and suspended) for daily flow (mg/l)
  = 15.5 mg/l (average of Table D-4 values)
- C_{ID} = average dissolved organic carbon concentration for the daily inflow (mg/l)
  = 7.9 mg/l (average of Table D-4 values)
- C_{IS} = average suspended carbon concentration for the daily flow (mg/l)
  = 7.6 mg/l (difference between C_I and C_{ID})

Degradable mass outflow. This term is expressed

$$[Q_o \cdot C_o]_{outflow} = 5.44 \times 10^6 \text{ L/day} \cdot 20.1 \text{ mg TOC/L}$$
$$= 1.10 \times 10^8 \text{ mg TOC/day}$$
$$= 110 \text{ kg TOC/day}$$

in which

- Q_O = average daily outflow (liters/day)
  = 5.44×10^6 (liters/day)
- C_O = average organic carbon concentration (dissolved and suspended) for daily outflow
  = 20.1 mg/l (Table D-5 average)
- C_{OD} = average dissolved organic carbon concentration for the daily outflow mg/l
  = 9.3 mg/l (Table D-5 average)
- C_{OS} = average suspended carbon concentration for the daily flow (mg/l)
  = 10.8 mg/l (difference between C_O and C_{OD})

Conversion to free energy of formation in glucose equivalents is

$$[Q_o \cdot \Delta F_{fo}]_{outflow} = 110 \frac{\text{kg TOC}}{\text{day}} \times \frac{\text{mole algae}}{.096 \text{ kg algae TOC}} \times \frac{900 \text{ kcal}}{\text{mole}}$$
$$= 1.03 \times 10^6 \text{ kcal/day}$$

Volume storage change. This term is expressed:

$$\left[\frac{dV}{dt} \cdot \overline{C}_p\right]_{\substack{\text{volume} \\ \text{storage} \\ \text{change}}} = (Q_I - Q_o) \cdot \overline{C}_p$$
$$= (26.9 \times 10^6 - 5.44 \times 10^6) \text{L/day} \cdot 22.8 \text{ mg TOC/l}$$
$$= 4.90 \times 10^8 \text{ mg TOC/day}$$
$$= 490 \text{ kg TOC/day}$$

The pond depth measurement did not show discernible change (Table D-5), however, we take $dV/dt \neq 0$, because $Q_I >> Q_o$.

Conversion to free energy of formation in algae equivalents is:

$$\left[\frac{dV}{dt} \cdot \Delta F_f\right]_{\substack{\text{volume} \\ \text{storage}}} = 490 \frac{\text{kg TOC}}{\text{day}} \times \frac{\text{mole algae}}{.096 \text{ kg TOC}} \times \frac{900 \text{ kcal}}{\text{mole}} = 4.60 \times 10^6 \text{ kcal/day}$$

Aerobic degradation rate. We consider here only the degradation of *dissolved* organics. The term is expressed as a first order kinetic formulation. This is justified by assuming all factors, except substrate, are constant between the flask reactor containing pond water, and the pond water at the time of the 24 hour measurements.

$$\left[\frac{dC_{PD}}{dt}\right]_{\substack{\text{aerobic} \\ \text{metabolism}}} \cdot V = -k\, C_{PD} \cdot V$$
$$= -.057 \text{ hr}^{-1} \cdot 23.7 \text{ mg TOC/l} \cdot 6.06 \times 10^8 \text{ L}$$
$$= -8.18 \times 10^8 \text{ mg TOC/hr}$$
$$= -19,600 \text{ kg TOC/day}$$

in which

- k = kinetic rate constant determined for glucose C^{14} uptake in flask reactor using pond water from surface
  = .057 hr^{-1} (Table 4)
- C_{PD} = average dissolved organic carbon in pond water
  = 23.7 mg/l (Table D-6, organic carbon after Millipore filtration)

Conversion to standard state free energy of formation (glucose equivalents) is:

$$\left[\frac{d\Delta F_f}{dt} \cdot V\right]_{\substack{\text{aerobic} \\ \text{metabolism}}} = -19,600 \text{ kg TOC/day} \times \frac{\text{mole glucose}}{.072 \text{ kg glucose TOC}} \times \frac{217.5 \text{ kcal}}{\text{mole}}$$
$$= -59.0 \times 10^6 \text{ kcal/day}$$

Algae synthesis. The algae synthesis rate is one eighth the rate of utilization of carbon dioxide (resulting from bicarbonate conversion). We formulate this term as first order with respect to bicarbonate, as justified previously.

$$\left[\frac{d\Delta F_f}{dt}\cdot V\right]_{\substack{\text{algae}\\ \text{synthesis}\\ 0\text{-}3'}} = k_{HCO_3^-}\cdot[HCO_3^-]_{0\text{-}3}\cdot V_{0\text{-}3}$$

$$= .078\ hr^{-1}\times 213\ \frac{mg\ HCO_3}{L}\times\frac{172\ mg\ algae}{488\ mg\ HCO_3}\times 3.60\times 10^8\ L$$

$$= 21.1\times 10^8\ mg\ algae/hr$$

$$= 2110\ kg\ algae/hr$$

Now from Figure 13, saturation light intensity[1] is available as follows:

13.5 hr - surface
10.5 hr - 1 ft.
9.0 hr - 2 ft. = 9.5 hr avg. saturation exposure 0-3' depth
5.0 hr - 3 ft.

$$\therefore \left[\frac{dC_A}{dt}\right]_{\substack{\text{algae}\\ \text{synthesis}\\ 0\text{-}3'}}\cdot V = 20,100\ kg/day\ (\text{for } 10.5\ hr\ day)$$

in which

k_{HCO_3} = kinetic rate constant determined for bicarbonate C^{14} uptake in flask reactor using pond water from surface
= .078 hr^{-1} (Table 4)

$[HCO_3^-]_{0\text{-}3}$ = average of all bicarbonate measurements from zero to 3 ft. depth during 24 hour test
= 213 mg/l

$V_{0\text{-}3}$ = volume of pond from surface to 3' depth
= 3.60×10^8 L

Now the remaining depth to 5'6" is calculated on the basis of the bottom measurement with a rate constant $k_{3\text{-}5}$ = 0.025 hr^{-1} (Table 5). Let us use the same average $[HCO_3^-]$ concentration, and $V_{3\text{-}5}$ = 2.48×10^8 L, and calculate

$$\left[\frac{dC_A}{dt}\right]_{\substack{\text{algae}\\ \text{synthesis}\\ 0\text{-}5}}\cdot V_{3\text{-}5} = 2110\ \frac{kg\ algae}{hr}\times\frac{2.48}{3.60}\times\frac{.025}{.078}$$

$$= 470\ kg\ algae/hr$$

It appears from Figure 13 that the light sufficient for this reaction rate is available for an average time of about 6 hours in the 3-5' depth range. Thus:

$$\left[\frac{dC_A}{dt}\right]_{\substack{\text{algae}\\ \text{synthesis}\\ 0\text{-}3}}\cdot V_{3\text{-}5} = 2800\ kg\ algae/day$$

So for the whole pond:

$$\left[\frac{dC_A}{dt}\cdot V\right]_{\substack{\text{algae}\\ \text{synthesis}}} = \left[\frac{dC_A}{dt}\ V\right]_{0\text{-}3} + \left[\frac{dC_A}{dt}\ V\right]_{3\text{-}5}$$

$$= 20,100 + 2800$$

$$= 22,900\ kg\ algae/day$$

$$= 12,600\ kg\ algae\ TOC/day$$

This value converts to 7.7 tons/acre/mo.

$$\left(22,900\ \frac{kg\ algae}{day}\times\frac{1}{9.7\ acres}\times\frac{30\ day}{mo}\times\frac{lb}{.454\ kg}\times\frac{ton}{2000\ lb}\right)$$

—which compares to a value of 5.0 tons/acre/mo. reported by Oswald and Gotaas (1957) for a pond at Richmond, California.

Conversion to standard state free energy of formation is as follows:

$$\left[\frac{d\Delta F_f^o}{dt}\cdot V\right]_{\substack{\text{algae}\\ \text{synthesis}}} = 22,900\ \frac{kg\ algae}{day}\times\frac{mole\ algae}{.172\ kg\ algae}\times\frac{900\ kcal}{mole}$$

$$= 120\times 10^6\ kcal/day$$

Restating Equation (9), in terms of organic carbon gives:

$$\left[\frac{dC_P}{dt}\cdot V\right]_{net} = [Q_I\cdot C_I]_{inflow} - [Q_o\cdot C_o]_{outflow} + \left[\frac{dV}{dt}\cdot\overline{C}_P\right]_{\substack{\text{vol.}\\ \text{storage}}} + \left[\frac{dC_{PD}}{dt}\cdot V\right]_{\substack{\text{aerobic}\\ \text{metabolism}}} + \left[\frac{dC_A}{dt}\cdot V\right]_{\substack{\text{algae}\\ \text{synthesis}}} + \left[\frac{dC}{dt}\cdot V\right]_{\substack{\text{anaerobic}\\ \text{metabolism}}}$$

Substituting numerical values, in terms of TOC, gives:

$$0 = (415) - (110) + (490) + (-19,600) + (22,900) + \left[\frac{dC}{dt}\ V_B\right]_{\substack{\text{anaerobic}\\ \text{metabolism}}}$$

or

$$\left[\frac{dC}{dt}\cdot V_B\right]_{\substack{\text{anaerobic}\\ \text{metabolism}}} = -4,200\ kg\ TOC/day$$

[1] Figure 4 outlines the manner in which the saturation light intensity was evaluated; any light of greater intensity does not cause further increase in algae production rate.

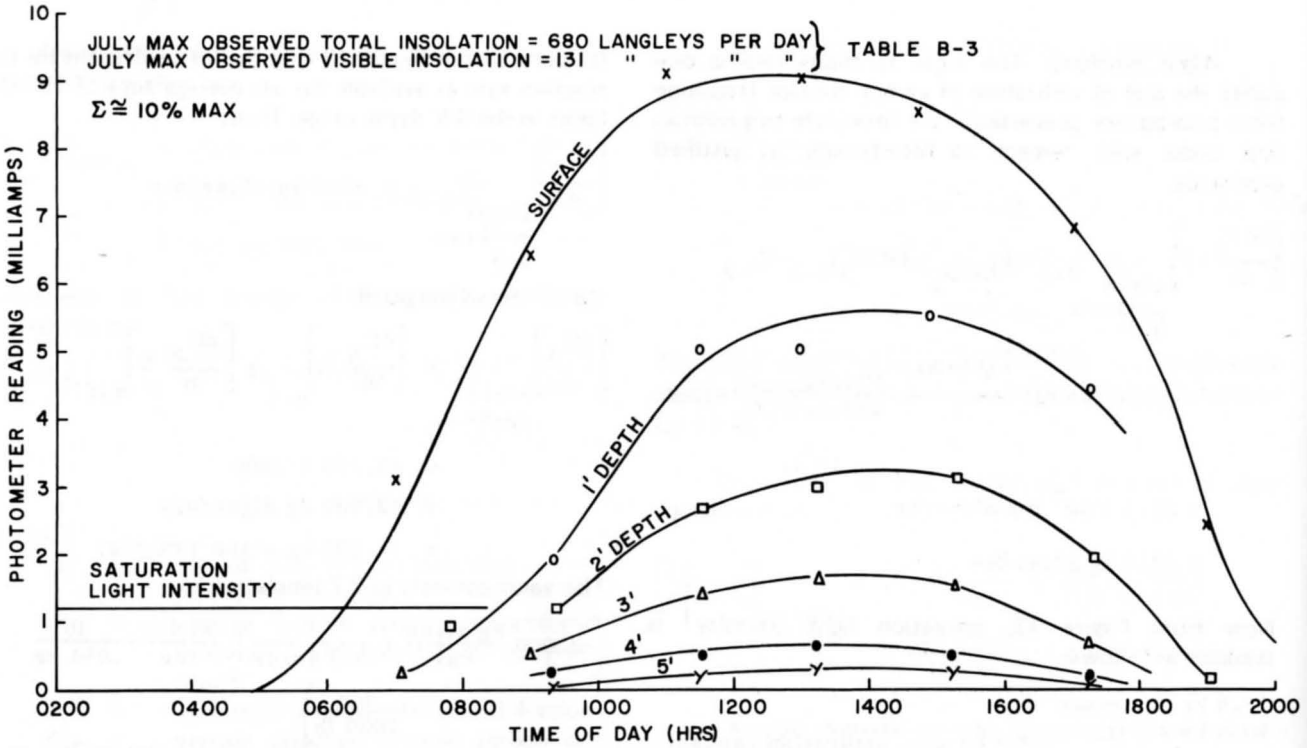


Figure 13. Photometer readings in south primary pond, Logan, Utah, July 8, 1970.

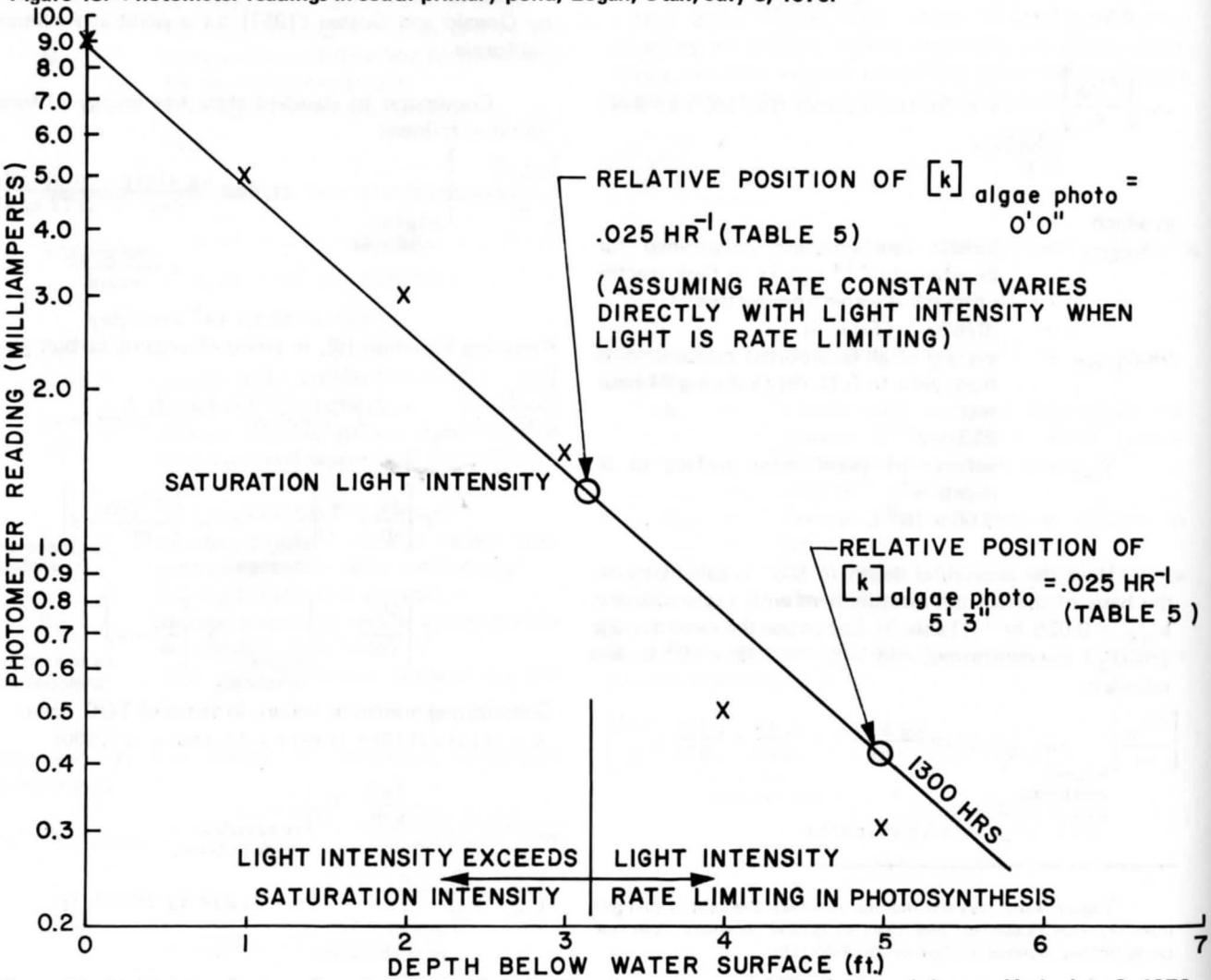


Figure 14. Light intensity as a function of depth in south primary waste stabilization pond, Logan, Utah, July 8, 1970.

Table 6 compares our measurements of algae synthesis rate with values reported by others and with the "algae production potential" for this latitude. Although our production rate measurements are higher than others reported, they are consistent with the other results in the table. The maximum production rate is seen to be about four times the rate based upon our measurements.

Anaerobic degradation. We did obtain a rate constant for anaerobic degradation of glucose; the determination is shown in Appendix E, Table E-3 and Figure E-3. The value obtained .066 hr^{-1} compares with an aerobic rate constant of 0.20 hr^{-1}, Table E-1, using bottom muds in each case for the experimental batch reactor. The anaerobic rate constant cannot be utilized to evaluate the anaerobic kinetic term, however, since the aerobic zone in the pond went essentially to the bottom. The muds were undoubtedly anaerobic as they contained much gas which was continuously released as bubbles and could be dislodged easily in large quantities. However, obtaining a substrate concentration for use in the anaerobic kinetic term is indeterminate and nebulous when bottom muds are the primary reaction medium.

A daily rate value for the anaerobic kinetic term probably is a misconception anyway without a liquid anaerobic zone. To be more rigorous and correct, the term in Equation (9) designated "anaerobic degradation" should be retitled "anaerobic degradation and storage" since the substrate in the mass balance equation will first go to storage in the muds and then undergo reaction subsequent to creation of a suitable anaerobic environment.

Summary of mass balance reactor formulation

Table 7 summarizes the results of applying Equation (9) in a gross manner to the whole south primary pond, over a one day period for July 1970 conditions. These results are given in three equivalent forms: (1) mass weight of the organic species being degraded or synthesized, (2) chemical oxygen demand COD equivalent (from Table 3), and (3) the standard state free energy of formation contained in the respective organic mass being degraded or synthesized. All of the foregoing are expressed as daily rates.

As with Figures 4 and 5, Table 7 has poignant implications. First it is clear, using column (1) units, that the flux out, which should legitimately include the storage change term, is 600 kg TOC/day versus 415 kg TOC/day in. Thus the product water is worse; it contains more in degradable mass than does the raw sewage. Next we see in the algae synthesis term a prolific rate of synthesis 22,900 kg algae/day, or 12,600 kg TOC/day, this compares with about 300 kg algae/day needed to supply the stoichiometric amounts of oxygen to the inflow. This rate of algae synthesis is consistent with our July 8 observations of dissolved oxygen in the pond, which showed large oxygen amounts throughout the pond. The rate of degradation -19,600 kg TOC/day (as glucose), is in excess of the rate of synthesis, 22,900 kg/day; it should also be realized that our determination for the degradation term is undoubtedly very high compared with actual rates, since glucose is so easily metabolized. The deficit in TOC is -6,205[2] kg/day which will continue to accrue partially as suspension (in pond storage) and partially as debris which will fall to the benthos for eventual anaerobic degradation. The comparisons reveal a surplus of carbon compounds if either COD or free energy of formation stored in the molecules in question is used as a basis for the comparisons.

[2]The number of significant figures used is intended to be used only in tracing calculations and is not intended to imply accuracy.

Table 6. Comparison of algae production rates from several sources.

Investigator	Algae Production Rate	
	gm algae/m^2/day	tons algae/acre/mo.
Fitzgerald & Rohlich (1958) (laboratory)	10	1.33
Israelsen (pilot pond)	21	2.7
Oswald and Gotaas (1957)—July	37	5.0
Algae Prod. Pot (Fig. 3 and Table B-3)—July	202	27.0
This work—July (measured in Logan S. primary)	57	7.7
Stoichiometric algae production needed to provide oxygen for 415 kg waste inflow to Logan S. primary	0.303	0.040

Table 7. Summary of results of evaluations for each term in Equation (9), in different units, for the Logan south primary pond.

Term		Rate of change of organic molecules		
		kg TOC/day	kg COD/day	kcal/day[d]
Net effect	$\left[\frac{dC_p}{dt} \cdot V\right]$	0	0	0
Inflow	$[Q_I \cdot C_I]$	+415[d]	+1100	$+(-1.25 \times 10^6)$
Outflow	$-[Q_o \cdot C_o]$	-110[b]	-294	$-(-0.103 \times 10^6$
Storage changes	$\left[\frac{dV}{dt} \cdot C_p\right]$	+490[b]	+1300	$+(-4.60 \times 10^6)$
Aerobic degradation	$\left[\frac{dC_{pD}}{dt} \cdot V\right]$	-19,600[b]	-20,900	$-(59.0 \times 10^6)$
Algae synthesis	$\Sigma\left[\frac{dC_D}{dt} \cdot V\right]$	+12,600[ce]	+34,200	$+(-120 \times 10^6)$
Surplus or deficit to anaerobic storage[c]	$\left[\frac{dC}{dt} \cdot V_B\right]$	-6,205	+15.406	$+(-65.82 \times 10^6)$

[a]Measured TOC of incoming waste (considered glucose in calculations).

[b]Measured TOC of pond water (considered algae in calculations) is basis for calculation.

[c]The amount of algae produced is 22,900 kg.

[d]The stoichiometric daily amount of algae needed to provide sufficient oxygen to aerobically degrade this quantity of waste is:

$$\frac{19.8 \text{ gm algae (Fig. 4)}}{68 \text{ gm dissolved glucose (Fig. 4)}} \times 415 \text{ kg TOC (as glucose) inflow} \times \frac{180}{72} = 300 \text{ kg algae cells}$$

$$= 167 \text{ kg algae TOC}$$

[e]Algae production potential, AGP (July, Logan, Utah) = 202 gm algae/m^2/day (Figure 3). For the whole pond having a surface of 396,000 m^2, the AGP is:

AGP = 0.202 kg algae/m^2/day (Table 6) x 396,000 m^2

= 7.93×10^4 kg algae/day

= 79,300 kg algae/day

Also this amount of algae will produce $\frac{32.2 \text{ gm } O_2}{19.8 \text{ gm algae}} \times$ 793,000 kg algae = 1.29×10^6 kg oxygen per day, which compares with a daily COD demand by the incoming waste of 443 kg/day.

SUMMARY AND CONCLUSIONS

1. Comparing inflow and outflow TOC concentrations, the effluent from the pond is essentially no better than the influent. Results showed:

	TOC(mg/L)		
	Total	Dissolved	Suspended
influent stream	20.1	9.3	10.8
effluent stream	15.4	7.9	7.6

2. The stoichiometric amount of algae needed to provide sufficient oxygen to degrade 415 kg TOC/day incoming waste (as glucose) is 167 kg TOC/day (as algae cells). Even in this limited sense the trade is only moderately favorable.
3. The potential algae production for the pond studied, and for the date of the experiments, is 44,000 kg TOC/day (as algae).
4. The actual algae production, as determined by kinetic studies, was 12,600 kg TOC/day. This is about one-third of the upper limit and almost 100 times the stoichiometric amount needed.
5. The aerobic degradation rate, as determined by kinetic studies, was 19,600 kg TOC/day. This was for a glucose substrate, however, which probably has a considerably faster reaction rate constant than substrates actually metabolized in the pond. If so, the rate of synthesis is probably in near balance with the rate of degradation.
6. Comparing the rates of algae synthesis, degradation, and influent TOC flux, it is evident that a vast energy overturn exists in relation to that needed stoichiometrically. Further, in examining TOC flux out and contribution to storage, all this is to no avail.
7. Although we were not able to quantify the importance of the anaerobic zone, a preliminary kinetic study did show a rate constant about one-third that of the aerobic zone (this has only nominal significance). Also the profuse amount of gas evolving from the benthic muds suggests that the anaerobic zone must have an important role. Probably the anaerobic zone bears the brunt of the waste degradation finally achieved.
8. The primary waste stabilization pond studied was grossly inefficient during the time period it was studied.

RECOMMENDATIONS

1. The other cells in the pond system should be studied to determine the final disposal of the algae contained in the primary pond effluent.
2. The study should be continued over an annual cycle where Equation (9) is formulated or summed over a longer time period.
3. The loading rates to an anaerobic pond need to be determined by kinetic studies, in situ.
4. An anaerobic pilot pond should be built and monitored over an annual cycle.
5. The area loading criteria should be examined critically; a volume loading criteria should be reviewed as a possible better design basis.

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APPENDIX A

THERMODYNAMIC CALCULATIONS OF POND REACTIONS

1. Oxidation of glucose

The reaction is:

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

ΔF_f^o (glucose = - 217.56 kcal/mole (Williams, 1967)

$$\Delta F_R^o = (6)(-92.31) + (6)(-56.69) - (1)(-217.56) - (6)(0)$$

$$= -676.44 \text{ kcal}$$

2. Aerobic bacterial synthesis reaction

The following equation is adapted from a larger empirical equation given by Schroeder and Busch (1966). The bacterial formulation is also given by McCarty (1965).

$$6C_6H_{12}O_6 + 4NH_3 + 16O_2 \longrightarrow 4C_5H_7O_2N + 16CO_2 + 28H_2O \quad \text{(A-1)}$$

$$4\Delta F_f^o(C_5H_7O_2N) = .55\,\Delta F_R^o \quad \text{(A-2)}$$

$$\Delta F_R^o = (4)(\Delta F_f^o(\text{cells}) + (16)(-92.31) + (28)(-56.69) - (6)(-217.56) - (4)(-19.00) \quad \text{(A-3)}$$

Solving for ΔF_f^o (cells) and ΔF_R^o gives:

ΔF_R^o (cells) = -520 kcal/mole cells

ΔF_R^o = -3760 kcal

We should note that Burkhead and McKinney (1969) propose the reaction:

$$C_6H_{12}O_6 + 1.26\,O_2 + 0.96\,NH_3 \longrightarrow 0.96\,C_5H_{11}O_4N + 1.20\,CO_2 + 2.16\,H_2O$$

3. Photosynthesis reaction

(a) Empirical formula determination for algae:

	%[a]	Atomic weight	%/atomic weight	Empirical elemental composition
Carbon	55.5	12	4.62	8.1
Hydrogen	8.0	1	8.00	14.0
Oxygen	28.5	16	1.78	3.1
Nitrogen	8.0	14	0.57	1.0

[a] Reference (Foree, 1968).

Thus the empirical formula is: $(C_8H_{14}O_3N)_x$ and if x = 1 then molecular weight = 172 gm/mole; the corresponding oxygen requirement to oxidize the eight carbon atoms would be 256 gm COD/mole

(b) The stoichiometry for the simplified photosynthesis reaction is:

$$NH_3 + 8.0\,CO_2 + 4.5\,H_2O \longrightarrow C_8H_{14}O_3N + 8.75\,O_2 \quad \text{(A-4)}$$

Using the ratio of oxygen produced per gram of algae synthesized of 1.6 from Bartsch (1961), we arrive at the stoichiometry shown in Equation (A-4).

The energy of a compound is stored primarily in the chemical bonds. Algae have 7 carbon to carbon bonds and the bacteria have 4 carbon to carbon bonds. Therefore we calculate the free energy of formation of the algae as follows:

$$\Delta F_{f(\text{algae})}^o = (7/4)(-520) = -900 \text{ kcal/mole}$$

$$\Delta F_R^o = [(1)(-900) + 8.75(0)] - [(1)(-19.00) + (8)(-92.31) + 4.5(-56.69)]$$

$$= +112 \text{ kcal/mole algae}$$

$$= +0.44 \text{ kcal/gm COD}$$

4. Anaerobic reactions

(a) Calculation of free energy of formation of organic sludge deposit.

Using the amounts of organic matter delivered to the sludge deposit shown in Figure 2, we calculate the psuedo free energy of formation by the weight average of the conglomerate material. Also Foree and McCarty (1968) indicate that only 60 percent of the algae decomposes under anaerobic conditions. Individual ΔF_f^o values for each material is from Table 2.

$$\Delta F_f^o \text{ (organic sludge)}$$

$$= \frac{\overset{\text{glucose}}{(180)(-217.56)} + \overset{\text{bacteria}}{(285)(-520)} + \overset{\text{algae}}{(.6)(19.8)(-900)}}{.516}$$

$$= -438 \text{ kcal/mole}$$

A weighted average molecular weight is:

$$MW \text{ (organic sludge)} = \frac{26(180) + 28.5(113) + .6 \cdot 19.8(172)}{66.3}$$

$$= 166$$

And a composite COD is:

$$COD \text{ (organic sludge)}$$

$$= \frac{(26/180)(192) + (285/113)(160) + (.6 \times 19.8/172)(256)}{.516}$$

$$= 168$$

(b) Free energy of reaction for acid formation

The 66.3 gm of degradable sludge resulting from the original 94 gm glucose (suspended and dissolved), as shown in Figure 2, contains -223 kcal, as shown in Figure 4. It is beyond the scope of this work to pursue the biochemistry of the fermentation reaction for each sludge component (glucose, bacteria, algae), especially since we have been unable to find such reactions in the literature, after nominal search. Therefore we simplify the sludge fermentation reaction by assuming glucose fermentation is representative of the sludge. Thus the -223 kcal is equivalent to (-223 kcal of sludge/-217.56 kcal/mole glucose), or 1.03 moles glucose sludge, or 184 gm glucose sludge.

(c) Acid formation from sludge in terms of its glucose equivalent

The next step is to formulate the acid fermentation stage. McCarty (1965, p. 179) gives such a reaction (among many) that would appear to serve our purposes well:

$$\text{glucose} \longrightarrow 1.33 \text{ propionate} + .67 \text{ acetate} + .67 CO_2 \quad \Delta F_R^o = -421 \text{ cal/gm glucose COD}$$

$$= 81.0 \quad \ldots\ldots\ldots \quad \text{(A-5)}$$

For this reaction he gives the cell yield as $f_s = 0.250$,

where $f_s = \dfrac{\text{COD of net mass of cells formed}}{\text{original COD of substrate utilized}}$

$\therefore$ COD of cell mass formed = .250 × 192 gm COD/mole of glucose

= 48 gm COD

= -78 kcal (Table 3 conversion)

= 0.30 moles (Table 3 conversion)

= 39 gm cells (Table 3 conversion)

Therefore we hypothesize the following reaction for acid fermentation and cell synthesis:

$$\text{glucose} \longrightarrow \underset{\text{(cells)}}{0.60\, C_5H_9O_3N} + 1.33 \text{ proprionate} + 0.67 \text{ acetate} + 0.67\, CO_2, \quad \Delta F_R^o$$

$$\ldots\ldots\ldots\ldots \quad \text{(A-6)}$$

Rewriting Equation (A-6) in terms of molecular formula, and whole number coefficients, and adding an N source, we have:

$$15 C_6H_{12}O_6 + 9NH_3 \longrightarrow \underset{\text{(cells)}}{9 C_5H_4O_3N} + \underset{\text{(prop.)}}{20 C_3H_6O_2} + \underset{\text{(acetic)}}{10 C_2H_4O_2} + 10 CO_2, \quad \Delta F_R^o$$

$$\ldots\ldots\ldots\ldots \quad \text{(A-7)}$$

Equation (A-7) is not balanced as it stands, however, so there is little point in calculating ΔF_R^o.

Acid fermentation

Again the complete gross reaction including cell synthesis is not given in the literature. According to McCarty (1968), of 100 gm COD substrate, 72 gm will go through acetic acid to methane, and 13 gm through propiomic to methane and 15 gm through other intermediates. The acetic acid fermentation is the simplest to write, and is (continuing the .67 yield from Equations A-5, A-6):

$$.67 CH_3COOH \longrightarrow .67 CH_4 + .67 CO_2 \quad \Delta F_R^o$$

$$= \frac{-106 \text{ cal} \times .67}{\text{gm COD as glucose}}$$

$$= 13.8 \text{ kcal} \quad \ldots\ldots\ldots\ldots \quad \text{(A-8)}$$

The growth yield for this reaction is, $f_s = 0.060$ (McCarty, 1965, p. 180); thus the cell yield would be .67 x .06 x 192 = 7.7 gm COD of methane formers = -13 kcal = 0.05 moles of methane bacteria = 6.6 gm cells.

Rather than try to construct a synthesis reaction for methane formers, let us abandon this approach and consider its reference value.

(d) One step simplified reaction

Rather than pursue the two stage approach further, let us write the gross fermentation reaction as suggested by McCarty (1965):

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \longrightarrow 3\,CH_4 + 3\,CO_2 \qquad \Delta F_R^o = \frac{-532 \text{ cal}}{\text{gm glucose COD}}$$

$$= -102 \text{ kcal} \qquad \ldots\ldots\ldots\ldots \text{(A-9)}$$

also

$$\Delta F_R^o = [3(-12.14) + 3(-94.26)] - [(-217.56)]$$

$$= -102 \text{ kcal}$$

(The difference between (-81 kcal + -13 kcal) and the -102 kcal is not apparent.) The cell yields from these two reactions for one mole of glucose, are: 39 gm acid formers (-78 kcal), and 6.6 gm methane bacteria (-13 kcal). Since the 94 gm of original glucose resulted in 184 gm of sludge in glucose equivalents, or 1.03 moles, we multiply the above by 1.03 to get the yields for Figure 4.

(e) Methane escape of methane into the atmosphere

When the methane escapes from the pond to the atmosphere, the amount of free energy of formation contained in the methane leaves the system. This also diminishes the potential free energy of oxidation, ΔF^o_{ox} in the pond system as illustrated in Figure 3. The oxidation equation and the free energy of oxidation calculation is shown below:

$$3\,CH_4 + 6\,O_2 \longrightarrow 3\,CO_2 + 6\,H_2O$$

$$\Delta F_R^o = 3(-92.31) + (6)(-56.69) - (3)(-12.14)$$

$$= -580 \text{ kcal per mole of glucose sludge degraded}$$

APPENDIX B

MISCELLANEOUS PILOT EXPERIMENTS

This appendix contains the following:

1. Influent and effluent COD's and results of light and dark bottles gross productivity experiments during September 1969
2. Insolation data
3. Pilot data from the pond during June 1970, giving oxygen and temperature profiles
4. Correlation between instrument and conventional alkalinity titrations for inorganic carbons
5. Ascertaining importance of acid washing Millipore filters prior to use
6. Ascertaining the proportions of dissolved and suspended organic carbon, and the proportion of algae and bacteria sizes to the suspended portion
7. An analysis of bottom muds to find volatile solids composition of a grab sample
8. Sample calculation of pond outflow from current meter data and saturation dissolved oxygen table for converting instrument readings in percent saturation to mg/l dissolved oxygen.

These results answer many preliminary questions and form the basis for developing a better intuitive "feel" for pond conditions.

These results serve as a source of background information about pond conditions and behavior. They are used both directly and indirectly in assessing the nature and consistency of data used in the numerical mass balance calculations. In addition, such information adds to the intangible "feel" for a system that must be developed for guiding experiments.

Table B-1. COD values of influent and effluent of south primary pond (after Andrew, 1970)[a]

Date	Influent COD (mg/l)	Effluent COD (mg/l)
9/4	175	36
9/5	149	51
9/6	30	20
9/8	51	41
9/10	100	10
9/11	330	50
9/12	30	40
9/18	220	5
9/19	180	20
9/22	120	30
9/23	60	20
9/25	137	29
Ave.	132	29

[a]During this period of tests the pond water was very clear with the pond bottom clearly visible; all algae had sunk to the bottom.

Table B-2. Gross O_2 production and energy conversion efficiency for algae[d] during September in Logan primary ponds (after Andrew, 1970).

Date	Gross O_2[a] Production gm O_2 /M^2/day	Energy[b] Utilized cal/M^2/day	(F)[c] Energy Utilized Energy Available
		(X 10^3)	
9/4	17.38	63.96	.078
9/6	11.53	42.43	.052
9/10	4.58	16.85	.021
	7.87	28.96	.035
9/11	10.06	37.02	.045
	10.43	38.38	.047
9/12	11.35	41.76	.051
	6.77	24.91	.030
	6.95	25.58	.031
	3.66	13.47	.016
9/18	7.69	28.30	.035
	0.18	0.66	.001
	11.90	43.79	.053
	0.91	3.35	.004
9/19	0.37	1.36	.002
	0.18	0.66	.001
	1.10	4.05	.005
	1.10	4.05	.005
9/22	4.94	18.18	.022
	10.98	40.41	.049
	5.12	18.84	.023
	7.87	28.96	.035
9/23	6.22	22.89	.028
	13.72	50.49	.061
	2.01	7.40	.009
	13.72	50.49	.061
9/25	8.78	32.31	.039
	2.74	10.08	.012
	10.61	39.04	.048
	7.32	26.94	.033
Mean	6.94	25.52	.031

[a]Measurement of O_2 production by the light-dark bottle method described by Ryther (1957).

[b]Energy utilized was computed by: E.U. = (O_2 production) (energy needed to produce O_2). It has been reported Oswald et al. (1957) that 3680 calories are used to produce 1 gram O_2 by algae. E.U. = (3680) (gms O_2/M^2/day) = cal/M^2/day.

[c]F is the amount of light energy that is actually used by the algae. Oswald and Gotaas (1955) call it the energy conversion efficiency. The average daily light energy available during September was 82 Langleys/day (Table B-3, this appendix) which is 8.2 x 10^5 cal/M^2/day. F was computed by dividing the energy utilized by 8.2 x 10^5 cal/M^2/day.

[d]Algae samples were picked up from bottom for use in experiments.

Table B-3. Insolation and maximum algae production south primary waste stabilization pond, Logan, Utah (after Andrew, 1970).

Lat. 41°44N
El. 4430 msl.

Month		Calculated[a] Total Insolation	Observed[b] Total Insolation		Calculated[a] Visible Insolation	Observed[b] Visible Insolation	Algae[c] Production Potential x 10^3
		(Langleys/Day)					(gm/m^2/day)
Jan.	max	276	102	max	71	36	55
	min	t 97		min	25		
Feb.	max	400	280	max	124	54	83
	min	151		min	47		
Mar.	max	525	475	max	179	76	117
	min	270		min	92		
April	max	669	513	max	246	99	152
	min	372		min	125		
May	max	788	612	max	295	121	186
	min	438		min	164		
June	max	840	602	max	310	141	217
	min	487		min	181		
July	max	824	680	max	299	131	202
	min	477		min	174		
Aug.	max	725	578	max	264	106	164
	min	436		min	149		
Sept.	max	591	427	max	204	82	126
	min	313		min	109		
Oct.	max	444	257	max	150	59	91
	min	202		min	68		
Nov.	max	312	152	max	87	40	62
	min	121		min	35		
Dec.	max	264	116	max	58	27	42
	min	84		min	20		

[a] Interpolated from Table B-3 for Logan, Utah.

[b] Values from instrument located on roof of F-Z Building, Utah State University.

[c] Potential algae production per day (gm/cm^2/day) = $\frac{V \cdot E}{[\Delta F_R]_{algae}}$

in which

V = observed visible Langleys per day (gm-cal/cm^2/day)

$[\Delta F_R]_{algae}$ = free energy of reaction for the photosynthesis reaction (0.65 real per gram of algae

E = energy conversion efficiency = 10% maximum (Oswald and Gotaas, 1957).

Table B-4. Solar radiation; [a] probable average values of insolation—direct and diffuse—on a horizontal surface at sea level, in Langleys [b] per day (after Oswald and Gotaas, 1955).

North Latitude		Month																				North Latitude	
Degree	Range	January		February		March		April		May		June		July		August		September		October		November		December		Range	Degree
		vis[c]	tot[d]	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot	vis	tot		
0	max[e]	255	685	266	700	271	703	266	690	249	645	236	626	238	630	252	666	269	690	265	691	256	683	253	667	max	0
	min[f]	210	580	219	583	206	536	188	462	182	480	103	274	137	368	167	452	207	533	203	530	202	513	195	527	min	
2	max	250	670	263	693	271	706	267	697	253	655	241	642	244	646	255	673	269	693	262	688	251	666	249	646	max	2
	min	206	560	213	560	201	534	183	461	181	481	108	288	141	375	169	442	206	541	200	523	195	526	189	505	min	
4	max	244	650	259	688	270	701	268	701	258	665	247	656	250	657	258	678	269	695	260	680	246	659	244	628	max	4
	min	200	540	206	543	202	532	187	466	187	492	113	300	146	385	171	448	201	529	196	513	191	510	183	489	min	
6	max	238	630	254	675	268	702	270	705	262	675	252	668	255	669	261	683	269	697	256	670	240	631	238	619	max	6
	min	193	520	199	530	200	530	186	467	189	500	118	310	150	395	172	452	202	524	191	500	185	494	176	469	min	
8	max	230	610	249	665	267	700	270	709	266	685	258	678	260	680	263	688	267	695	252	660	231	616	231	590	max	8
	min	187	495	192	510	196	523	185	467	191	506	124	329	151	405	174	456	200	518	186	486	182	478	169	449	min	
10	max	223	595	241	655	261	694	271	711	270	691	262	688	265	690	266	693	266	693	248	650	228	600	225	570	max	10
	min	179	475	184	490	193	513	183	464	192	512	129	330	158	414	176	460	196	510	181	474	176	462	162	429	min	
12	max	216	572	239	645	262	690	271	710	273	702	267	700	269	700	267	697	264	691	241	649	221	585	217	559	max	12
	min	172	455	176	470	189	500	181	462	193	518	133	343	161	421	176	461	193	502	176	462	169	446	154	400	min	
14	max	208	555	233	620	258	680	271	709	276	710	272	710	273	708	269	700	262	685	240	627	214	567	209	536	max	14
	min	163	430	167	450	181	487	179	460	194	524	137	351	164	429	177	467	189	496	170	449	162	430	146	389	min	
16	max	200	530	226	610	255	670	272	707	279	718	276	720	277	715	270	703	259	681	231	615	206	554	200	520	max	16
	min	154	400	159	430	180	473	177	456	194	528	141	363	167	435	177	469	185	489	164	434	154	410	138	369	min	
18	max	192	515	220	590	250	661	272	705	282	725	280	728	280	723	272	705	256	680	220	595	198	533	192	500	max	18
	min	141	380	150	410	174	459	174	452	194	530	145	375	170	442	177	471	180	479	157	418	146	390	129	340	min	
20	max	183	500	213	575	246	652	271	703	284	730	281	738	282	729	272	706	252	671	224	596	190	520	182	484	max	20
	min	134	360	140	390	168	440	170	447	191	532	148	383	172	450	177	472	176	467	150	400	138	370	120	320	min	
22	max	174	480	206	560	241	641	270	701	286	734	286	747	285	736	273	707	248	668	218	582	183	500	172	469	max	22
	min	123	335	132	370	162	426	167	440	193	530	152	392	173	451	176	472	170	455	143	389	128	350	110	309	min	
24	max	165	460	200	545	236	625	268	697	288	738	290	753	287	742	273	708	241	659	212	568	175	480	161	449	max	24
	min	111	310	123	340	156	410	161	433	191	525	155	403	176	459	174	471	165	443	136	369	119	326	101	280	min	
26	max	156	410	192	530	230	615	266	690	288	741	292	760	288	749	273	706	249	652	205	552	166	460	149	420	max	26
	min	90	280	114	310	149	390	160	425	189	518	158	409	177	463	172	469	169	429	128	352	109	309	90	260	min	
28	max	146	420	181	510	224	603	261	683	289	743	294	764	283	755	272	701	236	635	190	547	157	440	138	400	max	28
	min	87	250	106	290	142	373	156	415	187	506	161	418	178	467	169	466	154	415	120	310	99	278	80	230	min	
30	max	136	400	176	490	218	587	261	675	290	744	296	768	289	759	271	702	231	625	192	524	148	420	126	389	max	30
	min	76	220	96	260	131	362	151	405	181	490	163	425	178	469	166	462	147	399	113	290	90	259	70	210	min	
32	max	126	380	169	470	212	570	258	663	290	741	296	772	289	761	269	700	226	615	185	510	158	460	114	360	max	32
	min	63	180	87	240	126	340	146	395	181	475	166	431	178	472	163	455	140	385	101	270	80	224	60	181	min	
34	max	114	360	160	450	204	553	254	657	290	743	297	775	289	763	267	696	221	602	178	490	128	350	101	338	max	34
	min	53	155	78	215	118	320	141	385	176	462	168	439	178	472	159	448	134	368	96	250	70	202	47	158	min	
36	max	103	335	150	430	196	538	250	650	288	741	298	776	289	765	261	690	215	590	170	470	118	340	88	311	max	36
	min	41	133	70	200	111	300	136	375	172	441	170	443	177	470	155	438	127	350	88	250	60	180	39	134	min	
38	max	90	310	140	415	189	520	246	640	287	738	298	778	288	766	262	684	210	576	162	450	106	336	77	290	max	38
	min	36	120	62	180	103	289	131	365	166	428	171	448	175	464	152	429	120	330	80	216	50	158	30	111	min	
40	max	80	280	130	390	181	500	241	630	286	732	298	778	288	765	258	680	203	562	152	430	95	313	66	270	max	40
	min	30	105	53	160	95	270	125	355	162	415	173	450	172	455	147	416	112	310	72	202	42	134	24	91	min	
42	max	68	255	119	370	172	485	236	618	283	728	298	777	287	761	254	670	196	517	141	410	84	280	56	244	max	42
	min	24	90	45	140	88	250	120	341	157	405	174	451	167	442	143	403	105	290	65	187	31	112	19	78	min	
44	max	55	228	106	319	165	470	230	607	280	722	296	777	285	755	250	659	189	530	132	369	72	265	47	218	max	44
	min	20	80	37	130	80	230	114	325	153	395	175	453	161	430	139	389	98	270	58	173	28	98	15	62	min	
46	max	45	200	91	315	156	450	224	593	278	716	293	776	281	749	245	650	181	512	122	370	61	238	39	191	max	46
	min	16	74	30	110	72	210	108	315	150	385	175	455	161	420	131	371	90	250	52	158	23	86	11	48	min	
48	max	35	180	82	290	149	430	218	582	274	710	297	776	282	740	241	610	171	485	111	350	50	210	32	170	max	48
	min	12	61	25	99	61	190	102	307	146	378	176	458	158	410	129	358	81	230	45	141	18	70	9	37	min	
50	max	23	161	70	265	141	410	210	568	271	703	297	776	280	733	236	625	165	480	100	329	40	183	26	141	max	50
	min	10	51	19	80	58	173	97	300	141	371	176	458	155	403	125	342	73	210	40	130	15	60	7	30	min	
52	max	22	140	60	240	131	390	202	555	267	695	296	776	278	725	232	615	158	460	87	307	32	160	21	121	max	52
	min	8	45	14	62	51	158	92	295	111	366	176	460	153	398	120	326	65	190	31	120	12	53	4	27	min	
54	max	16	120	50	215	126	370	191	542	263	687	296	776	276	720	221	602	150	440	76	285	25	110	16	90	max	54
	min	6	40	11	45	46	115	88	280	139	360	176	460	150	391	116	312	58	170	29	105	9	43	3	23	min	
56	max	12	102	43	200	120	350	188	528	258	680	295	775	273	714	218	587	141	420	64	261	20	120	12	78	max	56
	min	4	35	8	35	41	132	85	283	136	352	175	460	148	390	110	297	51	150	24	95	7	36	2	18	min	
58	max	9	89	37	170	113	330	182	516	254	670	294	774	270	710	212	575	131	402							max	58
	min	3	28	6	28	37	118	82	277	134	346	175	460	146	385	106	285	44	132							min	
60	max	7	61	32	150	107	310	176	500	249	660	294	773	268	708	205	556	126	338							max	60
	min	2	20	4	20	33	105	79	270	132	340	174	460	141	380	100	270	58	116							min	

[a] Calculated from data published by the Weather Bureau. Approximate corrections for elevation up to 10,000 ft: (1) Total radiation = (total at sea level) (1 + 0.0185 x El.), and (2) Visible radiation = ("visible" at sea level) (1 + 0.00925 x El.). Correction for cloudiness (approximate): Min + [(max - min) cl.] in which cl. is the fraction of time the weather is clear.

[b] Gram calories per square centimeter.

[c] "Visible" = radiation of wave lengths of 4,000 A° to 7,000 A° penetrating a smooth water surface.

[d] "Total" = radiation of all wave lengths in the solar spectrum.

[e] Value which will not normally be exceeded.

[f] Value based on, or extrapolate from, lowest values observed for indicated month and latitude during ten years of record.

3. Pilot data for June 1970

Table B-5. Dissolved oxygen and temperature profiles at various stations in the south primary pond, Logan City sewage system.

Date: Monday, June 15, 1970, 1000 hours.

Station[a]	Depth	Temp. (°C)	Dissolved Oxygen (% Sat.)	D.O. Conc. (mg/l)
D2	Surface	25.8	>200%	>13.56
	2′	22.0	>200%	>14.62
	3′	16.8	91%	7.42
	5½′	16.7	8%	0.65
C2	Surface	26.6	>200%	>13.36
	2′	22.2	150%	10.92
	3′	17.1	103%	8.34
	5½′	16.9	9%	0.73
B2	Surface	25.7	>200%	>13.60
	2′	21.8	160%	11.64
	3′	17.2	103%	8.32
	5½′	16.8	11%	0.90

[a]Station designations are identified in Figure 5.

Observations:

1. Oxygen and temperature vary markedly with depth from two feet depth to the bottom.
2. These data indicate reasonable areal consistency.
3. A good algal bloom was present, thus giving credence to the high oxygen saturation levels through the first 3 feet of water surface.

4. Correlation between TOC and titration for inorganic carbons

Purpose

The purpose of this experiment was to determine whether or not the reading obtained in the inorganic carbon channel of the Beckman No. 915 Total Organic Carbon Analyzer would correlate with the values determined titratametrically for alkalinity and dissolved CO_2 in four individual depth samples from the Logan City Sewage Ponds.

Procedure

All titration methods employed followed those outlined in *Standard Methods*.

All TOC measurements followed the procedure outlined in Appendix C.

Results

(a) free CO_2
Quantity of sample used: 25 ml
Titrant: 0.0454 M NA_2CO_3

Station	Ml. of Titr. Used	mg/l CO_2 as $CaCO_3$
Surface to 1 ft	0.0	0.0
2′	0.0	0.0
3′	0.0	0.0
4′	0.0	0.0

(b) Alkalinity determinations
(1) Phenolphalein Alkalinity
Quantity of sample: 25 ml
Titrant: 0.02 NH_2SO_4

Station	Ml. of Titr. Used	P. alk. mg/l as $CaCO_3$
Surface to 1 ft	2.5	100
2′	2.0	80
3′	1.9	76
4′	1.6	64

(2) Methyl Orange Alkalinity
Quantity of sample: 25 ml
Titrant: 0.02 NH_2SO_4

Station	Ml. of Titr. Used	T. alk. mg/l as $CaCO_3$
Surface to 1 ft	7.4	296
2′	6.6	264
3′	6.6	264
4′	6.4	256

(c) TOC values
Infrared gain = 414

Station	Total Carbon (mg/l) Initially	Total Carbon (mg/l) AMF[a]	Inorganic Carbon (mg/l)	Organic Carbon (mg/l) Initially	Organic Carbon (mg/l) AMF[a]
Surface to 1′	66	49	42	24	25
2′	62	49	41	21	28
3′	64	50	40	24	26
4′	66	53	41	25	12

[a]AMF means "after Millipore filtration."

(d) Algebraic sample calculations for inorganic carbon determination

(1) $HCO_3^{=} = (T - 2P)\ (61/50)$

$$\therefore \text{Carbon (mg/l)} = (T-2P) \times 61/50 \times 12 \text{ mg carbon}/61 \text{ mg } CaCO_3$$
$$= (T-2P) \times 12/50$$

(2) $CO_3 = (1.2P)$

$$\text{Carbon (mg/l)} = (1.2P(12/60))$$

(3) Carbon (mg/l = (12/44) (mg/l CO_2)

(e) Inorganic carbon determinations (calculated as shown in (d))

(1) Surface to 1′ samples
C as CO_2 = 0.0
C as $CO_3^{=}$ = 24.0
C as HCO_3^{-} = 19.2
T alk = 43.2

(2) 2′ depth samples
C as CO_2 = 0.0
C as $CO_3^{=}$ = 19.2
C as HCO_3^{-} = 25.0
T alk = 44.2

(3) 3′ depth samples
C as CO_2 = 0.0
C as $CO_3^{=}$ = 18.2
C as HCO_3^{-} = 26.9
T alk = 45.1

(4) 4′ depth samples
C as CO_2 = 0.0
C as $CO_3^{=}$ = 15.4
C as HCO_3^{-} = 32.0

5. The contribution of unwashed Millipore filter discs to organic carbon present in the filtrate

Millipore filters, in themselves, will contribute to the organic carbon present in the filtrate (Cohn, 1967). Thus it is necessary to wash the filter with acid prior to use. We ascertain here the importance of the washing operation.

A. Washing Procedure

The Millipore filter discs were washed with 70 ml of 0.1N HCl and then washed with 10 volumes (700 ml) of distilled water.

B. Results—using a water sample from the south primary pond

	Total Carbon mg/l	Inorganic Carbon mg/l	Organic Carbon mg/l
Non-Washed Filter	62.0	40.0	22.0
Acid Washed Filter	54.5	39.0	14.5

C. Observation

The organic carbon contribution of the Millipore filter, per se, to the solution which passes through its pores is approximately 7 - 8 mg/l.

6. A comparison of sewage samples after various filtration procedures

A. Purpose

The purpose of this experiment was to ascertain the quantity of organic carbon contributed by the various suspended solids and organisms found in several sewage pond samples.

B. Experimental Procedure

The samples were initially analyzed for total and inorganic carbon. To prepare the samples for such analysis in the TOC Analyzer, fifty (50 ml) milliliter aliquots of the samples were blended in a Waring Blender (liquify setting) for 30 seconds. Three successive 0.02 ml injections were made into each channel and the total organic carbon was determined by the procedure outlined in Appendix C.

The algae and large suspended solids were then Buchner filtered on Whatman No. 1 filter paper. The 50 ml of filtrate was then examined with the TOC (0.02 ml aliquots) in order to determine the quantity of organic materials still present in the sample.

Table B-6. Dissolved oxygen and temperature profiles at station C2 in the south primary pond on various sampling days.

Date	Depth (ft.)	Temp. (°C)	D.O. (% Sat.)	D.O. (mg/l)
6/16/70 (1000 hrs.)	Surface to 1 ft.	26.6	> 200%	> 13.36
	2	22.2	150%	10.92
	3	17.1	103%	8.34
	4	---	---	---
	5½	16.9	9%	0.73
6/19/70 (1000 hrs.)	Surface to 1 ft.	24.0	> 200%	> 14.06
	2	---	> 200%	---
	3	---	> 200%	---
	4	17.7	11%	0.88
	5½	17.0	4%	0.32
6/22/70 (1000 hrs.)	Surface to 1 ft.	27.0	> 200%	> 13.26
	2	22.4	> 200%	> 14.50
	3	19.6	> 200%	> 14.36
	4	18.4	15%	1.18
	5½	17.4	2%	0.16
6/25/70[a] (1000 hrs.)	Surface to 1 ft.	23.4	> 200%	> 14.22
	2	22.9	> 200%	> 14.36
	3	22.9	> 200%	> 14.36
	4	18.7	36%	2.82
	5½	17.9	3%	0.24

[a]Surface to water was very choppy; current movements were noted in south to north direction.

In order to further remove all bacterial, unicellular algae, and other solids which could pass through the pores of the filter paper, 50 ml of the water samples were passed through a Millipore filter which had been previously washed with 70 ml of 0.1 NHCl, followed by washing with 700 ml of distilled water (see Part 4 of this appendix). These filtrates (50 ml) were then also analyzed for carbon content.

C. Results

A comparison of sewage samples after various filtration procedures. Date of Samples June 22, 1970.

Observations

1. The 0 - 3 ft. depth range is supersaturated (> 200%) with oxygen.
2. A distinct zonation of oxygen and temperature is distinguishable below 3 ft. depth.
3. The breakpoint in oxygen concentration is in the 3 ft. to 4 ft. zone.
4. Wind will cause greater oxygen concentrations at 4 ft. depth.

D. Observations

1. It can readily be concluded that Millipore filtration following a filter paper filtration has little, if any, effect on the removal of organic carbon (as bacteria) from a water sample. Thus, the bacteria present must contribute very little on a weight basis to the quantity of organic carbon present in the pond water samples.
2. The quantity of organic carbon exiting through the outlet is comparable to that entering the pond (in solution).
3. The organic carbon content of the various depth samples is reasonably consistent.

Table B-7. Distribution of carbon in dissolved and two sizes of suspended solids for both organic and inorganic phases from several pond samples.

Source		Total Carbon (mg/l)	Inorganic Carbon (mg/l)	Organic Carbon (mg/l)
Inlet	Initial	98	62	34
	After Buchner Filt.	63	51	14
	After Millipore Filt.	64	52	12
Outlet	Initial	79	48	31
	After Buchner Filt.	62	41	21
	After Millipore Filt.	58	41	17
1' depth	Initial	74	38	36
sample	After Buchner Filt.	51	34	17
	After Millipore Filt.	50	30	20
2' depth	Initial	66	38	28
sample	After Buchner Filt.	53	35	18
	After Millipore Filt.	49	34	15
3' depth	Initial	74	42	32
sample	After Buchner Filt.	53	38	15
	After Millipore Filt.	51	37	14
4' depth	Initial	82	52	30
sample	After Buchner Filt.	57	38	19
	After Millipore Filt.	--	--	--
5½'	Initial	85	55	30
depth	After Buchner Filt.	69	51	18
sample	After Millipore Filt.	65	43	22

7. Bottom Sample Analysis

Materials

Eckman Dredge, Wildlife Supply Company, Inc., Saginaw, Michigan.

Labline Oven, Labline Instruments, Inc., Malrose Park, Illinois.

Lindberg High Temperature Furnace, Type B-2, source of origin unknown.

Mettler Balance, Model P160 Balance.

Methods

Fifty milliliters of the dredge sample previously collected by Eckman Dredge Apparatus for an anaerobic kinetic experiment was placed into a weighed procelain evaporation dish. The solution was evaporated at 110°C for 24 hours in a Labline Oven. Following this evaporation procedure, the dish was reweighed and then placed into a Lindberg High Temperature Furnace for contents incineration over a 24 hour period. The dish was then removed, allowed to cool and reweighed.

Bottom sample analysis

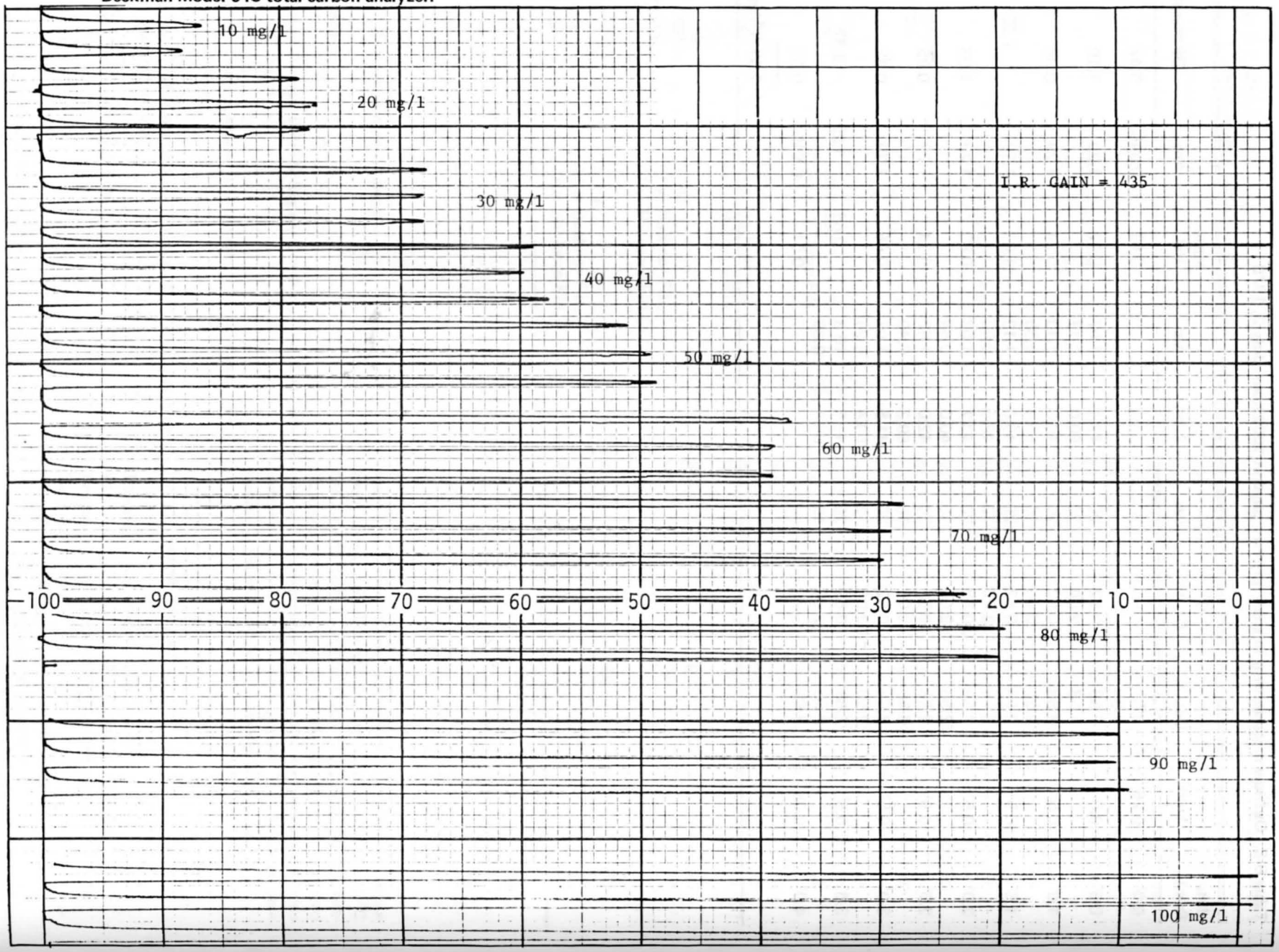
Porcelain Dish (empty) = 88.3 gm
Porcelain Dish + 50 ml sample dried = 92.7 gm
Porcelain Dish + ash from incinerated sample = 91.2 gm
Sample Analysis:

I. 0.615 gm/l solid material present
II. % Volatile solid in sample
1.5 gm/4.4 gm X 100 = 31.8%

Table B-8. Outflow measurement in south primary pond at 1000 hours July 3, 1970.

Int. Point	Width	Depth	0 Depth	Rev.	Time	Vel.	Mean Vel.	Area	Discharge
0.0′	0.25′	2.58	--	--	--	--	0.65	0.645	0.42
		2.58	--	--	--	--			
0.5′	0.5′	2.58	0.52	20	42.1	1.07	0.92	1.29	1.19
		2.58	2.06	20	60.0	0.77			
1.0′	0.5′	2.58	0.52	20	40.0	1.13	0.74	1.29	0.95
		2.58	2.06	10	69.8	0.35			
1.5′	0.5′	2.58	0.52	20	40.0	1.13	1.03	1.29	1.33
		2.58	2.06	20	48.5	0.93			
2.0′	0.5′	2.58	0.52	20	43.7	1.03	0.80	1.29	1.03
		2.58	2.06	10	40.0	0.58			
2.5′	0.5′	2.58	0.52	20	42.0	1.07	0.74	1.29	0.95
		2.58	2.06	10	60.3	0.40			
3.0′	0.5′	2.58	0.52	20	41.5	1.07	0.65	1.29	0.82
		2.58	2.06	5	53.4	0.24			
3.5′	0.5′	2.58	0.52	20	43.5	10.3	0.68	1.29	0.87
		2.58	2.06	10	68.0	0.36			
4.0′	0.25′	2.58	--	--	--	--	0.51	0.645	0.33
									7.89 ft^3/sec

Figure B-1. Instrument measurements to obtain standard curve (0-100 mg/l) for total carbon as determined by the Beckman Model 915 total carbon analyzer.



APPENDIX C

OPERATING INSTRUCTIONS FOR THE TOTAL ORGANIC CARBON ANALYZER

1. Introduction

Organic carbon micro analysis of water samples is accomplished easily and rapidly with the Beckman Model 915 Total Organic Carbon Analyzer. Total carbon contained in the samples is analyzed by the low temperature mode of the instrument, while the high temperature mode of the instrument determines the inorganic (bicarbonate) content of the sample. A subtraction of the inorganic from the total carbon value then yields directly the organic carbon present in the sample. Also, the instrument may be operated in any one of several concentration ranges, thus permitting the analysis of samples with either excessive (up to 5000 mg/l) or minute carbon content (to 1 mg/l).

Stepwise procedures are outlined in the following section for: (1) instrument settings, (2) calibration, and (3) analysis of sample.

2. Instrument Settings

a. Model 915 TOC Analyzer

(1) Low Temperature System (Inorganic Carbon)
- (a) Temperature setting: 150°C
- (b) Carrier Gas Gauge Setting (Oxygen Tank): 10 psi
- (c) Pressure Regulator: 4 psi
- (d) Flow Control Valve Setting: 140 cc/min.
- (e) Sample Select Valve: Inorganic Carbon

(2) High Temperature System (Total Carbon)
- (a) Temperature setting: 950°C
- (b) Carrier Gas Gauge Setting (Oxygen Tank): 10 psi
- (c) Passive Regulator: 4 psi
- (d) Flow Control Valve Setting: 140 cc/min.
- (e) Sample Select Valve: (Total Carbon)

b. Beckman 10" Linear Recorder
- (1) Range Setting: 100 milliamperes
- (2) Chart Speed: Variable, depending preference of user

c. Beckman Model 215A Infrared Analyzer
- (1) Gain: Variable; in range 430-450 (see section 3)
- (2) Range Setting: 1(0-100 mg/l)

3. Calibration of the instrument

Prior to analyses of unknown samples, the instrument must be calibrated using samples of known concentration. Though the instrument is linear in the 0-100 mg/l range, preparation of a standard curve gives the operator the opportunity to check not only the operating efficiency of the instrument but also his adeptness at injection of samples.

Since both channels can measure the *inorganic carbon* present in a water sample, a *working standard* of *inorganic carbon* should be prepared in CO_2 free water. The most convenient working standard concentration to prepare is 1000 mg/l. This solution is prepared using 4.404 gm anhydrous sodium carbonate plus 3.497 gm of anhydrous sodium bicarbonate diluted to 1000 ml using CO_2 free water. Appropriate dilutions for use in calibration can then be made using the dilution table given below. To prepare a 100 mg/l standard solution, which is used for both inorganic carbon and total carbon calibrations, we add 100 ml of 1000 mg/l working standard solution to a 1000 ml volumetric flask, and then dilute to volume (1000 ml) with CO_2 free water.

Desired ppm (mg/l) in Calibration Solution	Required ml of 100 mg/l Stock Solution for Dilution to 100 ml with CO_2 Free Water
100	100
90	90
80	80
70	70
60	60
50	50
40	40
30	30
20	20
10	10

The first stage in preparing the actual curve involves finding the exact gain setting on the I.R. Analyzer which causes the recorder pen to deflect to full scale when

analyzing a 100 mg/l sample. This procedure is accomplished by injecting successive 20 micro liter samples into the selected channel and adjusting the duodial until repeatable results are obtained within 1 percent. Record this value and use this setting for both channels (if the value changes on the switching of channels some compensation may have to be made).

Once the 100 percent deflection is set and the instrument zero has been determined, the actual injection of calibration solutions (20 micro liter quantities) may be carried out. Three injections of each solution should be made and an average taken of their recorded values. (A typical standard curve deflection series is depicted in Figure B-1.) These values are then plotted in graphical form. Alternatively, the channel selection should be changed to the other mode of analysis and the previously described procedure should be carried out in this channel. (Changes in instrument zero may be made, but the gain setting should remain constant.)

4. Procedures of analysis

The water samples are generally analyzed in the total carbon range first. Three 20 micro liter samples are injected successively into the T.C. furnace port (care should be exercised that the teflon plug is not removed from the port except during sample injections). If samples are found to contain suspended solids, i.e. algae and debris, they should be mixed first in a blender before injection into the instrument. It is more convenient to analyze all samples in one range, rather than switching ranges for the same sample, but care must be exercised to keep all samples cold during this interval.

Following the determination of the total carbon concentration in all samples, the channel selector is switched to the inorganic channel and the appropriate zero setting is made (if required). All samples are then analyzed for inorganic carbon following the procedure outlined above.

If in either case, the deflection of the recorder is such that an off scale reading (> 100 mg/l) is obtained, dilutions of the sample must be done. It is recommended that in making dilutions, volumetric flasks and only CO_2 free distilled water be used. After obtaining the concentration of carbon (in mg/l) in the diluted sample, this value must be adjusted by a dilution factor.

After ascertaining the inorganic and total carbon values for a sample (which are averages of three determinations), a determination of organic carbon present can be made by using the formula:
Organic carbon (mg/l) = Total Carbon (mg/l) - Inorganic carbon (mg/l).

APPENDIX D

DIURNAL MEASUREMENTS

All measurements and resulting graphical displays for the 24 hour cycle field study of the Logan south primary pond are given in this appendix. This includes sewage inflow data, pond outflow, and measurements of TOC, inorganic carbon, organic carbon, temperature, dissolved oxygen, pH, alkalinity, carbon dioxide, and light transmission; these measurements were taken at two hour intervals at the following locations: inlet, outlet, and pond depths of 0', 1', 2', 3', 4', and 5' at pond station C2.

Table D-1. Flow measurements from Parshall flume located in sewer tank line. Sewer Inlet Flow Data 0530 7/8/70 to 0500 7/9/70

Time (hr)	Ave. Flow Rate through Parshall Flume (ft^3/sec)	Total Gallons
0530-0820	21.0	1,600,000
0820-0940	21.6	780,000
0940-1100	23.6	850,000
1100-1345	21.4	1,590,000
1345-1525	27.8	1,250,000
1525-1730	23.4	1,320,000
1730-1909	22.8	1,010,000
1909-2130	23.2	1,470,000
2130-2340	21.5	1,260,000
2340-0140	22.5	1,210,000
0140-0337	20.0	1,050,000
0337-0500	21.7	810,000

Total gallons to both primary ponds during the 23.5 hr. period = 14,200,000 gallons
Volume amount to south primary pond 7,100,000 gallons = 26,900,000 liters
Average flow to south primary pond during 23.5 hr. period 11.2 ft^3/sec = 42.5 liters/sec
1 gallon = 3.7853 liters

Table D-2. Pond outflow measurements, 0615 7/8/70 to 0530 7/9/70.

Time (hr)	Flow (ft^3/sec)	Flow (liters/sec)
0615-0645	8.49	70.0
0935-0955	8.62	72.0
1340-1356	8.27	68.6
1653-1717	8.77	73.5
2102-2106	8.12	68.0
0100-0120	8.35	69.9
0508-0530	8.56	72.0

Average outflow = 8.49 ft^3/sec (70.9 liters/sec)

Total gallons per 23.5 hr. period = 1,582,020 (5,440,000 liters)

Table D-3. Chemical and physical parameters of the south primary pond measured during the diurnal cycle.

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
INLET	0550	14.1	5.2	7.4	16	0	268	
	0800	14.2	4.05	7.4	20	0	260	
	0925	14.6	2.9	7.3	41	0	268	
	1100	15.0	2.42	7.2	40	0	272	
	1327	15.0	2.3	7.6	40	0	280	
	1525	15.0	2.03	7.4	9	0	270	
	1732	14.9	3.48	7.4	48	0	282	
	1905	--	--	7.2	28	0	266	
	2130	14.4	4.36	7.5	--	0	272	
	2340	14.4	3.7	7.4	32	0	270	
	0140	14.5	4.96	7.3	40	0	270	
	0337	14.6	4.02	7.3	40	0	280	
	0500	14.0	3.64	7.4	30	0	260	

Table D-3. Continued.

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
OUTLET	0545	23.0	8.45	8.5	0	28	252	
	0815	22.6	8.9	8.6	0	52	208	
	0952	23.1	10.6	8.4	0	44	246	
	1140	23.6	10.0	8.2	0	40	228	
	1340	25.6	10.75	8.4	0	56	232	
	1535	28.1	10.6	8.4	0	48	196	
	1755	29.4	9.7	8.4	0	72	178	
	1936	--	--	8.6	0	60	220	
	2145	28.2	8.9	9.1	0	88	172	
	2330	23.6	10.8	8.8	0	72	218	
	0120	25.0	9.5	9.6	0	80	200	
	0330	24.5	7.8	9.0	0	100	180	
	0556	24.0	6.2	9.1	0	100	180	

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
SURFACE TO 1'	0520	23.2	9.8	8.6	0	60	260	Surf = 0 1' = 0
	0746	23.1	10.9	8.6	0	8	252	Surf = 100% 1' = --
	0923	23.4	10.65	8.6	0	24	256	Surf = 100% 1' = 29%
	1135	24.1	12.6	8.5	0	48	232	Surf = 100% 1' = 55%
	1258	24.0	14.0	8.4	0	60	220	Surf = 100% 1' = 55.5%
	1455	26.8	13.3	8.6	0	60	190	Surf = 100% 1' = 64.5%
	1721	27.9	13.0	8.6	0	68	180	Surf = 100% 1' = 57.5%
	--	--	--	8.7	0	64	216	Surf = 100% 1' = 71%
	2124	28.4	13.0	9.0	0	100	166	Surf = 100% 1' = 22.7%
	2320	26.5	10.9	9.0	0	80	200	Surf = 0 1' = 0
	0115	25.9	11.5	9.1	0	80	200	Surf = 0 1' = 0
	0325	25.6	10.4	8.9	0	80	200	Surf = 0 1' = 0
	0544	25.6	8.05	8.9	0	100	180	Surf = 0 1' = 0

Table D-3. Continued.

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
2 FEET OF DEPTH	0525	23.5	9.25	8.5	0	60	220	0
	0745	23.1	10.9	8.5	0	40	234	33%
	0922	23.4	10.6	8.6	0	40	240	18.7%
	1134	24.0	12.6	8.6	0	40	220	30.8%
	1316	23.5	14.2	8.5	0	68	212	33.4%
	1517	24.7	13.8	8.3	0	60	180	36.4%
	1720	25.5	13.6	8.6	0	84	154	28%
	1930	--	--	8.9	0	72	308	3%
	2122	25.9	13.5	9.1	0	88	194	10%
	2318	25.5	13.6	9.1	0	80	200	0
	0113	25.9	12.0	9.0	0	80	200	0
	0323	25.3	11.2	8.9	0	60	220	0
	0545	26.1	8.1	9.2	0	80	200	0

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
3 FEET OF DEPTH	0527	23.5	9.2	8.4	0	44	232	0
	0710	23.1	10.5	8.4	0	48	232	8.7%
	0905	23.2	10.2	8.5	0	44	186	6.9%
	1133	23.6	12.0	8.4	0	60	230	15.4%
	1315	23.0	14.3	8.6	0	76	204	17.8%
	1515	23.7	14.1	8.5	0	72	168	17.7%
	1718	24.5	12.5	8.6	0	80	160	9%
	1929	--	--	9.0	0	48	192	1.3%
	2120	25.0	13.8	9.1	0	140	90	4%
	2316	24.5	14.0	9.0	0	80	200	0
	0111	24.9	13.9	9.0	0	80	200	0
	0321	25.3	11.2	9.2	0	80	210	0
	0540	25.0	9.6	9.0	0	100	180	0

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
4 FEET OF DEPTH	0529	23.0	3.65	8.5	0	56	212	0
	0743	23.0	5.9	8.5	0	40	240	3.23%
	0920	23.0	8.8	8.4	0	40	240	3.44%
	1131	23.4	11.6	8.6	0	60	190	5.5%
	1314	22.7	14.5	8.5	0	60	220	7.1%
	1513	23.0	11.0	8.6	0	64	184	6.1%
	1716	24.3	12.5	8.5	0	96	134	4%
	1928	--	--	9.0	0	80	200	5%
	2117	23.7	13.5	9.1	0	88	156	1.6%
	2314	23.4	10.5	9.0	0	60	220	0
	0109	24.0	12.6	9.1	0	68	212	0
	0319	24.4	11.3	9.1	0	100	180	0
	0538	24.4	9.5	8.9	0	100	180	0

Table D-3. Continued.

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
5 FEET OF DEPTH	0530	22.5	.69	8.4	0	44	236	0
	0715	22.9	.61	8.6	0	52	218	.23%
	0910	22.4	1.38	8.6	0	28	272	.1%
	1130	23.1	5.0	8.5	0	32	250	1.65%
	1312	22.2	5.1	8.5	0	48	232	3.68%
	1511	22.7	3.6	8.4	0	40	230	2.94%
	1716	23.4	--	8.4	0	60	200	1.92%
	1926	--	--	9.1	0	80	200	.25%
	2115	23.2	3.2	9.0	0	44	236	1.1%
	2312	22.6	2.66	8.6	0	20	280	0
	0107	23.1	2.2	8.7	20	0	310	0
	0317	23.0	3.0	8.9	0	120	170	0
	0536	23.5	4.7	9.1	0	100	180	0

Station	Time of Observation	Temperature (Centigrade)	Dissolved Oxygen (mg/l)	pH	CO_2 (mg/l)	Carbonate Ion (mg/l)	Bicarbonate Ion (mg/l)	% Light Transmission
BOTTOM	0535	22.5	.47	8.2	12	0	304	0
	0717	22.6	.36	8.4	12	32	284	.13%
	0912	22.2	.87	8.7	0	8	252	.1%
	1127	22.9	1.0	8.2	0	24	260	.2%
	1310	21.7	2.28	8.6	0	40	260	1.1%
	1509	22.7	2.45	8.6	0	60	200	1.18%
	1715	23.4	--	8.5	0	28	238	.59%
	1924	--	--	8.7	0	20	260	.83%
	2115	22.9	2.22	8.8	0	72	210	.55%
	2310	22.5	1.8	8.7	0	28	292	0
	0105	22.6	1.58	8.7	0	0	340	0
	0315	22.6	1.45	8.6	0	0	400	0
	0534	22.7	2.38	9.0	0	88	192	0

Table D-4. Carbon analysis of samples taken at the inlet to the Logan sewage pond system.

Time of Sample	Total Carbon (mg/l)	Inorganic Carbon (mg/l)	Organic Carbon (mg/l)
0515	55.0	49.0	6.0
	[a]AMF 53.0		[a]AMF 4.0
0801	62.0	51.5	10.5
	[a]AMF 56.0		[a]AMF 4.5
0925	79.0	57.5	21.5
	[a]AMF 68.0		[a]AMF 10.5
1100	75.5	53.5	22.0
	[a]AMF 62.0		[a]AMF 8.5
1325	73.0	54.5	18.5
	[a]AMF 65.5		[a]AMF 11.0
1526	75.5	52.0	23.5
	[a]AMF 65.0		[a]AMF 13.0
1730	66.5	52.0	14.5
	[a]AMF 56.0		[a]AMF 4.0
1905	67.5	43.0	24.5
	[a]AMF 61.5		[a]AMF 18.5
2130	70.0	53.5	16.5
	[a]AMF 61.5		[a]AMF 9.0
2340	73.0	54.5	18.5
	[a]AMF 59.5		[a]AMF 5.0
0140	64.0	53.0	11.0
	[a]AMF 57.5		[a]AMF 4.5
0337	61.5	53.0	8.5
	[a]AMF 58.5		[a]AMF 5.5
0505	57.0	49.0	6.0
	[a]AMF 53.5		[a]AMF 4.5

[a]AMF = After Millipore Filtration.

Table D-5. Carbon analysis of samples taken at the outlet of the south primary pond.

Time of Sample	Total Carbon (mg/l)	Inorganic Carbon (mg/l)	Organic Carbon (mg/l)
0530	76.0	51.0	25.0
	[a]AMF 64.0		[a]AMF 13.0
0815	69.5	50.5	19.0
	[a]AMF 62.5		[a]AMF 12.0
0952	68.0	49.5	18.5
	[a]AMF 59.0		[a]AMF 9.5
1140	68.5	44.0	24.5
	[a]AMF 57.5		[a]AMF 13.5
	65.5	41.0	24.5
	[a]AMF 55.5		[a]AMF 14.0
1535	62.0	46.0	16.0
	[a]AMF 53.5		[a]AMF 7.5
1755	65.5	49.5	16.0
	[a]AMF 55.5		[a]AMF 6.0
1936	64.5	48.5	16.0
	[a]AMF 53.5		[a]AMF 5.0
2145	65.0	47.5	17.5
	[a]AMF 56.0		[a]AMF 8.5
2330	78.0	51.0	19.0
	[a]AMF 59.0		[a]AMF 8.0
0120	71.5	43.5	28.5
	[a]AMF 54.5		[a]AMF 11.5
0330	66.0	46.5	19.5
	[a]AMF 52.5		[a]AMF 6.0
0556	65.5	45.0	20.5
	[a]AMF 52.5		[a]AMF 7.0

[a] AMF = After Millipore Filtration.

Table D-6. Carbon measurements of samples taken at depths within the south primary pond.

Depth ft.	Collection Time (hr.)	Total Carbon (mg/l)	Inorganic Carbon (mg/l)	Organic Carbon (mg/l)	Total Carbon (mg/l) after Millipore Filt.	Organic Carbon (mg/l) after Millipore Filt.
1	0746 7/8	75.5	51.0	24.5	70.0	19.0
2	0745 7/8	71.0	48.0	23.0	69.5	21.5
3	0744 7/8	71.5	47.5	24.0	64.5	17.0
4	0735 7/8	74.5	51.5	23.0	71.0	19.5
5	0742 7/8	70.0	48.0	22.0	67.0	19.0
Bottom[a]	0741 7/8	160.0	68.0	92.0	113.5	45.5
1	1317 7/8	74.0	51.5	23.5	72.5	21.0
2	1316 7/8	75.0	51.0	25.0	73.5	22.5
3	1315 7/8	74.5	52.5	25.0	73.5	21.0
4	1314 7/8	74.5	51.5	26.0	71.0	19.5
5	1312 7/8	76.0	52.0	24.0	72.5	20.5
Bottom[a]	1311 7/8	99.0	57.5	43.5	84.5	27.0
1	1931 7/8	72.0	52.5	19.5	73.0	20.5
2	1930 7/8	66.5	45.5	21.0	65.5	20.0
3	1929 7/8	69.0	48.0	21.0	68.5	20.5
4	1928 7/8	79.5	54.5	21.0	73.5	19.0
5	1926 7/8	75.5	52.5	23.0	74.0	21.5
Bottom[a]	1924 7/8	125.0	63.0	62.0	100.0	37.5
1	0115 7/8	68.5	49.5	19.0	66.5	17.0
2	0113 7/8	71.5	52.0	19.5	68.5	16.5
3	0111	67.5	45.5	22.0	64.5	19.0
4	0109	79.5	50.0	29.5	75.0	25.0
5	0107	76.0	51.5	24.5	72.5	21.0
Bottom[a]	0105	690.0	235.0	455.0	395.0	160.0

[a] Bottom sample contained varying quantities of bottom muds and debris.

APPENDIX E

DETERMINATION OF KINETIC RATE CONSTANTS

Kinetic rate constants were determined for (1) rate of aerobic uptake of glucose C^{14}, (2) rate of algae production using bicarbonate C^{14}, (3) rate of anaerobic uptake of glucose C^{14}.

These rate constants were conducted in batch reactors (one liter Erlenmeyer flasks) containing pond water taken from a specified depth and reimmersed to that depth after adding a known amount of C^{14} substrate.

Measurements of samples were in terms of counts resulting from C^{14} contained in residue on a Millipore filter. The amount of C^{14} measured in these residues was due to substrate uptake. For first order kinetic rate constant determination it was necessary to convert these readings of C^{14} contained in the residue samples to concentration of substrate in solution. Table E-1, E-2, and E-3 show these calculations for aerobic uptake of glucose, algae synthesis, and anaerobic uptake of glucose, respectively. Figures E-1, E-2, and E-3 show how the first order rate constant is determined using these data, reduced to substrate concentration ratios at various times of sampling.

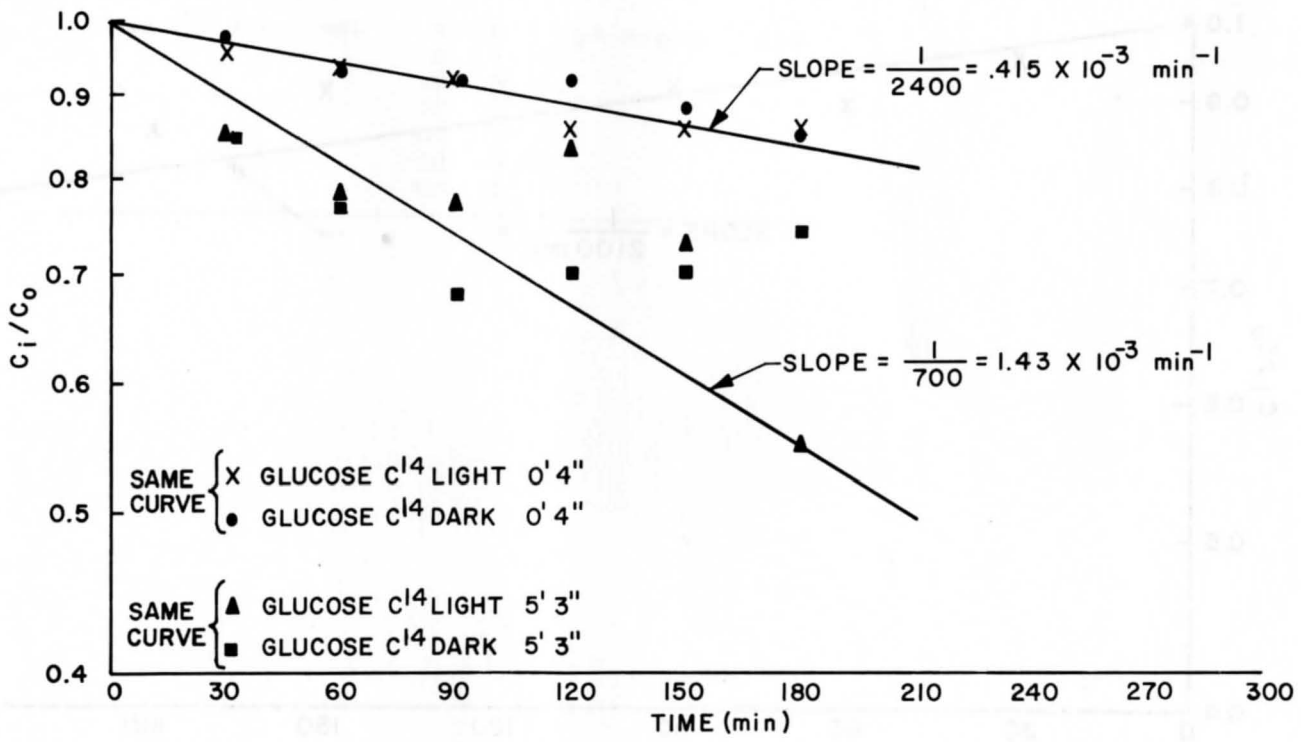


Figure E-1. Depletion of glucose in aerobic reactors.

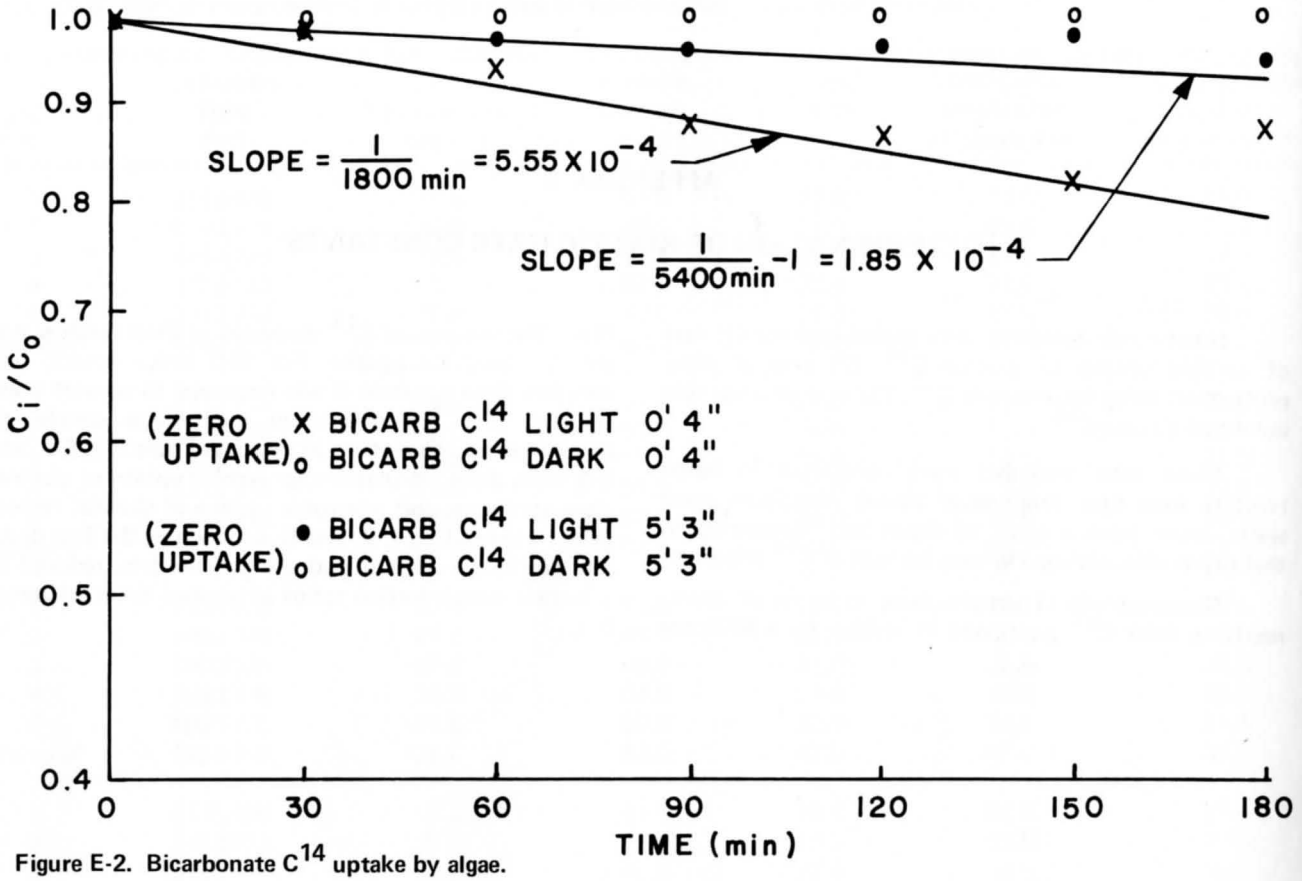


Figure E-2. Bicarbonate C^{14} uptake by algae.

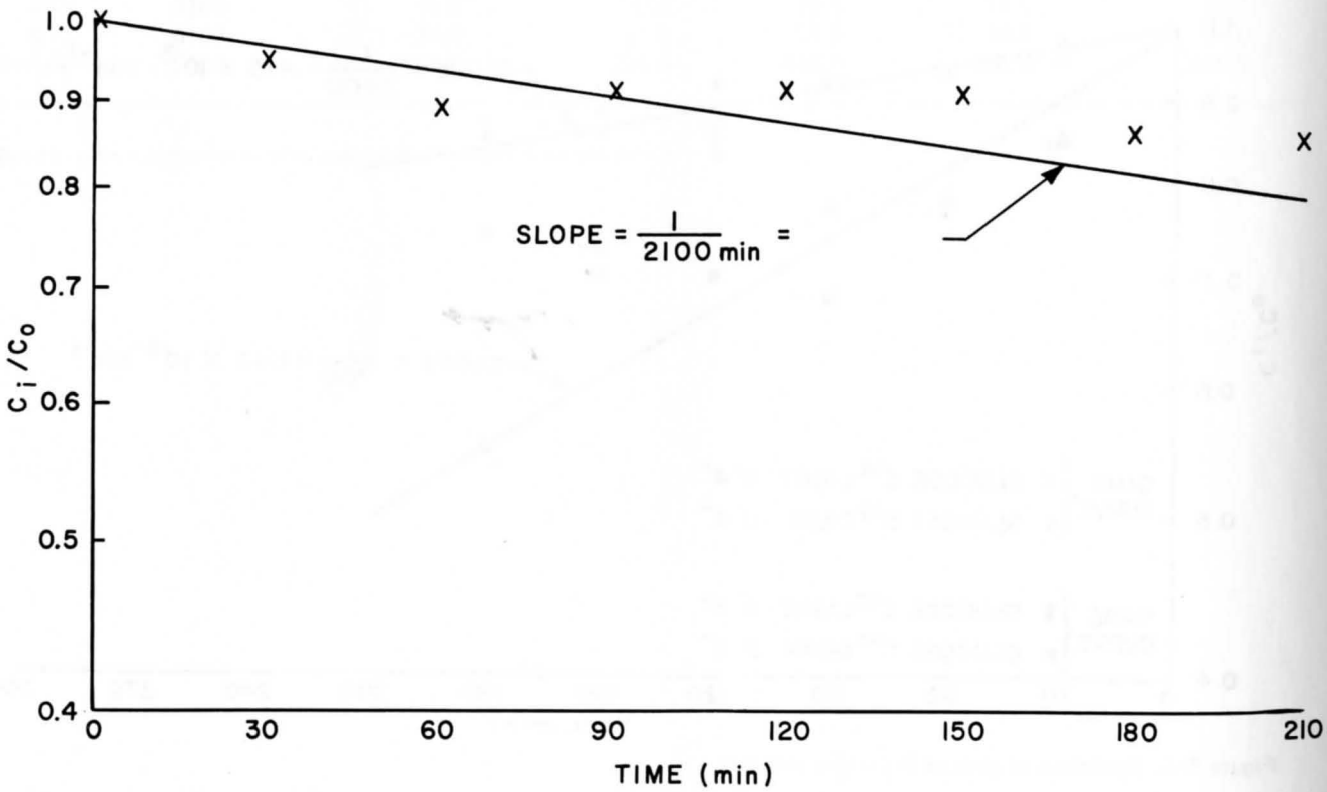


Figure E-3. Depletion of glucose in anaerobic reactors.

Table E-1. Glucose C^{14} uptake during aerobic substrate degradation experiments using pond water.

Radioactive substrate	Depth of sample	Color of bottle	Elapsed time (min)	i	C_o (μM/L)	V_o (L)	$C_o \cdot V_o$ (μM) (CPM)	Counts[a] per minute	$\overline{X}_{si}$[b] (μM)	V_s (L)	$[\overline{X}_i]$[c] (μM/L)	V_i (L)	$\overline{X}_i$[d] (μM)	$\sum^{i} X_{si}$ (μM)	C_i (est) (μM/L)	$C_i \cdot V_i$ (μM)	$\sum^{i} C_i V_i$ (μM)	$C_i V_i$ (μM)	C_i[e] (μM/L)	C_i/C_o	Slope[f] (min^{-1})	k[g] (min^{-1})	k (hr^{-1})
Glucose C^{14}	0'4"	light	0	0	9.56×10^{-2}	0.250	2.39×10^{-2}	0	0	.01	0	.25	0	0	9.56×10^{-2}	0	0	0	9.56×10^{-2}	1.0	4.15×10^{-4}	9.55×10^{-4}	.057
			30	1	9.56×10^{-2}	0.250	2.39×10^{-2}	4,360	3.7×10^{-5}	.01	3.7×10^{-3}	.24	0.89×10^{-3}	3.7×10^{-5}	9.15×10^{-2}	9.15×10^{-4}	9.15×10^{-4}	220×10^{-4}	9.15×10^{-2}	0.955			
			60	2	9.56×10^{-2}	0.250	2.39×10^{-2}	6,620	5.6×10^{-5}	.01	5.6×10^{-3}	.23	1.29×10^{-3}	9.3×10^{-5}	9.00×10^{-2}	9.00×10^{-4}	18.15×10^{-4}	207×10^{-4}	9.00×10^{-2}	0.940			
			90	3	9.56×10^{-2}	0.250	2.39×10^{-2}	8,840	7.5×10^{-5}	.01	7.5×10^{-3}	.22	1.65×10^{-3}	16.8×10^{-5}	8.80×10^{-2}	8.80×10^{-4}	27.0×10^{-4}	194×10^{-4}	8.80×10^{-2}	0.920			
			120	4	9.56×10^{-2}	0.250	2.39×10^{-2}	10,750	9.1×10^{-5}	.01	9.1×10^{-3}	.21	1.91×10^{-3}	25.9×10^{-5}	8.70×10^{-2}	8.70×10^{-4}	35.2×10^{-4}	172×10^{-4}	8.20×10^{-2}	0.860			
			150	5	9.56×10^{-2}	0.250	2.39×10^{-2}	15,870	13.4×10^{-5}	.01	13.4×10^{-3}	.20	2.68×10^{-3}	39.3×10^{-5}	8.00×10^{-2}	8.00×10^{-4}	43.2×10^{-4}	165×10^{-4}	8.20×10^{-2}	0.860			
			180	6	9.56×10^{-2}	0.250	2.39×10^{-2}	16,950	14.4×10^{-5}	.01	14.4×10^{-3}	.19	2.74×10^{-3}	53.7×10^{-5}	8.00×10^{-2}	8.00×10^{-4}	51.2×10^{-4}	155×10^{-4}	8.20×10^{-2}	0.860			
Glucose C^{14}	0'4"	dark	0	0	9.56×10^{-2}	0.250	2.39×10^{-2}	0	0	.01	0	.25	0	0	9.56×10^{-2}	0	0	0	9.56×10^{-2}	1.0	4.15×10^{-4}	9.55×10^{-4}	.057
			30	1	9.56×10^{-2}	0.250	2.39×10^{-2}	3,330	2.8×10^{-5}	.01	2.8×10^{-3}	.24	0.67×10^{-3}	2.8×10^{-5}	9.20×10^{-2}	9.20×10^{-4}	9.20×10^{-4}	223×10^{-4}	9.30×10^{-2}	0.980			
			60	2	9.56×10^{-2}	0.250	2.39×10^{-2}	6,940	5.9×10^{-5}	.01	5.9×10^{-3}	.23	1.36×10^{-3}	8.7×10^{-5}	9.00×10^{-2}	9.20×10^{-4}	18.4×10^{-4}	206×10^{-4}	8.95×10^{-2}	0.935			
			90	3	9.56×10^{-2}	0.250	2.39×10^{-2}	8,540	7.2×10^{-5}	.01	$7.2z10^{-3}$	.22	1.58×10^{-3}	15.9×10^{-5}	8.80×10^{-2}	8.80×10^{-4}	27.2×10^{-4}	194×10^{-4}	8.80×10^{-2}	0.920			
			120	4	9.56×10^{-2}	0.250	2.39×10^{-2}	10,200	8.6×10^{-5}	.01	8.6×10^{-3}	.21	1.80×10^{-3}	24.5×10^{-5}	8.60×10^{-2}	8.60×10^{-4}	33.8×10^{-4}	185×10^{-4}	8.80×10^{-2}	0.920			
			150	5	9.56×10^{-2}	0.250	2.39×10^{-2}	14,200	12.0×10^{-5}	.01	12.0×10^{-3}	.20	2.40×10^{-3}	36.5×10^{-5}	8.60×10^{-2}	8.60×10^{-4}	42.4×10^{-4}	169×10^{-4}	8.45×10^{-2}	0.885			
			180	6	9.56×10^{-2}	0.250	2.39×10^{-2}	16,950	14.4×10^{-5}	.01	$14.4z10^{-3}$	.19	2.73×10^{-3}	50.9×10^{-5}	8.40×10^{-2}	8.40×10^{-4}	50.8×10^{-4}	156×10^{-4}	8.20×10^{-2}	0.855			
Glucose C^{14}	5'3"	light	0	0	9.56×10^{-2}	0.250	2.39×10^{-2}	0	0	.01	0	.25	0	0	9.56×10^{-2}	0	0	0	9.56×10^{-2}	1.0	14.3×10^{-4}	32.9×10^{-4}	0.20
			30	1	9.56×10^{-2}	0.250	2.39×10^{-2}	16,120	13.7×10^{-5}	.01	13.7×10^{-3}	.24	3.3×10^{-3}	13.7×10^{-5}	8.20×10^{-2}	8.20×10^{-4}	8.20×10^{-4}	194×10^{-4}	8.10×10^{-2}	0.850			
			60	2	9.56×10^{-2}	0.250	2.39×10^{-2}	23,300	19.8×10^{-5}	.01	19.8×10^{-3}	.23	4.55×10^{-3}	43.5×10^{-5}	7.80×10^{-2}	7.80×10^{-4}	16.2×10^{-4}	173×10^{-4}	7.50×10^{-2}	0.783			
			90	3	9.56×10^{-2}	0.250	2.39×10^{-2}	25,600	21.7×10^{-5}	.01	21.7×10^{-3}	.22	4.77×10^{-3}	65.2×10^{-5}	7.00×10^{-2}	7.00×10^{-4}	23.2×10^{-4}	162×10^{-4}	7.40×10^{-2}	0.775			
			120	4	9.56×10^{-2}	0.250	2.39×10^{-2}	17,870	15.1×10^{-5}	.01	15.1×10^{-3}	.21	3.18×10^{-3}	80.3×10^{-5}	7.00×10^{-2}	7.00×10^{-4}	30.2×10^{-4}	169×10^{-4}	8.00×10^{-2}	0.837			
			150	5	9.56×10^{-2}	0.250	2.39×10^{-2}	29,370	24.9×10^{-5}	.01	24.9×10^{-3}	.20	5.00×10^{-3}	$105.\times10^{-5}$	7.20×10^{-2}	7.20×10^{-4}	37.4×10^{-4}	141×10^{-4}	7.00×10^{-2}	0.730			
			180	6	9.56×10^{-2}	0.250	2.39×10^{-2}	27,100	23.0×10^{-5}	.01	23.0×10^{-3}	.19	4.37×10^{-3}	$128.\times10^{-5}$	7.00×10^{-2}	7.00×10^{-4}	44.4×10^{-4}	100×10^{-4}	5.25×10^{-2}	0.550			
Glucose C^{14}	5'3"	dark	0	0	9.56×10^{-2}	0.250	2.39×10^{-2}	0	0	.01	0	.25	0	0	9.56×10^{-2}	0	0	0	9.56×10^{-2}	1.0	14.3×10^{-4}	32.9×10^{-4}	0.20
			30	1	9.56×10^{-2}	0.250	2.39×10^{-2}	16,680	14.1×10^{-5}	.01	14.1×10^{-3}	.24	3.39×10^{-3}	14.1×10^{-5}	8.50×10^{-2}	8.50×10^{-4}	8.50×10^{-4}	195×10^{-4}	8.10×10^{-2}	0.850			
			60	2	9.56×10^{-2}	0.250	2.39×10^{-2}	20,400	17.3×10^{-5}	.01	17.3×10^{-3}	.23	4.00×10^{-3}	31.4×10^{-5}	7.80×10^{-2}	7.80×10^{-4}	16.3×10^{-4}	170×10^{-4}	7.40×10^{-2}	0.773			
			90	3	9.56×10^{-2}	0.250	2.39×10^{-2}	35,450	30.0×10^{-5}	.01	30.0×10^{-3}	.22	6.60×10^{-3}	61.4×10^{-5}	7.40×10^{-2}	7.40×10^{-4}	23.7×10^{-4}	143×10^{-4}	6.50×10^{-2}	0.680			
			120	4	9.56×10^{-2}	0.250	2.39×10^{-2}	33,330	28.2×10^{-5}	.01	28.2×10^{-3}	.21	5.93×10^{-3}	89.6×10^{-5}	7.20×10^{-2}	7.20×10^{-4}	30.9×10^{-4}	140×10^{-4}	6.70×10^{-2}	0.700			
			150	5	9.56×10^{-2}	0.250	2.39×10^{-2}	32,250	27.3×10^{-5}	.01	27.3×10^{-3}	.20	5.47×10^{-3}	$117.\times10^{-5}$	7.20×10^{-2}	7.20×10^{-4}	38.1×10^{-4}	134×10^{-4}	6.70×10^{-2}	0.700			
			180	6	9.56×10^{-2}	0.250	2.39×10^{-2}	28,550	24.1×10^{-5}	.01	24.1×10^{-3}	.19	4.60×10^{-3}	$141.\times10^{-5}$	6.70×10^{-2}	6.70×10^{-4}	44.8×10^{-4}	134×10^{-4}	7.05×10^{-2}	0.740			

Table E-2. Bicarbonate C^{14} uptake during photosynthesis experiments using pond water.

Time of day	Radioactive substrate	Depth of sample	Color of bottle	Elapsed time (min)	i	C_o (μM/L)	V_o (L)	C_oV_o (μM)	Counts per minute	$\overline{X}_{si}$ (μM)	V_s (L)	$[\overline{X}_{si}]$ (μM/L)	V_i (L)	$\overline{X}_i$ (μM)	ΣX_{si} (μM)	C_i (est) (μM/L)	ΣC_iV_s (μM)	C_iV_s (μM)	C_iV_i (μM)	C_i (μM/L)	C_i/C_o	Slope (min^{-1})
11 AM	Bicarb. C^{14}	0'4"	light	0	0	1.19	.250	$2975x10^{-4}$	0	0	0	0	.250	0	0					1.19	1.00	$5.55x10^{-4}$
				15	1	1.19	.250	2975x10-4														
				30	2	1.19	.250	2975x10-4	231	$1.05x10^{-4}$	.005	$210x10^{-4}$	.245	$50x10^{-4}$	$1.05x10^{-4}$	1.00	.005	.005	.2875	1.17	0.985	
				60	4	1.19	.250	$2975x10^{-4}$	862	$3.91x10^{-4}$	.005	$780x10^{-4}$	.240	$180x10^{-4}$	$3.96x10^{-4}$	1.10	.0055	.0105	.2700	1.12	0.94	
				90	6	1.19	.250	$2975x10^{-4}$	1,725	$7.82x10^{-4}$	.005	$1560x10^{-4}$	.235	$343x10^{-4}$	$11.8x10^{-4}$	1.10	.0055	.0160	.2460	1.05	0.88	
				120	8	1.19	.250	$2975x10^{-4}$	2,080	$9.45x10^{-4}$	.005	$1890x10^{-4}$	.230	$400x10^{-4}$	$21.2x10^{-4}$	1.10	.0055	.0215	.2340	1.02	0.86	
				150	10	1.19	.250	$2975x10^{-4}$	2,565	$11.6x10^{-4}$	.005	$2320x10^{-4}$	.225	$465x10^{-4}$	$32.8x10^{-4}$	1.10	.0055	.0270	.2207	0.98	0.82	
1 PM				180	11	1.19	.250	$2975x10^{-4}$	1,922	$8.75x10^{-4}$	.005	$1750x10^{-4}$	.220	$332x10^{-4}$	$41.5x10^{-4}$	1.10	.0055	.0325	.2277	1.03	0.87	
	Bicarb. C^{14}	0'4"	dark	0	0	1.19	.250	$2975x10^{-4}$	0	0	0	0	.250	0	0					1.19	1.00	
				30	1	1.19	.250	$2975x10^{-4}$	92	$0.40x10^{-4}$	.005	$80x10^{-4}$	.245	$19x10^{-4}$	$0.40x10^{-4}$	1.20	.006	.006	.2890	1.18	0.99	0
				60	2	1.19	.250	$2975x10^{-4}$	125	$0.57x10^{-4}$	.005	$114x10^{-4}$	.240	$27x10^{-4}$	$0.97x10^{-4}$	1.20	.006	.012	.2827	1.18	0.99	
				90	3	1.19	.250	$2975x10^{-4}$	147	$0.67x10^{-4}$	.005	$134x10^{-4}$	.235	$31x10^{-4}$	$1.64x10^{-4}$	1.20	.006	.018	.2760	1.17	0.985	
				120	4	1.19	.250	$2975x10^{-4}$	178	$0.81x10^{-4}$	.005	$162x10^{-4}$	.230	$37x10^{-4}$	$2.45x10^{-4}$	1.20	.006	.024	.2696	1.17	0.985	
				150	5	1.19	.250	$2975x10^{-4}$	183	$0.83x10^{-4}$	.005	$166x10^{-4}$	.225	$37x10^{-4}$	$3.28x10^{-4}$	1.20	.006	.030	.2635	1.17	0.985	
				180	6	1.19	.250	$2975x10^{-4}$	167	$0.76x10^{-4}$	.005	$152x10^{-4}$	.220	$33x10^{-4}$	$4.04x10^{-4}$	1.20	.006	.036	.2578	1.17	0.985	
	Bicarb. C^{14}	5'3"	light	0	0	1.19	.250	$2975x10^{-4}$	0	0	0											
				30	1	1.19	.250	$2975x10^{-4}$	325	$1.48x10^{-4}$	.005	0	.250	0	0					1.19	1.00	$1.85x10^{-4}$
				60	2	1.19	.250	$2975x10^{-4}$	350	$1.59x10^{-4}$	.005	$300x10^{-4}$	.245	$73x10^{-4}$	$1.48x10^{-4}$	1.20	.006	.006	.2841	1.16	0.975	
				90	3	1.19	.250	$2975x10^{-4}$	540	$2.45x10^{-4}$	.005	$320x10^{-4}$	.240	$77x10^{-4}$	$3.07x10^{-4}$	1.20	.006	.012	.2775	1.15	0.970	
				120	4	1.19	.250	$2975x10^{-4}$	497	$2.25x10^{-4}$	.005	$490x10^{-4}$	.235	$115x10^{-4}$	$5.52x10^{-4}$	1.10	.0055	.0175	.2680	1.14	0.960	
				150	5	1.19	.250	$2975x10^{-4}$	327	$1.49x10^{-4}$	.005	$450x10^{-4}$	.230	$107x10^{-4}$	$7.77x10^{-4}$	1.10	.0055	.023	.2630	1.14	0.960	
				180	6	1.19	.250	$2975x10^{-4}$	606	$2.75x10^{-4}$	.005	$300x10^{-4}$	.225	$68x10^{-4}$	$9.26x10^{-4}$	1.20	.006	.029	.2608	1.16	0.975	
												$550x10^{-4}$	.220	$120x10^{-4}$	$12.01x10^{-4}$	1.20	.006	.035	.2493	1.13	0.950	
	Bicarb. C	5'3"	dark	0	0	1.19	.250	2975x10	0	0	0											
				30	1	1.19	.250	2975x10	134	0.60x10	.005	$120x10^{-4}$	.245	$29x10^{-4}$	$0.60x10^{-4}$	1.20	.006	.006	.2886	1.18	0.99	0
				60	2	1.19	.250	$2975x10^{-4}$	169	$0.80x10^{-4}$	.005	$160x10^{-4}$	.240	$38x10^{-4}$	$1.40x10^{-4}$	1.20	.006	.012	.2816	1.17	0.985	
				90	3	1.19	.250	$2975x10^{-4}$	194	$0.90x10^{-4}$	.005	$180x10^{-4}$	.235	$42x10^{-4}$	$2.30x10^{-4}$	1.20	.006	.018	.2751	1.17	0.985	
				120	4	1.19	.250	$2975x10^{-4}$	224	$1.04x10^{-4}$	.005	$208x10^{-4}$	.230	$48x10^{-4}$	$3.34x10^{-4}$	1.20	.006	.024	.2684	1.17	0.985	
				150	5	1.19	.250	$2975x10^{-4}$	187	$0.85x10^{-4}$	.005	$170x10^{-4}$	.225	$38x10^{-4}$	$4.19x10^{-4}$	1.20	.006	.030	.2633	1.17	0.985	
				180	6	1.19	.250	$2975x10^{-4}$	149	$0.68x10^{-4}$	.005	$140x10^{-4}$	.220	$31x10^{-4}$	$4.87x10^{-4}$	1.20	.006	.036	.2579	1.17	0.985	

Table E-3. Glucose C^{14} uptake during anaerobic substrate degradation experiments using pond water.

Radioactive substrate	Depth of sample	Color of bottle	Elapsed time (min)	i	C_o (μM/L)	V_o (L)	C_oV_o (μM)	Counts per minute (CPM)	$\overline{X}_{Ri}$ (μM)	V_D (L)	V_R (L)	$\overline{X}_{si}$ (μM/L)	V_s (L)	$[\overline{X}_i]$ (μM)	V_i (L)	$\overline{X}_i$ (μM)	ΣX_{si} (μM)	C_i (est) (μM/L)	C_iV_s (μM)	ΣC_iV_s (μM)	C_iV_i (μM)	C_i (μM/L)	C_i/C_o	Slope (min^{-1})
Glucose C	Bottom muds & water	dark	0	0	2.31	.260	$6000x10^{-4}$	0	0	.250	.005	0	.010	0	.260	0	0					2.31	1.00	$4.75x10^{-4}$
			30	1	2.31	.260	$6000x10^{-4}$	2,610	$2.21x10^{-5}$	.250	.005	$60x10^{-5}$	.010	.12	.255	.030	$60x10^{-5}$	2.25	.0112	.0112	.558	2.19	0.95	
			60	2	2.31	.260	$6000x10^{-4}$	2,940	$2.62x10^{-5}$	.250	.005	$131x10^{-5}$	.010	.26	.250	.065	$191x10^{-5}$	2.10	.0105	.0217	.511	2.05	0.89	
			90	3	2.31	.260	$6000x10^{-4}$	2,590	$2.19x10^{-5}$	.250	.005	$110x10^{-5}$	.010	.22	.245	.054	$301x10^{-5}$	1.90	.0095	.0312	.515	2.10	0.91	
			120	4	2.31	.260	$6000x10^{-4}$	2,490	$2.10x10^{-5}$	.250	.005	$105x10^{-5}$	.010	.21	.240	.050	$406x10^{-5}$	2.10	.0105	.0417	.504	2.10	0.91	
			150	5	2.31	.260	$6000x10^{-4}$	2,350	$1.99x10^{-5}$	.250	.005	$100x10^{-5}$	.010	.20	.235	.047	$506x10^{-5}$	2.10	.0105	.0522	.496	2.10	0.91	
			180	6	2.31	.260	$6000x10^{-4}$	3,270	$2.77x10^{-5}$	.250	.005	$138x10^{-5}$	.010	.28	.230	.005	$644x10^{-5}$	2.10	.0105	.0627	.465	2.00	0.865	
			210	7	2.31	.260	$6000x10^{-4}$	3,840	$3.25x10^{-5}$	.250	.005	$162x10^{-5}$	.010	.32	.225	.072	$806x10^{-5}$	2.00	.0100	.0727	.447	1.98	0.855	
			240	8	2.31	.260	$6000x10^{-4}$	3,750	$2.33x10^{-5}$	.250	.005	$167x10^{-5}$	.010	.35	.220	.077	$973x10^{-5}$	2.00	.0100	.0827	.431	1.96	0.850	