THE EFFECTS OF TEMPERATURE ON THE RESPONSE OF A LABORATORY WASTE STABILIZATION POND

By

C. HENRY GLAUS

Bachelor of Science

Tennessee Technological University

Cookeville, Tennessee

1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May 1971

THE EFFECTS OF TEMPERATURE ON THE RESPONSE OF
A LABORATORY WASTE STABILIZATION POND

Thesis Approved:

Don F Kincannon
Thesis Adviser

Echol E. Cook

Richard N. DeVries

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGMENT

The author would like to take this opportunity to express his sincere appreciation to the many people who had such a large part in making this course of study possible.

To Dr. Don F. Kincannon for his continuous faith and guidance as thesis adviser and friend throughout my eleven months at Oklahoma State University.

To Dr. E. E. Cook and Dr. R. N. DeVries for their encouragement and advice during the course of study and their careful evaluation of this manuscript.

To Mrs. Morrison for accurately typing this manuscript and to Mr. Peter F. Johnson for drawing the figures contained herein.

To the Tennessee Department of Public Health for their financial support and to my co-workers in the Division of Sanitary Engineering for their moral support.

To my parents for their constant faith in my abilities, which has been an everpresent source of strength to me.

Last, but most important, to my wife, Nancy, for her willingness to sacrifice so much, yet never utter a word of dissatisfaction, and for providing the love and understanding that only she could provide.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

A waste stabilization pond may be described as a man-made earthen basin used for the retention and treatment of domestic and industrial wastewaters under the conditions of natural purification or by incorporating certain mechanical processes to enhance the natural process. The contents of the waste stabilization pond consist of heterogeneous mixtures of algal and bacterial populations growing in a symbiotic relationship and whose function is to biologically degrade the pollutional material contained in the wastewater.

The efficiency of a waste stabilization pond in removing the pollutants from a waste stream is dependent on many factors. A portion of these factors are within present technical and economical realms to control, and a portion are not. Controllable factors include such parameters as the pond geometry and the hydraulic and organic loading of the pond. However, the engineer has not yet devised an economical way of controlling the forces of nature, including such things as sunlight intensity and duration, wind currents and velocities, or the ever-changing temperature. All of these things considered directly influence and dictate the effectiveness of the waste stabilization pond as a wastewater treatment device.

General acceptance of waste stabilization ponds as treatment units has gained popularity over the years since its recognition around 1924.

Many factors have contributed to the now widespread usage of these ponds in all sections of the world. The most heard of and read about factor is that of economics. In practically every piece of literature available in the pollution control field today which concerns itself with research or operation of waste stabilization ponds, a statement will be made similar to the following, "Waste stabilization ponds offer an economical means of waste treatment which is, in essence, as good as any other method available." Now the question which must be asked about such statements is, are they true?

In order to answer this question in light of present day stream pollution laws and stream standards, such things as "per cent removal" of biochemical oxygen demand, chemical oxygen demand, phosphates, etc., must be reexamined and the actual concentrations of the discharged pollutants must be considered. It has been observed that from many ponds claiming 85-95% BOD removal the effluent from these ponds has had the appearance of "green pea soup." Admittedly, the "green pea soup" may not contribute as large a pollutional load as the raw wastewater, and land in an area may be relatively inexpensive, and the city may not have to hire a fulltime, skilled man to operate the pond, but is this beautiful green effluent of the quality to prevent further degradation of streams and lakes where children of the future may enjoy the pleasures of today. As has been the case many times in the past, engineers have been fooled by wastewater treatment processes that create a false economy. The time has arrived when capital investment must not overshadow the quality effluents required for a modern, densely populated world.

Most of the research that has been reported concerning waste stabilization ponds has dealt primarily with either explaining the mechanisms of photosynthesis or with developing rational design equations. Little or no experimental work on the effects of temperature on the effluent quality of waste stabilization ponds has been reported. With the need for such information in mind, this study was undertaken.

A laboratory scale waste stabilization pond was operated under continuous flow and diurnal light-dark conditions with temperature being the only variable. The temperatures investigated ranged from 5^{o} C to 35^{o} C and the effect of the temperature changes on the effluent quality from the pond was measured in terms of dissolved oxygen, pH, total phosphates, biological solids concentration, and soluble (filtrate) COD. A heterogeneous culture of bacteria and algae was maintained throughout the study.

CHAPTER II

LITERATURE RESEARCH

A. General Considerations

Previous literature on the history, development, and application of waste stabilization ponds as a means of wastewater treatment has been thoroughly reviewed by others (6, 7, 8, 9) and will not be repeated here.

Waste stabilization ponds have been classified into three main categories depending on their biological processes in removing pollutants from wastewaters (10). The three categories are (1) aerobic, (2) facultative, and (3) anaerobic, with the aerobic and facultative types being the most used. Since only the pond depths and loading rates are dissimilar between the two main types, and since the biological mechanisms in the first two or three feet of depth in the facultative pond are strictly aerobic, the aerobic pond will be considered in detail.

Figure 1 shows the interacting processes that occur when organic wastes are added to an aerobic pond on a continuous basis. The reactions indicated in Figure 1 point out one of the truly important ecological factors that influence any environment: that of the interrelationships of microbial species. Practically all environmental engineers recognize that the interdependence of the bacterial and algal species in a waste stabilization pond are essential for efficient pond

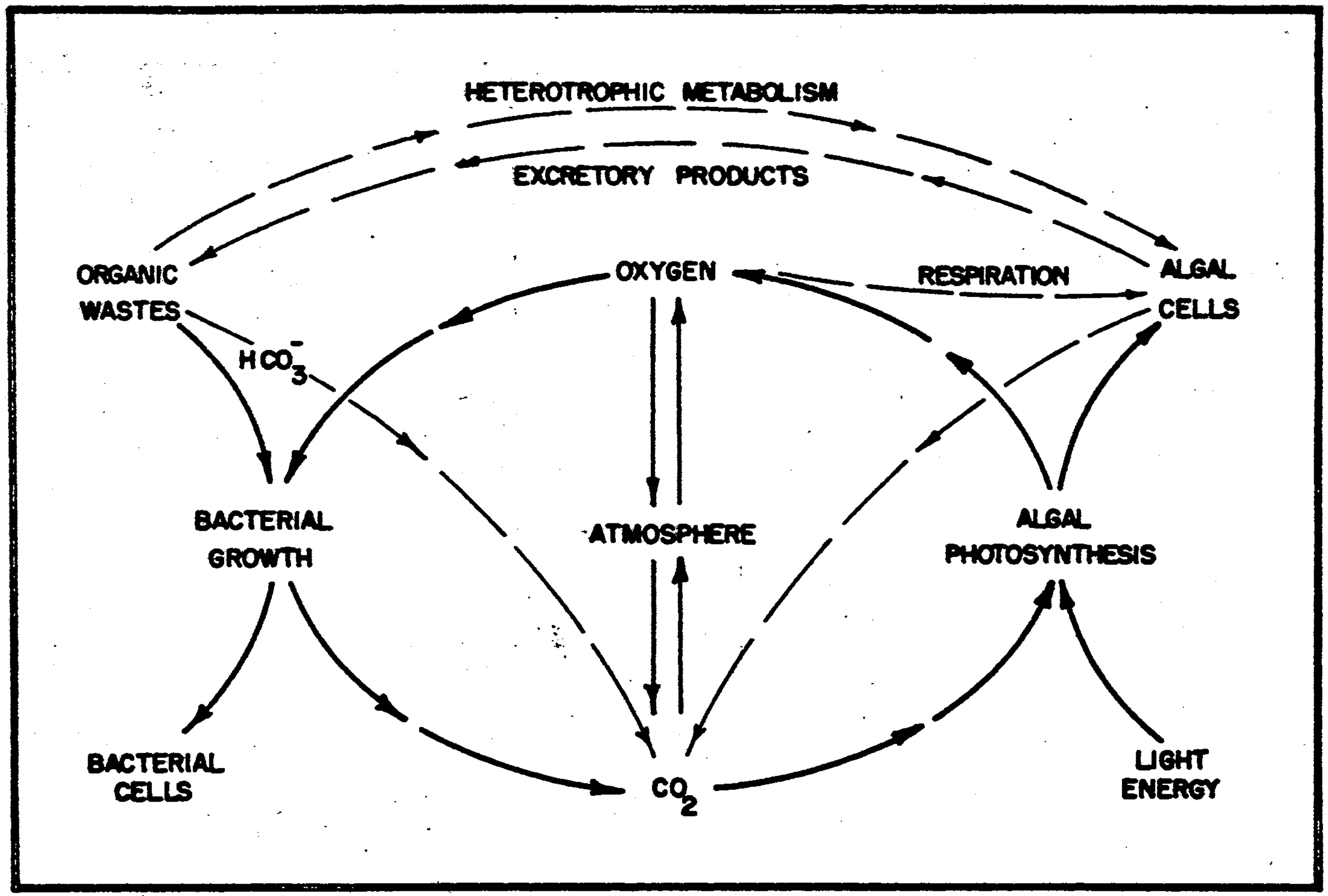

Figure 1. The Major Ecosystem Existing in an Aerobic Waste Stabilization Pond.

performance. Lange (12) found from his experiments with pure cultures of certain species of blue-green algae that for the removal of carbohydrates the presence of bacteria was necessary. Ludwig et al. (13) concluded from their work with cultures of *Euglena gracilis* that the treatment effected by an oxidation pond resulted from a complex symbiosis of bacteria and algae. Regardless, it must also be kept in mind that in certain circumstances the development of one partner (bacteria or algae) will proceed at the expense of the other, leading to the disturbance of the acquired balance (14). In essence the aerobic bacteria in a waste stabilization pond (some protozoa and fungi may be included also) feed directly on the organic compounds present in the wastewater, metabolizing these compounds eventually to carbon dioxide, water, and new cell material (11, 15, 6). The autotrophic organisms (algae) utilize the carbon dioxide by-product from bacterial metabolism as a carbon source, in the presence of sunlight, to build new cells and, in the process, liberate oxygen which can in turn be utilized by the aerobic bacteria.

During the night hours or on cloudy days, the algae themselves may exert an oxygen demand which can result in anaerobic conditions developing in the pond. Some algae may also utilize some portion of the organic waste material, thereby placing another strain on the dissolved oxygen (DO) resource (16).

B. Factors Affecting Pond Performance

1. Light Intensity and Duration

The light intensity necessary to drive the photosynthetic mechanisms in algae has been the object of much research in the past

(17, 18, 19, 20). Light intensity, like other environmental factors, is useful to algae within certain limits. The lower limit for growth appears to be around 20 foot-candles (17) and the upper limit, before oxygen production decreases, appears to be around 4500 foot-candles (19). Rodhe (21) concluded from his work with Ankistrodesmus ehlactus, at different temperatures and light intensities, that an intensity of 360 foot-candles gave optimum growth at temperatures of 15, 20, and 25^{o} C. Myers (59) concluded from his study of the growth characteristics of algae in mass culture that an intensity of 400 foot-candles was the minimum for optimum growth of Chlorella.

From the above discussion it can be surmised that under normal conditions a light intensity of 400 foot-candles is close to the optimum value.

Duration of the incident light necessary for optimum growth has not been studied as thoroughly as other factors, although Oswald (20) indicated that the ratio of light hours to dark hours was optimum at 0.5.

2. pH and Carbon Dioxide

The available amounts of carbon dioxide (CO_2) in waste stabilization ponds are dependent on bacterial decomposition of organic matter, pond alkalinity, pH, and temperature.

During the daylight hours when pond activity is at a maximum, bacteria may supply as much as 20 mg/l of CO_2 in a supersaturated state (22). The CO_2 is quickly utilized by the algae as a carbon source for synthesis reactions with subsequent liberation of oxygen. Carbon dioxide may also be transferred to the pond from the atmosphere. The concentration of CO_2 in the atmosphere is normally only 0.03 per cent, which is below the "saturation value." Laboratory and field studies have indicated that ponds relying primarily on atmospheric CO_2 as a carbon source have growth rates below those ponds supplied artificially with CO_2 (13, 23).

As the algae utilize the available CO_2 the pH of the pond increases (24, 25). In most ponds, during the daylight hours, at peak pond activity, the pH may increase to values as high as 11.0 (25, 39, 8). Most bacteria common to oxidation ponds cannot live at pH values of this magnitude, resulting in a reduction of available CO_2 for the algae (26). Thus an increase in pH will decrease the efficiency of the pond in removing the pollutants and the algae must rely on CO_2 sources from the atmosphere and pond alkalinity (principally the disassociation on HCO_3) to maintain its existence (25, 39).

Oswald (20) made some interesting observations concerning the pH effects on a culture of *Chlorella pyrenoidsa*. He observed that (1) "During light the rate of increase in pH and its ultimate magnitude were directly proportional to light intensity, and the length of time during which the pH level remained high was a function of duration of illumination, detention time, and increased light intensity; (2) intermittent variations in pH inhibited bacterial action, which in turn caused a shortage in CO_2, NH_3, and other nutrients essential to algal growth; and (3) during periods of high pH the algae became starved for nitrogen and their efficiency was impaired." He also found that by artificially increasing the CO_2 in the pond pH increases were less variable.

Pipes (25) investigated two different ways of controlling the pond pH, (1) by decreasing the pH of the influent and (2) by feeding the pond during the light hours only. His results indicated that loading the pond during the light hours only had no effect and that adjusting the pH of the influent downward did not prevent rapid pH increases, but that it did prevent the high pH levels from being reached. His data also showed that high-rate ponds which operate at a short detention time with resulting high pH levels did have a pronounced effect on BOD removal.

The work of Keefer and Meisel (27) on pH effects on activated sludge supports the same phenomena which occur in waste stabilization ponds. They showed that BOD reduction was best at pH 7.0-7.5 and that the bacterial activity decreased at pH 10.0.

C. The Role of Phosphorus

Within the field of environmental engineering, probably the naturally occurring element phosphorus has received more attention and concern during the last decade than any other inorganic substance. Perhaps the primary reason for such concern lies within the subject of eutrophication or the aging of rivers and lakes. Algae and other phytoplankton have been labeled as the major culprits in aiding in the early eutrophication of many rivers and lakes (10, 28, 29, 30, 31, 32).

Phosphorus is one of the major nutrient elements required for normal growth of algae (33). As in all living organisms, compounds containing phosphorus play important roles in nearly all phases of metabolism, particularly in energy transformation. Domestic wastewaters normally contain sufficient amounts of phosphorus to support both algal and bacterial growth. Therefore, waste stabilization ponds contain an abundance of this element.

Assenzo and Reid (29) found from their studies of several waste stabilization ponds in central Oklahoma that the raw wastewater contained an average of 2.7 mg/l of phosphorus. Stern and Dryden (30) reported average phosphate (PO_4) concentrations to be 45 mg/l from the effluent of a primary treatment plant at Lancaster, California. It can be concluded from this discussion that phosphorus is seldom a limiting nutrient for algal growth in waste stabilization ponds.

Some of the conditions affecting the uptake of inorganic phosphorus have been reviewed by Kuhl (33). Light intensity and duration, the phosphorus concentration in the medium, and pH, all affect phosphorus uptake. He also noted that some algal species, when provided with a sufficient supply of phosphorus, can store this element in quantities far in excess of their actual needs.

Azad and Borchardt (34) studied the green algal species <u>Chlorella</u> and <u>Scenedesmus</u> under phosphorus starvation conditions. They found that the algal growth rate depended only on concentrations from zero to 1.5 mg/l PO_4, i.e., zero to 3% PO_4 (as PO_4) by dry weight of algal cells. Furthermore, they defined the uptake of PO_4 from 1.5 to 5 mg/l PO_4 as "luxury uptake" and the uptake of PO_4 in excess of 5 mg/l PO_4 as "excess PO_4." They concluded that concentrations of phosphorus above 5 mg/l had no effect on the growth rate of phosphorus-starved algae.

Since algae have the ability to store large concentrations of excess phosphorus, waste stabilization ponds have been investigated and advocated to be the answer to phosphorus removal from watewaters. Assenzo and Reid (29) quoted removal efficiencies from 30-95% with an average of 1.7 mg/l PO_4 in the ponds' effluents. Neel and Hopkins (35) reported phosphate reductions by waste stabilization ponds but concentrations were not quoted. Bogan and Albertson (36) observed reductions in phosphates from an initial amount of 20 mg/l to a final amount of 5 mg/l in 4 hours. Herman and Gloyna (37) on studying pilot-ponds, observed increases in the effluent PO_4 concentration with increased detention time.

From the previous discussion, it is understood that the role of phosphorus in waste stabilization ponds is an important one, but that excessive amounts can cause pollution problems by being discharged into receiving streams.

D. The Theory and Relationship of Temperature to Pond Performance

Research data on the effects of temperature on the performance of waste stabilization ponds is conspicuously lacking in the published literature. To the author's knowledge there has been little or no research conducted in an effort to determine what effects the temperature parameter has on performance when other parameters are held constant.

1. Theory of Temperature Effects in General

Ferguson (28) states that the relative importance of temperature is known: "The rate of algal growth roughly doubles with every 20° F rise in water temperature between 32° F and 90° F." He did not give any data to support this hypothesis.

In general, most investigators approach the effect of temperature on biological systems from the theories of Arrhenius (38, 39). Arrhenius' relationships were developed for the reactions that occur in chemical systems, but since biological functions consist of a series of chemical reactions (enzymatic reactions) it has been generally observed that they indeed hold for biological systems as well (30, 40).

The Arrhenius' relationship (38) was given to be $\frac{k_1}{k_2} = e^{\alpha}$,

where $\alpha = \mu \left(\frac{1}{T_2} - \frac{1}{T_1}\right) \frac{1}{R}$

and: T_1 and T_2 are respectively the initial and final temperatures of the reaction in ^{o}K

R = gas constant

μ = energy function, characteristic of the reaction.

Phelps (38) further simplified the equation by explaining that when $\frac{1}{T_2} - \frac{1}{T_1}$ is in the form of $\frac{T_1 - T_2}{T_1 T_2}$, the product $T_1 T_2$ is approximately $(293)^2$ and the numerator would be merely the difference in the temperature in ^{o} C. By combining all constants the equation becomes $\frac{K_1}{K_2} = (\theta)^{(T_1 - T_2)}$.

He determined further $\theta = 1.047$. Sawyer and McCarty (39) stated that θ also represents the proportionate increase in rate per degree rise in temperature and therefore is also temperature dependent.

Gloyna (3) stated that from his studies on waste stabilization ponds θ was 1.085 when the ponds were operated at temperatures of 35, 24, 20 and 9^{o} C for a fixed organic loading. He had earlier, from other work, quoted a value of θ of 1.072 (40). Reasons for the discrepancies were not disclosed.

As a rule of thumb most investigators rely on the van't Hoff rule, which in essence states that for each 10^{o} C rise in temperature there is a doubling of the reaction rate over a restricted temperature range, to explain the effects of temperatures. This is also known as the Q_{10} relationship, defined as the ratio of the reaction rate at a particular temperature to the rate at 10^{o} C lower (39). It might also be said that, in order for the Q_{10} ratio to be 2, as many speculate, many conditions must be met. The Q_{10} ratio may be affected in numerous ways. A few include pH, light intensity and duration, culture densities, and the characteristics of the species in question.

Shih and Stack (41) studied the effect of temperature (6, 20, and 35^{o} C) on activated sludge and drew the following conclusions:

(1) The energy oxygen coefficient varies with the substrates oxidized and the temperature.

(2) Temperature may cause the difference in energy losses for the production of relatively undegradable extra cellular material.

(3) Predominant biological populations may change with temperature.

(4) The variation in biological growth may result in different energy oxygen requirements at different temperatures.

Kehrberger et al. (42) studied the effect of temperature on substrate utilization in BOD bottles and concluded that the temperature effect was controlled by the rate of diffusion of the substrate into the bacterial cells. For comparison they determined the Q_{10} for a stirred BOD bottle to be 1.73; for an unstirred BOD bottle, 1.35; for the Arrhenius equation, 2.23; and for the Streeter-Phelps equation, 1.58.

Jewel and McCarty (43) determined Q_{10} values that ranged from zero to 3.0 from their studies on algal decomposition. The temperatures used during the study ranged from 4 to 35° C. They concluded that a value of 2.0 was an appropriate approximation in most cases.

2. Effects of Temperature on Algae

Temperature may have varied effects on the algae common to waste stabilization ponds. Wolken (44) in his treatise on Euglena stated that temperature affects chlorophyll synthesis and that the organism is irreversibly bleached after continuous incubation at temperatures of 32 to 35° C. He also noted that, under normal growth conditions in the light, the rate of chlorophyll synthesis for Euglena increases with temperature to 38° C.

Marre (45) in a discussion on temperature stated that temperatures between 50° C and 70° C are, as a rule, only tolerated in nature under conditions of high light intensity and high CO_2 concentrations, but that both CO_2 and oxygen (O_2) solubility is limited with high temperatures.

Brock (46) has noted some interesting facts concerning temperature effects on microorganisms.

(1) Organisms will grow at any low temperature at which liquid water still exists.

(2) Many organisms that spend their entire lives at temperatures less than 10° C still often show temperature optimum for growth of around 20-25° C.

(3) The highest temperature at which living organisms are found is affected by pH and other environmental factors and in most favorable conditions is in the range of 85-88° C at which bacteria but not algae are found.

(4) The upper temperature limit for algae is 73° C.

Varma and Digiano (47) concluded that the oxygen uptake of dead algal cells was temperature dependent. The oxygen uptake of both young and old algal cells reached a maximum at 35^{o} C, then decreased sharply. This might have been due to bacterial suppression. The rate of oxygen uptake of old algal cells at 40^{o} C was approximately that at 20^{o} C. Jewel and McCarty's (43) results indicate similar findings but they also noted that algal decomposition was nearly completely inhibited at 4^{o} C.

3. Effects of Temperature on Phosphate Leakage

The effects of temperature on phosphate (PO_4) leakage, defined as the residual PO_4 in the pond effluent, by waste stabilization ponds has received little attention. Azad (48) from his work with cultures of Chlorella and Scenedesmus at temperatures of 15 and 25^{o} C detected no PO_4 in the filtrate at initial PO_4 concentrations of 2.0 and 1.5 mg/l, respectively. At a temperature of 30^{o} C and with a low algal cell density, PO_4 was detected in the filtrate. Since PO_4 residuals were not detected at the lower temperatures, he concluded that maximum leakage might occur at high temperatures. He also stated that higher PO_4 concentrations were required to grow equal masses at lower temperatures and lower PO_4 concentrations at higher temperatures. This would appear to be contrary to other observations of optimum algal growth at the higher temperatures which would in turn require greater concentrations of this essential nutrient.

Clare, Neel, and Monday (49) have presented two years of data on the performance of five single cell lagoons with the same surface areas and depths but which were subjected to different hydraulic and organic loadings. Most of the reduction in phosphorus occurred under the smaller organic loading conditions (less than 60 lbs/ac./day), and the temperature ranged from 2^{o} C in December to 31^{o} C in August. From the data presented, the greatest leakage of total phosphorus occurred during the months of December to April. The data presented by Assenzo and Reid (29) were in general agreement with that of Clare, Neel and Monday.

4. Effects of Temperature on Substrate Removal

Investigations into the effects of temperature on the efficiency of waste stabilization ponds in removing the organic materials from wastewaters have been few. When maintaining temperatures of 23 to 30^{o} C Canter et al. (1) observed BOD and COD removals of 90% and 80%, respectively, for loading rates up to 200 lbs BOD/acre/day. The work was done under laboratory conditions using a 15-gallon aquarium, a water depth of one foot, and a light intensity of 500 foot-candles.

Carpenter et al. (50) studied the effects of temperature on the treatability of pulp and paper mill wastes in an aerated lagoon. Actual waste was used as the substrate. The waste had a BOD of 200 mg/l and a BOD:N:P ratio of 100:5:1. With five days' aeration the results gave BOD removals of 70, 75, 82 and 85% at temperatures of 2, 10, 20, and 30^{o} C, respectively. Further investigations revealed an average deoxygenation constant (K_1) of 0.2 at 20^{o} C and an average temperature coefficient (θ) of 1.016. This value of θ disagrees somewhat with the previous value of 1.085 given by Gloyna (3).

Oswald et al. (51) made in depth studies on several pilot scale waste stabilization ponds. As a comparison, two of the ponds gave the following results:

	Pond 1	Pond 2
Month	August	January
Depth (inches)	8	36
Detention time (days)	3	10
Average temperature (^{o} C)	20	8
Raw BOD (mg/l)	117	109
Organic Loading (lbs/ac./day)	77	89
Final BOD (mg/l)	23	26

They concluded in part that it would be necessary to provide more pond area for winter operation than for summer operation in order to utilize maximum photosynthetic oxygenation.

Parker (52) studied the BOD removal efficiencies of waste stabilization ponds in Australia. His data for the same pond but at different times of the year gave the following results:

Average temperature	11^{o} C	18^{o} C
Raw BOD (mg/l)	272	172
Final BOD (mg/l)	83	44
Filt. Eff. BOD (mg/l)	65	14.8

From his data he states that the pond's efficiency is independent of temperature, algal population and other factors which might favor better summer performance. The data further indicated less substrate removal during the winter than in the summer despite lower organic loadings in the winter. Later in his conclusions he stated that if the same loading had been applied to the pond in both seasons the efficiency of BOD removal would have been the same.

Steffen (53) reported on the operation of an aerobic lagoon used as a polishing pond following anaerobic treatment of meat packing wastes. Reductions of 96% in summer to 70% in winter with temperatures as low as 2^{o} C were reported. The summer temperature was not given.

The data of Clare, Neel, and Monday (49) indicated more efficient BOD removals during the summer and fall months (temperature range of 12 to 31^{o} C) than in the winter and spring months with temperature ranges of 2 to 14^{o} C. The effluent still contained a 10-month average of 40 mg/l BOD. The pond loading was 60 lbs of BOD per acre per day.

El-Baroudi and Moawad (14) conducted a pilot-plant experiment with aerobic waste stabilization ponds in Egypt. The ponds were fed on an intermittent basis with pasteurized skim milk to which certain salts were added. The ponds were loaded at a rate of 70 lbs BOD/acre/day. In late summer when the temperature was 24 to 33^{o} C the BOD removal efficiency was 52 to 68% and during winter when the temperature was 12 to 25^{o} C the efficiency ranged from 87 to 91%. They explained that the difference was in the algal species. Blue-green algae predominated in the summer and the greens in the winter.

In contrast to the findings of recent investigations, Fitzgerald and Rohlich in 1958, from a search of the literature on field operations, concluded that a waste stabilization pond's performance was not greatly affected by temperature.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Apparatus

1. Experimental Pond

The experimental pond used in this study consisted of a rectangular aquarium constructed of 3/16-inch-thick Plexiglass with the following dimensions:

Length	49.28 cm.
Width	28.58 cm.
Depth	25.91 cm.
Surface Area	1408.42 $cm.^2$
Total Volume	36.5 liters

2. Water Bath

A larger tank constructed of galvanized iron and insulated with spun fiberglass was used as a water bath for the aquarium in order that the desired temprature could be maintained. The total volume of this tank was 120 liters.

3. Temperature Control System

The pond, when placed in the water bath, was held in place with a 3-inch strip of Plexiglass fastened by brackets to the sides of the water bath. The arrangement allowed water, which was previously heated or cooled to the desired temperature with a Precision Scientific

Company "Lo-Temptrol" water bath, to be continuously circulated around the exterior of the pond. The constant temperature water bath also prevented temperature stratification within the pond. A freeboard of 2 inches was maintained above the maximum water level in the pond.

4. Effluent Discharge

A 3/8-inch diameter port in one end of the pond was connected to a port of corresponding depth in the water bath, thus providing a positive overflow from the pond. A water depth of 13.4 cm. with a corresponding volume of 18.6 liters was maintained.

5. Illumination

Illumination was provided from eleven Grow-Lux fluorescent lamps (Sylvania) which were hung side by side above and parallel to the length of the pond. The light intensity at the operating water level of the pond was maintained at 375 foot-candles. A twelve hour light - twelve hour dark cycle was used for each twenty-four hour period throughout the study.

6. Substrate Feed System

A Milton Roy "mini-pump" (M-2-B-96R) was used to pump the substrate solution from 10 liter carboys to the pond. The pumps were in duplicate with the spare used to pump a weak chlorine solution through spare feed lines in order that bacterial growth would not clog the lines. Clean feed lines were substituted each time a new feed solution was made.

The feed solution was pumped to the end of the pond opposite the overflow and into a 3/8-inch diameter pipe that extended to the bottom of the pond. This arrangement eliminated short-circuiting within the pond.

The feed rate was calibrated initially and checked each time a fresh feed solution was prepared.

7. Mixing

Artificial mixing was not employed during this study except during the interim periods between temperature changes. Mixing in this case was used to insure uniform temperature changes in a relatively short time.

A schematic diagram of the individual units is shown in Figure 2.

B. Seeding Populations

1. Heterogeneous Microbial Seed

The heterogeneous microbial seed used for this study was obtained from the effluent of the primary clarifier of the Municipal Wastewater Treatment Plant located near Stillwater, Oklahoma.

2. Heterogeneous Algal Seed

The initial seeding population was obtained from an abandoned project located in one of the bioengineering laboratories at Oklahoma State University. A second seeding population used in the latter stages of the study was obtained from "Theta Pond," located on the campus of Oklahoma State University.

No attempt was made to maintain either a pure culture or one with a predominating species.

C. Synthetic Waste

The synthetic waste used throughout the experimental work had the following chemical make-up:

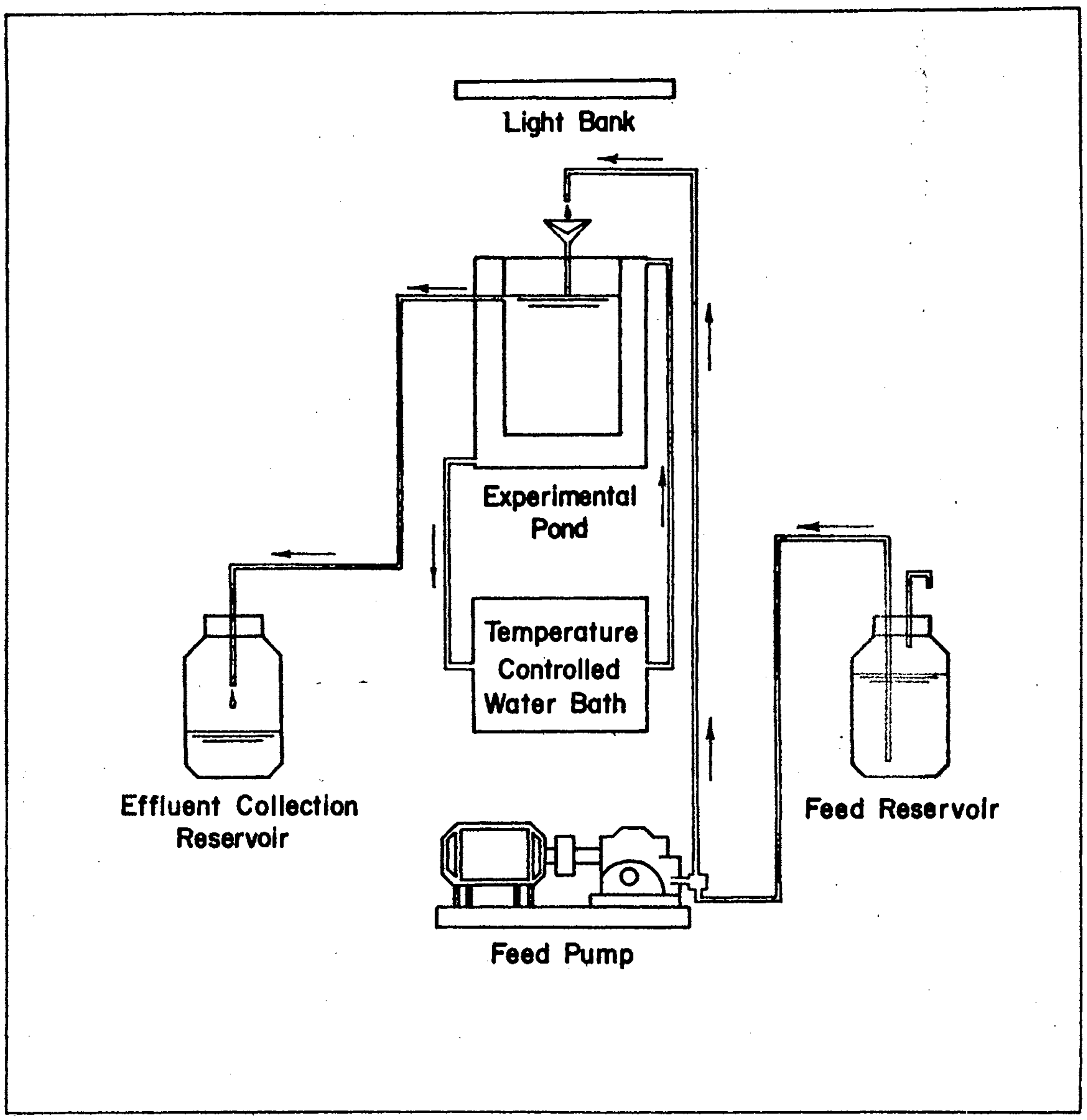

Figure 2. Schematic Diagram of the Experimental Apparatus.

Constituent	Concentration
Glucose	36.00 g/l
$(NH_4)_2\ SO_4$	21.20 mg/l
$KH_2\ PO_4$	1.68 mg/l
$MgSO_4 \cdot 7H_2O$	10.00 mg/l
$FeCl_3 \cdot 6H_2O$	0.05 mg/l
$MnSO_4 \cdot H_2O$	1.00 mg/l
$CaCl_2 \cdot 2H_2O$	0.75 mg/l
$NaHCO_3$	100.00 mg/l

The major constituents of the synthetic waste and their concentrations are based on those commonly used in the bioenvironmental laboratories at Oklahoma State University, with the exceptions being the glucose, $(NH_4)_2\ SO_4$, KH_2PO_4, and $NaHCO_3$ concentrations.

In making the preliminary investigations prior to the initiation of the project, a decision had to be reached concerning the organic loading to be applied to the pond. Canter et al. (1) in a recent article gave a summary of the various loading rates allowed in the 50 states for waste stabilization ponds. Based on the values given, it was decided to use a rate of 70 pounds of BOD per acre/day. It was then calculated, assuming that the BOD of a "typical" wastewater is normally 60% of the COD, that a glucose concentration of 36 g/l (COD of 90 mg/l) would be required.

In order for total phosphates to be used as a parameter of pond performance a monobasic-dibasic phosphate buffer system could not be used because of the high concentrations that would be encountered. Also the use of an artificial buffering system would not give a true picture of pH fluctuations in the pond. Furthermore, it was advantageous to limit the phosphate concentration to a reasonable level and thereby reduce the chances of leakage due only to over storage by the algae. Sawyer (2) reports that for a waste to be amenable to treatment by activated sludge the BOD:N:P ratio should be 150:5:1. Gloyna (3) indicated that for oxidation ponds the BOD:N:P ratio should be 100:20:1. It was decided for this study to use a C:N:P ratio of 100:20:1. This ratio would insure adequate growth factors yet would not give an excess of nutrients which would affect the validity of the experimental results. The concentrations of $(NH_4)_2SO_4$ and KH_2PO_4 used in the synthetic waste were based on a ratio of 100:20:1.

Sodium bicarbonate was included in the synthetic waste to act as a natural buffer and to provide an additional carbon source for the algae. It was necessary that the additional carbon source be available when high pH levels were reached and/or extreme temperatures limited the bacterial metabolism.

D. Experimental Procedures

Initially the pond was seeded with a large volume of primary wastewater and a small volume of algae from the sources indicated earlier. Batch feeding was then initiated until a prolific growth of algae was obtained. Analytical analysis of the pond contents indicated excessive amounts of phosphates. To reduce the high phosphate concentration, the pond contents were centrifuged with a Sharples continuous flow centrifuge which concentrated the algal cells. The cells were mixed for only a few seconds in a Waring Blendor and resuspended in the synthetic waste. This method proved to be successful in reducing the phosphate concentration. Batch feeding was continued until the pond had become acclimated.

Room temperature (21^{o} C) was chosen for the first study using the synthetic waste on a continuous flow basis. The feed rate of the synthetic waste was calibrated to be 3.5 liters per twenty-four hours, which gave a total detention time of 5.3 days. This same feed rate was held constant throughout the study. Samples for analytical analysis were collected at the end of each twelve hour light and dark period by siphoning from the top half-inch of the pond surface and from the end opposite the pond influent. Dissolved oxygen, biological solids, pH, total inorganic phosphate, and the filtrate COD were determined on the samples. An amount of liquid equal to that withdrawn from the pond was carefully replaced following sampling.

The same temperature was maintained for seven to eight days before it was changed. Normally twenty-four to thirty-six hours were required for the pond to acclimate to its new environment.

E. Analytical Procedures

1. Substrate Removal

Substrate removal was determined by analyzing the biological solids filtrate for COD as described in Standard Methods (4).

2. Biological Solids

The concentration of biological solids in the effluent of the oxidation pond was determined by filtering a known volume through a 0.45 μ Millipore Filter as described in Standard Methods (4).

3. pH

The pH of the pond effluent was determined by use of a Beckman "Zeromatic II" pH meter. The pH meter was standardized every experimental day to pH 7.0.

4. Dissolved Oxygen

Dissolved oxygen concentration was measured by the azide modification of the Winkler Method as described in Standard Methods (4).

5. Inorganic Phosphate

Inorganic phosphate was measured on the biological solids filtrate in accordance with the method developed by Fiske and Subbarow (5).

CHAPTER IV

RESULTS

The results of the experimental study are shown in Figures 3 through 8 and are summarized in Figures 9 through 12. The average values of the parameters that are plotted in Figures 9 through 12 are tabulated in the appendix.

All of the experimental work was performed under conditions of constant luminous intensity and organic and hydraulic loading with the only variable being that of the temperature. It was felt necessary to conduct the experimental work under these conditions to determine if certain trends of microbial activity could be observed at one temperature and to what extent these trends would be altered when the temperature was increased or decreased.

Figures 3 through 8 indicate the response of the experimental pond to temperatures of 21, 15, 10, 5 and 35^{o} C, respectively. From Figure 3 (21^{o} C) it can be seen that the microbial population was active in that the DO concentration varied considerably during the light and dark cycles. In some instances the microbial respiration during the dark cycle lowered the DO from above that of saturated DO (9 mg/l @ 21^{o} C) to levels below 5 mg/l. However, after four days of continuous operation the pond activity began decreasing. The reason for a decrease in activity is difficult to explain because the amount of the substrate

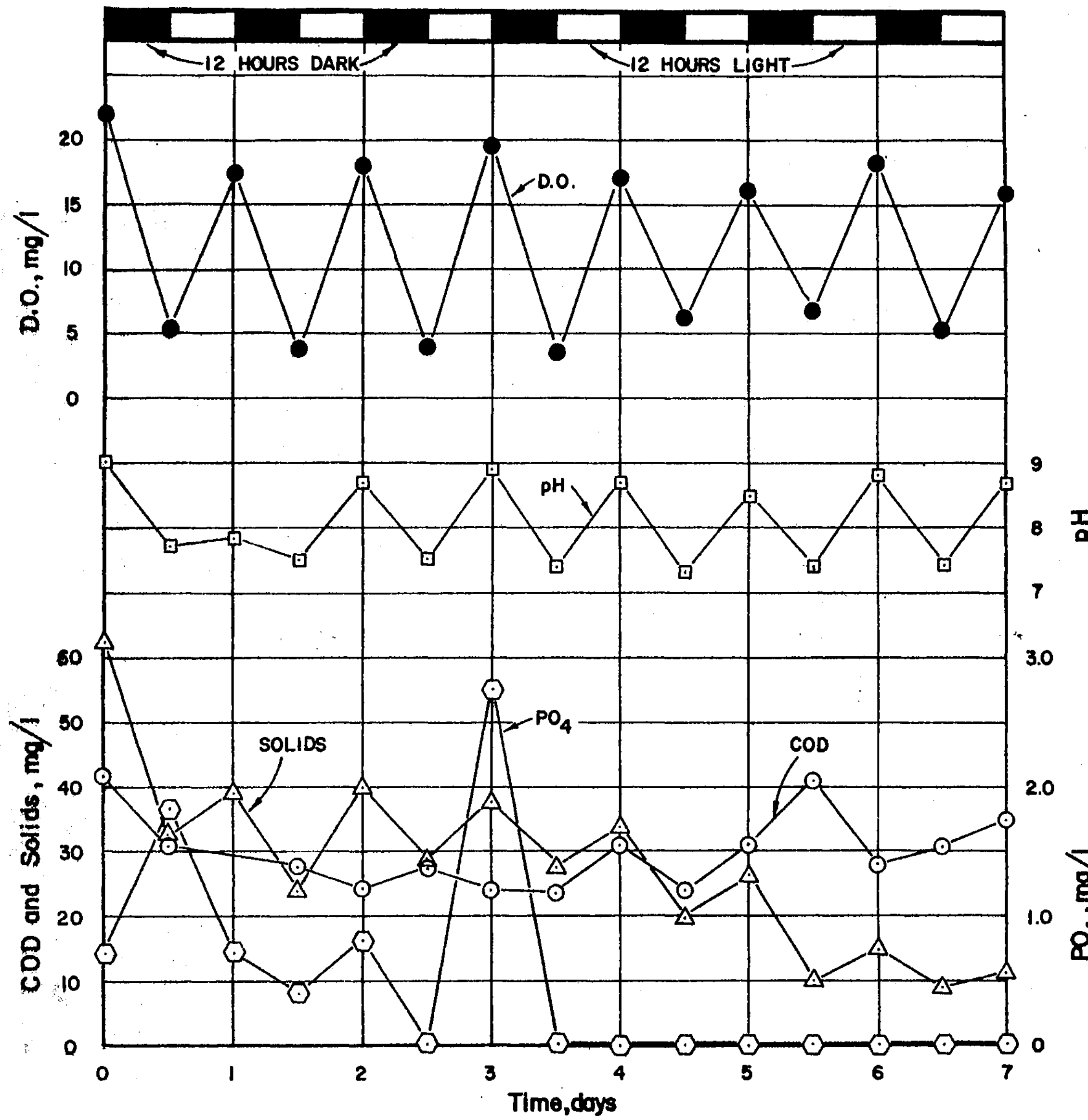

Figure 3. Pond Response to a Temperature of 21° C for the Continuous Flow Unit (Initial COD of 90 mg/l).

available for bacterial metabolism was constant throughout the experiment. Probably either the algae or the bacteria were producing some metabolic intermediate which was inhibiting growth, although no experimental evidence exists to substantiate this reasoning.

The possibility of an exogenous substrate is evidenced further by observing the steady decline in biological solids and a gradual increase in the soluble COD in the pond effluent.

A decrease in the solids concentration (Figure 3) would in part account for the low amounts of phosphate leakage. Since algae store phosphorus, a decrease in solids concentration in the effluent could mean that the algal cells had stored all the phosphate available from the synthetic waste.

Results of the experiment at 15^{o} C, as shown in Figure 4, indicate an increase in pond activity as compared to the preceding results at 21^{o} C. Since most investigators report optimum activity within a temperature range of 20-25^{o} C a decrease in pond response was expected at this temperature (3, 13, 20).

One primary indication of increased activity was that of a high algal yield or an increase in the biological solids concentration. It would appear that when the effluent of the pond contains greater concentrations of biological solids the filtrate COD will also be less; conversely, when the effluent solids are higher the effluent phosphate concentrations are higher.

Bacterial activity at 15^{o} C appeared to be dominated by the algae. Even though the average decrease in dissolved oxygen was 10.2 mg/l between the light-dark cycles, the combined respiration of the bacteria

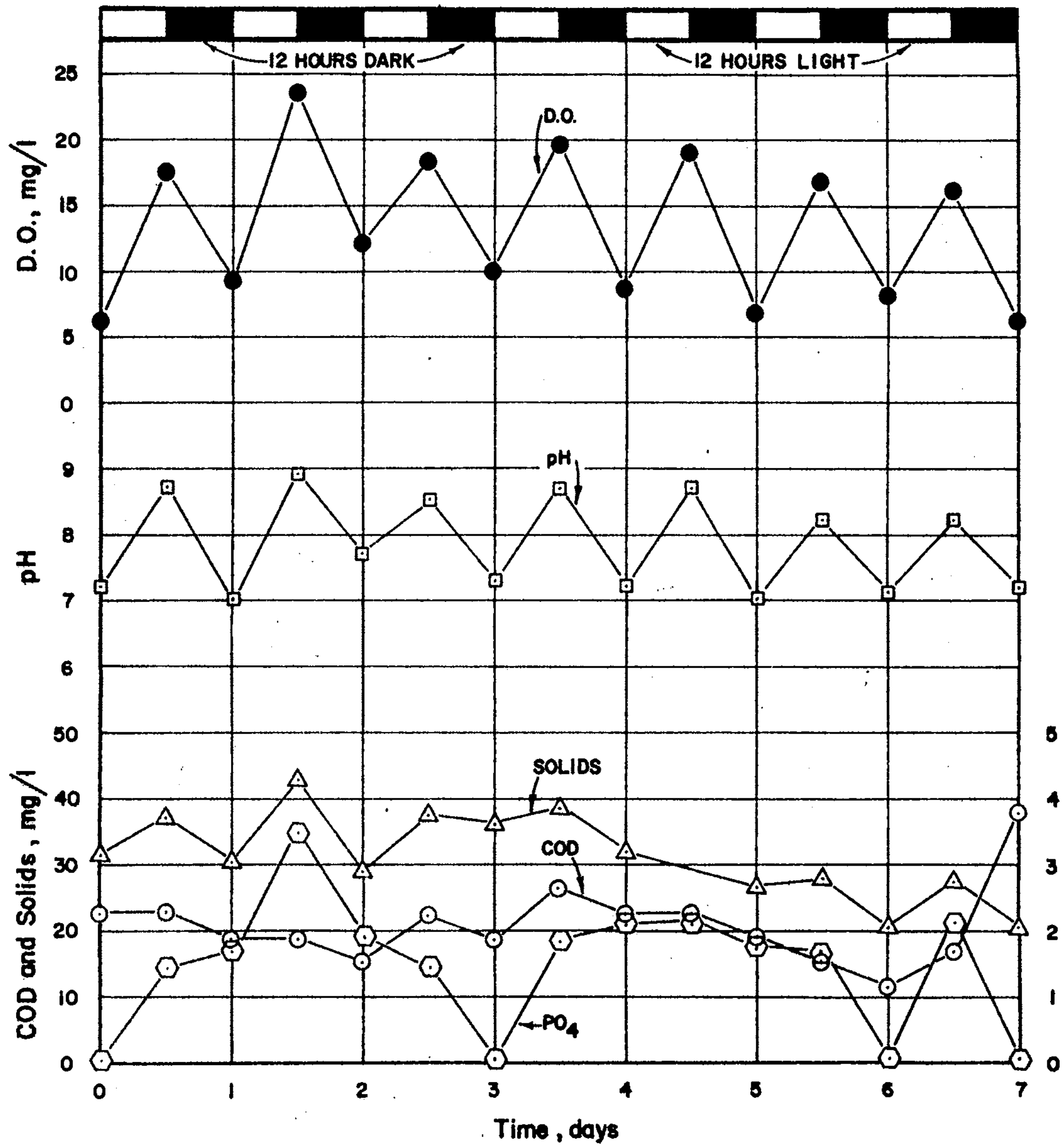

Figure 4. Pond Response to a Temperature of 15^{O} C for the Continuous Flow Unit (Initial COD of 90 mg/l).

and the algae in the dark barely lowered the DO level below that of saturation, which is 10.2 mg/l at 15^{o} C.

A decrease in microbial response to decreasing temperatures is indicated by the results shown in Figure 5 for the experiment at a temperature of 10^{o} C. Visual observations of the pond during this experiment indicated a tendency of the algae to accumulate in the lower portion of the pond. Only at this temperature did such a phenomenon occur. At all other temperatures the algal growth remained dispersed. Despite a lack of visible solids in the effluent, phosphate leakage remained evident. Possibly the algal population was experiencing predominance or growth changes such that the phosphates were being released into the medium. It is not believed that the algal population was dying, because of continued DO production. There was an initial decreasing trend in DO production, but toward the last three days of the experiment a gradual increase in DO was observed. The last twenty-four hours of the experiment (continuous lighting) saw the DO increase from a deficit of 3 mg/l to a supersaturated concentration of 14.2 mg/l.

The steady decrease in pH at this temperature (10^{o} C) would suggest that smaller quantities of CO_2 were available from bacterial metabolism and the algae were utilizing the "bicarbonate CO_2" as a carbon source.

Experimental results at the 5^{o} C temperature, as shown in Figure 6, indicate a marked decrease in overall microbial activity. Effluent DO concentrations varied insignificantly between the light and dark cycles and showed even less variation toward the end of the experiment. pH also showed very small light-dark variations and became constant beginning from the 4 1/2 day point and continuing until the 7th day.

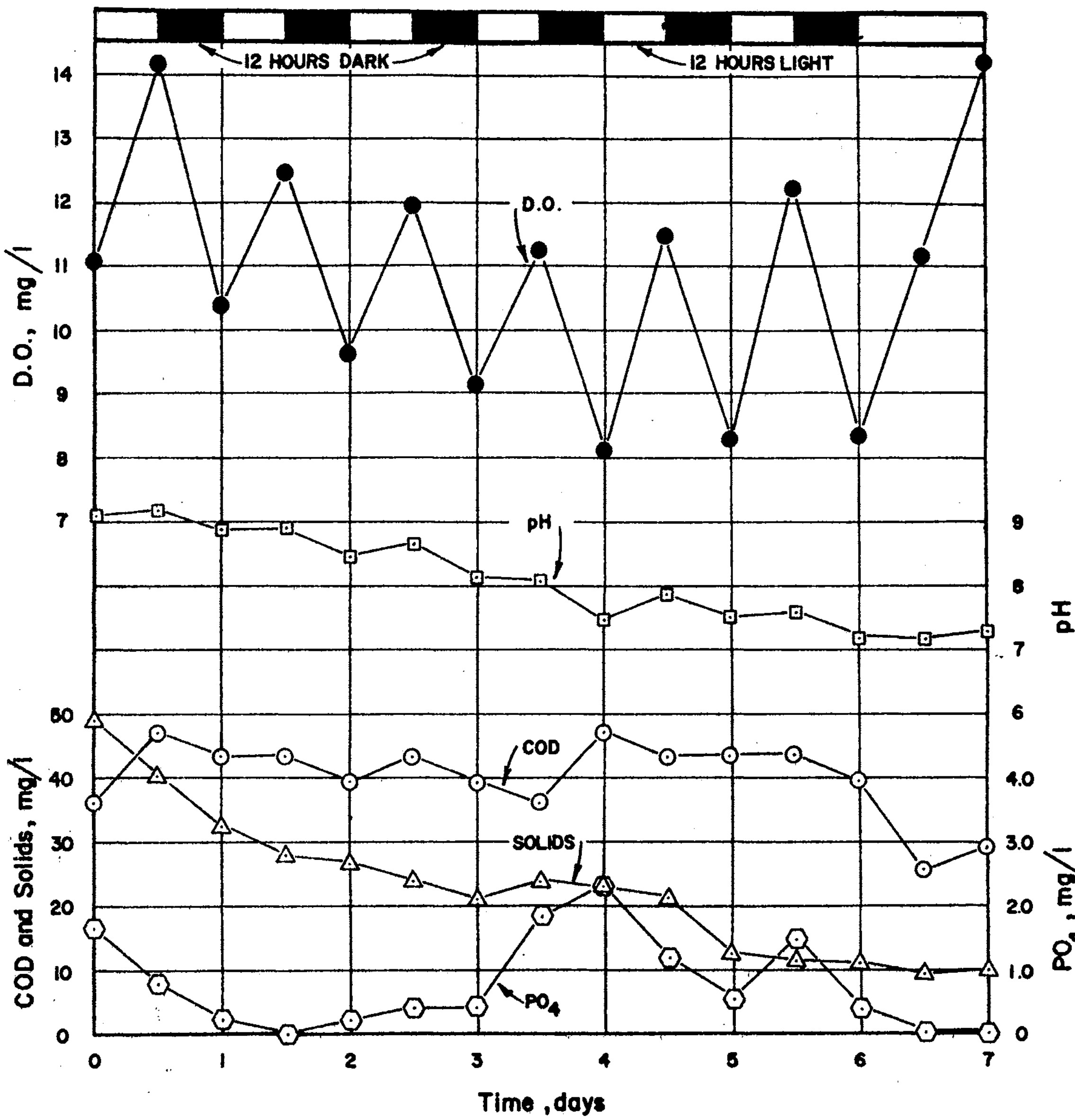

Figure 5. Pond Response to a Temperature of 10° C for the Continuous Flow Unit (Initial COD of 90 mg/l).

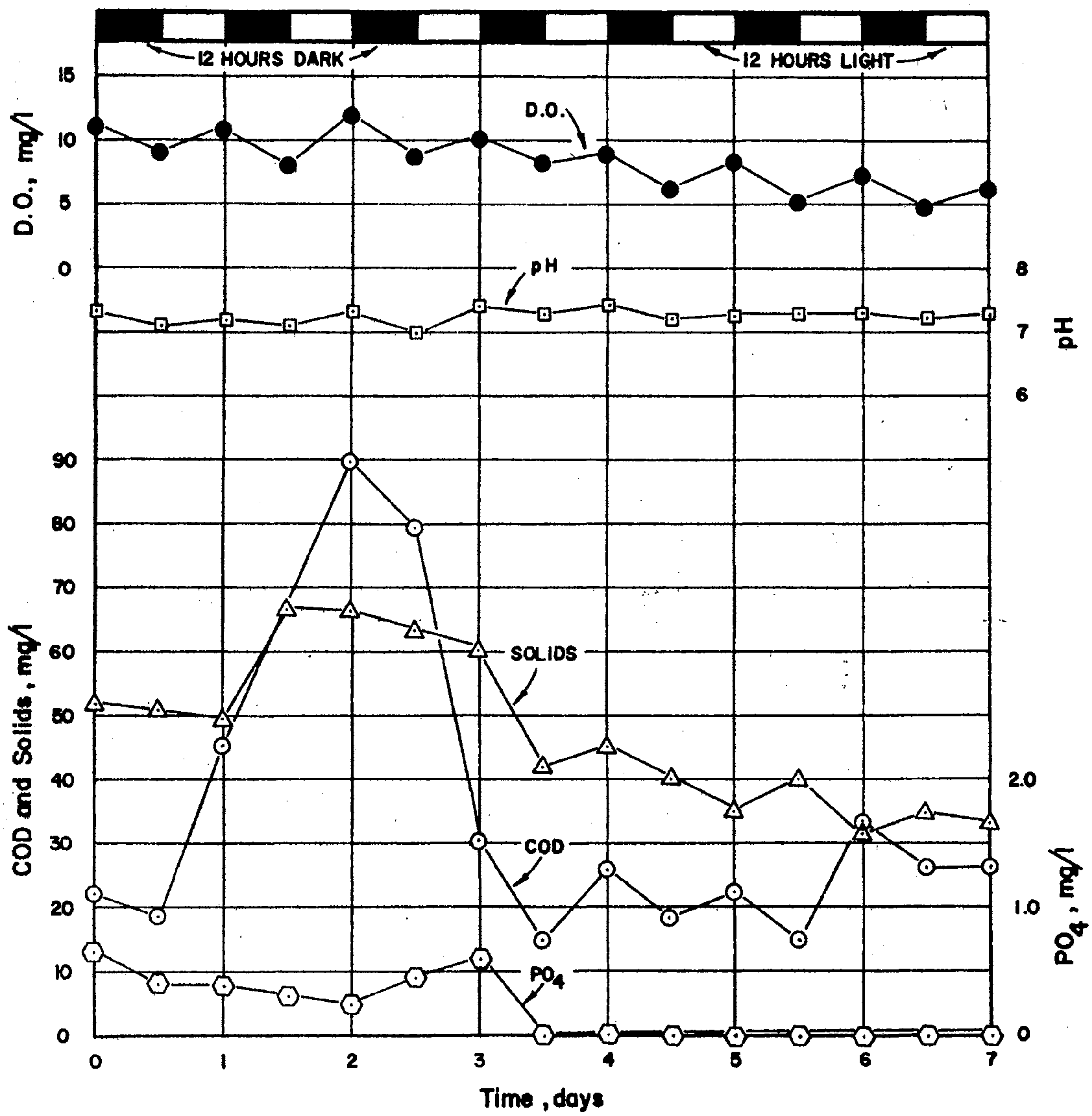

Figure 6. Pond Response to a Temperature of 5^0 C for the Continuous Flow Unit (Initial COD of 90 mg/l).

The concentration of biological solids in the effluent showed an increase from 50 to 67 mg/l with a corresponding increase in filtrate COD from 20 to 90 mg/l over the first thirty-six hours of operation. High concentrations of both solids and COD lasted for an additional 2 1/2 days before subsiding to more predictable levels. With the high initial biological solids a decrease in the soluble COD of the effluent would be expected, but from the data, the increasing trend in COD did not subside until the solids had begun to drop. This indicates that despite high solids or algae, as is the case here, the soluble organics increase. Stated in different terms, maximum algal cell yield does not necessarily mean maximum efficiency in removing the soluble organics.

It is interesting to note that there was continuous phosphate leakage during the period of high solids production, but this decreased below detectable levels after three days and remained so for the remainder of the experiment. An explanation as to why the parameters gave such unexpected values for the first three days of operation is not readily available. The time lapse between the time the temperature was lowered from 10 to 5^{o} C might not have been adequate for microbial acclimation or the algal population might have been undergoing a predominance change.

Before the last two experiments (Figures 7 and 8) were begun the contents of the pond were disposed of and a heterogeneous culture was prepared as outlined in Chapter III. A change in cultures was made in hopes of improving the predictability of the results at the higher temperatures over that at the lower temperatures.

The new culture was batch fed and mixed for one week starting at a temperature of 24^{o} C, after which the temperature was gradually

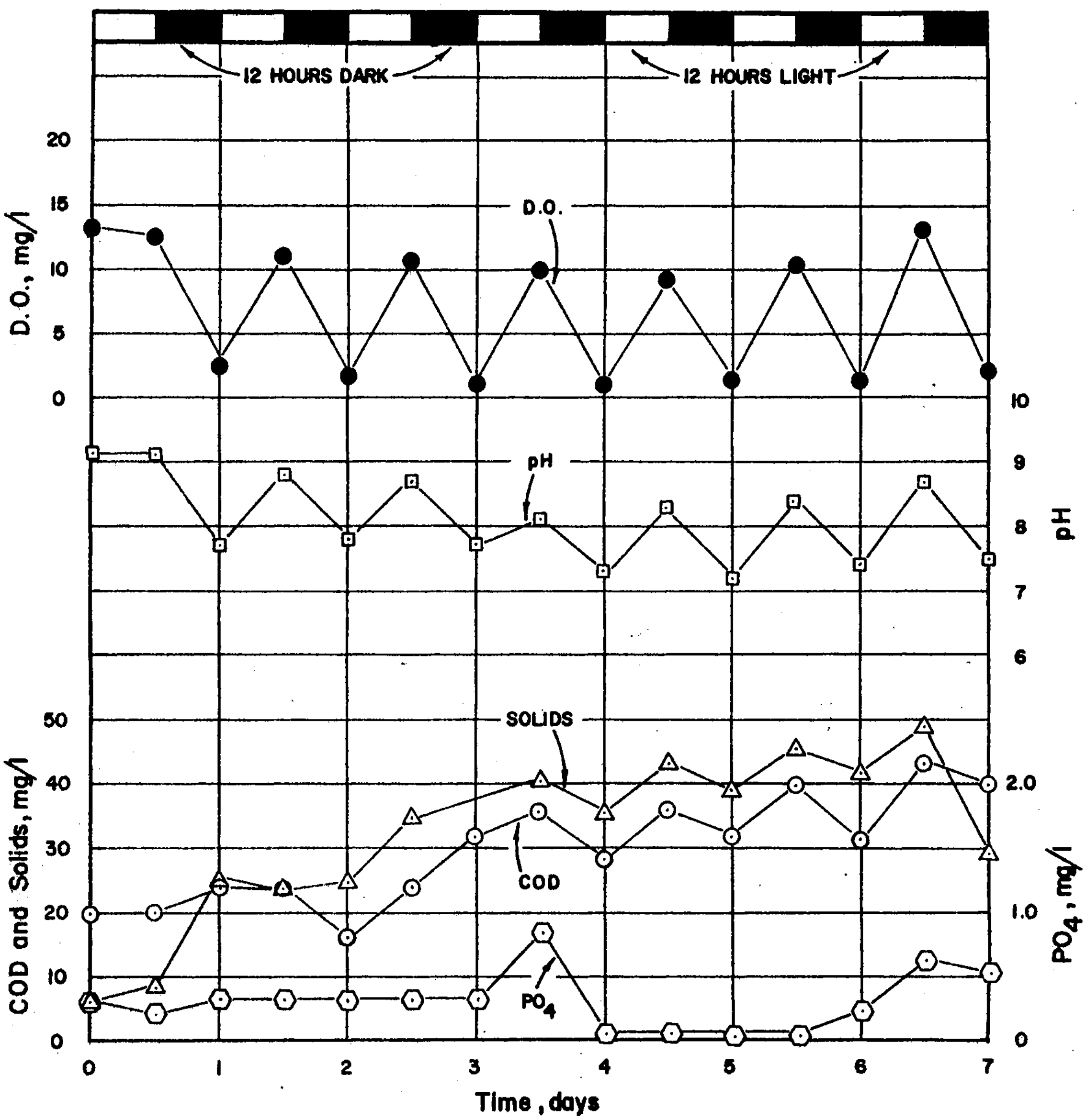

Figure 7. Pond Response to a Temperature of 30° C for the Continuous Flow Unit (Initial COD of 90 mg/l).

increased to 30^{o} C. Batch feeding was continued until the DO reached a consistent level while having the lights on continuously.

If at the 30^{o} C temperature, shown in Figure 7, all other parameters with the exceptions of DO and pH are ignored, the results would indicate an equilibrated system, existing in a near even balance between synthesis and respiration over the light and dark cycles; but equilibrium conditions of the actual microbial activity did not exist. From the data for the first two days of operation the system appeared to be existing in a lag phase. After this lag phase a continuous growing phase began and continued for the remainder of the experiment. Such a trend was evidenced not only by gradual increases in effluent solids concentration but by a gradual increase in the soluble COD. Apparently the change in the culture temperature of 24^{o} C to the experimental temperature of 30^{o} C, with an accompanying acclimation period, can account for the growth changes, although the increased growth did not commence for two days. At this temperature, as previously discussed, there was not a recognizable relationship between phosphate leakage and any of the parameters. One exception observed was that at most of the peaks where the filtrate COD increased the phosphate leakage also increased, which normally followed the twelve-hour light period. Indications would be that the release of phosphates by the algal population are unpredictable.

Increasing the temperature to 35^{o} C placed considerable strain on the pond's biological mechanisms as indicated in Figure 8. Using the saturation dissolved oxygen value (7.1 @ 35^{o} C) as a measure of pond activity indicates a failure in the system to produce an effluent saturated in dissolved oxygen during the light periods when

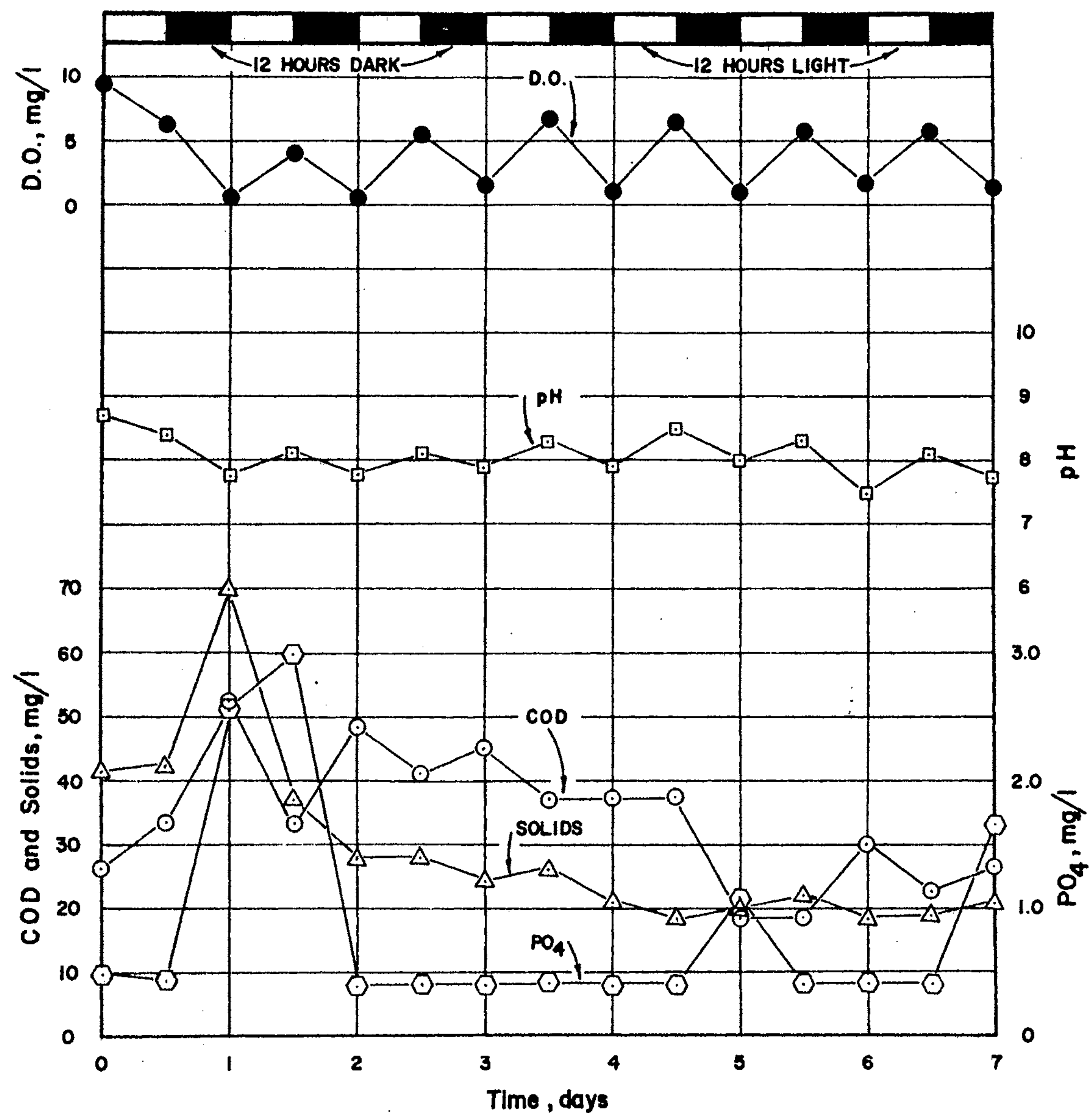

Figure 8. Pond Response to a Temperature of 35^{0} C for the Continuous Flow Unit (Initial COD of 90 mg/l).

photosynthetic oxygenation is at a maximum. One reason for the drop in algal activity is an apparent lack of sufficient amounts of substrate available for bacterial metabolism. The work by Oswald (11, 20) and Canter (1) have shown that, for temperatures above 25^{o} C and under light conditions, increasing amounts of organic carbon are required for optimum oxygenation, 100 lbs BOD/ac./day as compared with the 70 lbs BOD/ac./day used in this study. In comparing the results obtained from this temperature study with those previously discussed, it seems that smaller quantities of phosphorus are required for the microbial population. This is pointed out by observing (1) an abundant leakage of phosphates accompanied by an initial surge of biological solids being carried out the overflow and (2) continued phosphate leakage despite a decreasing trend in biological solids concentration.

In Figure 9 the average effluent concentrations of each parameter for each experiment are plotted against the corresponding temperatures. It is evident from Figure 9 that the 15^{o} C temperature was the most active as indicated by the high dissolved oxygen concentration, high biological solids, and the lowest average filtrate COD. Average phosphate concentration was lowest at the 5^{o} C temperature and highest at 15^{o} C. The high amount of phosphate leakage at 15^{o} C is to be expected since the culture was the most active at this temperature resulting in the release of more algal cells containing stored phosphate. An increase in phosphate leakage at 35^{o} C would tend to indicate lysing of the cells. Visual observations at this temperature gave indications that many of the algal cells had lost their chlorophyll content.

Figure 9 also indicates that there was very little variation in solids concentration and COD removal from either the 15, 21, 30, or 35^{o} C

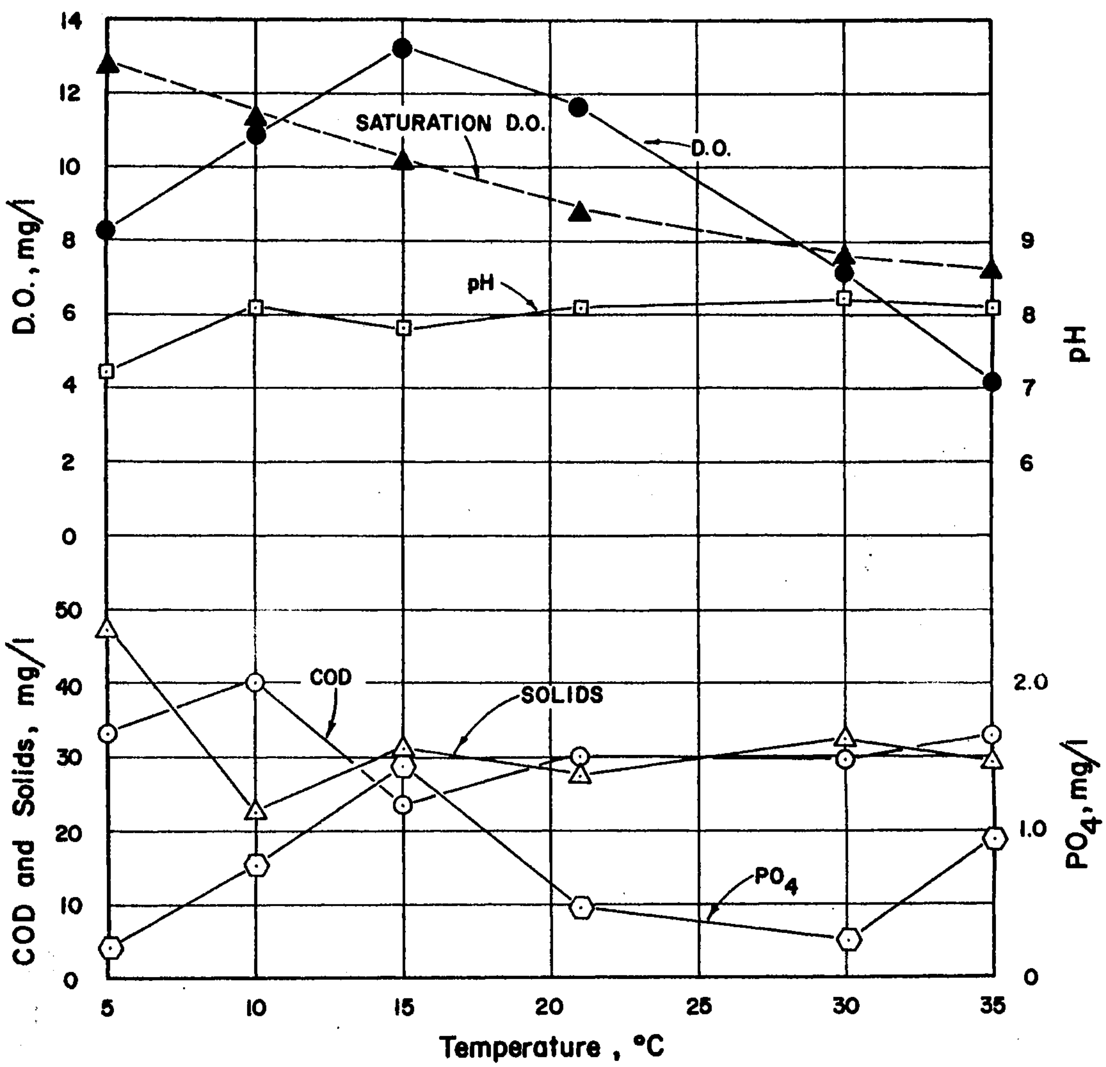

Figure 9. Response of the System to Various Temperatures for the Light and Dark Cycles.

experiments. Such was not the case with the dissolved oxygen as it indicated an increase from 8.2 mg/l at 5^{o} C to a high of 13.3 mg/l at 15^{o} C and then gradually decreased to an average of 4.1 at 35^{o} C. As an interesting comparison the saturation value for dissolved oxygen was plotted as a smooth curve at each of the temperatures investigated. Only at the 15 and 21^{o} C temperatures did the average oxygen production exceed the saturation values during the combined light and dark cycles.

Figures 10 and 11 give an indication as to what effect the twelve-hour dark cycles had on the response of the pond to temperature differences.

In comparing the light cycle with that in the dark, the effect of temperature is seen to have its greatest impact on the DO concentration, which is a function of the light itself. The light cycle produced saturated DO levels at all temperatures except at 5^{o} C, while during the dark cycles the DO decreased below saturation levels.

An interesting point can be made by comparing the DO deficits at each temperature. From Figure 11 it can be seen that the DO was higher at the 10^{o} C temperature than at any of the other temperatures, but if the comparison is based on the actual deficits then the DO production was actually greater at the 15^{o} C temperature. The deficit actually decreased from 5^{o} C to 15^{o} C, where it was at a minimum and then rapidly increased for the 21, 30, and 35^{o} C experiments. An explanation lies in the fact that more DO was produced at the 15^{o} C temperature, thus making more DO available for bacterial respiration which would result in less deficit.

The data in Figures 10 and 11 point out more completely that when the effluent contains a high concentration of solids it will in general

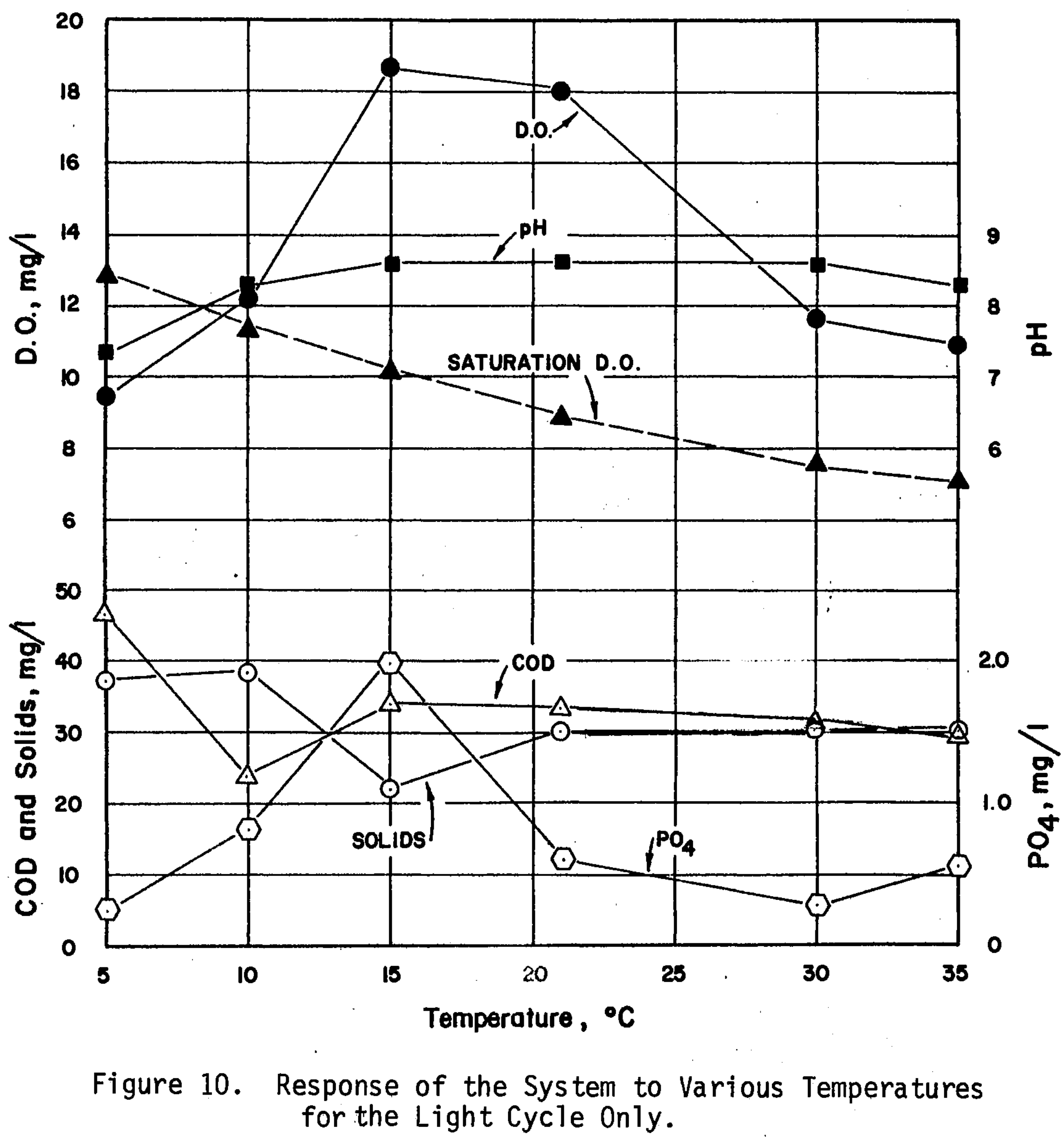

Figure 10. Response of the System to Various Temperatures for the Light Cycle Only.

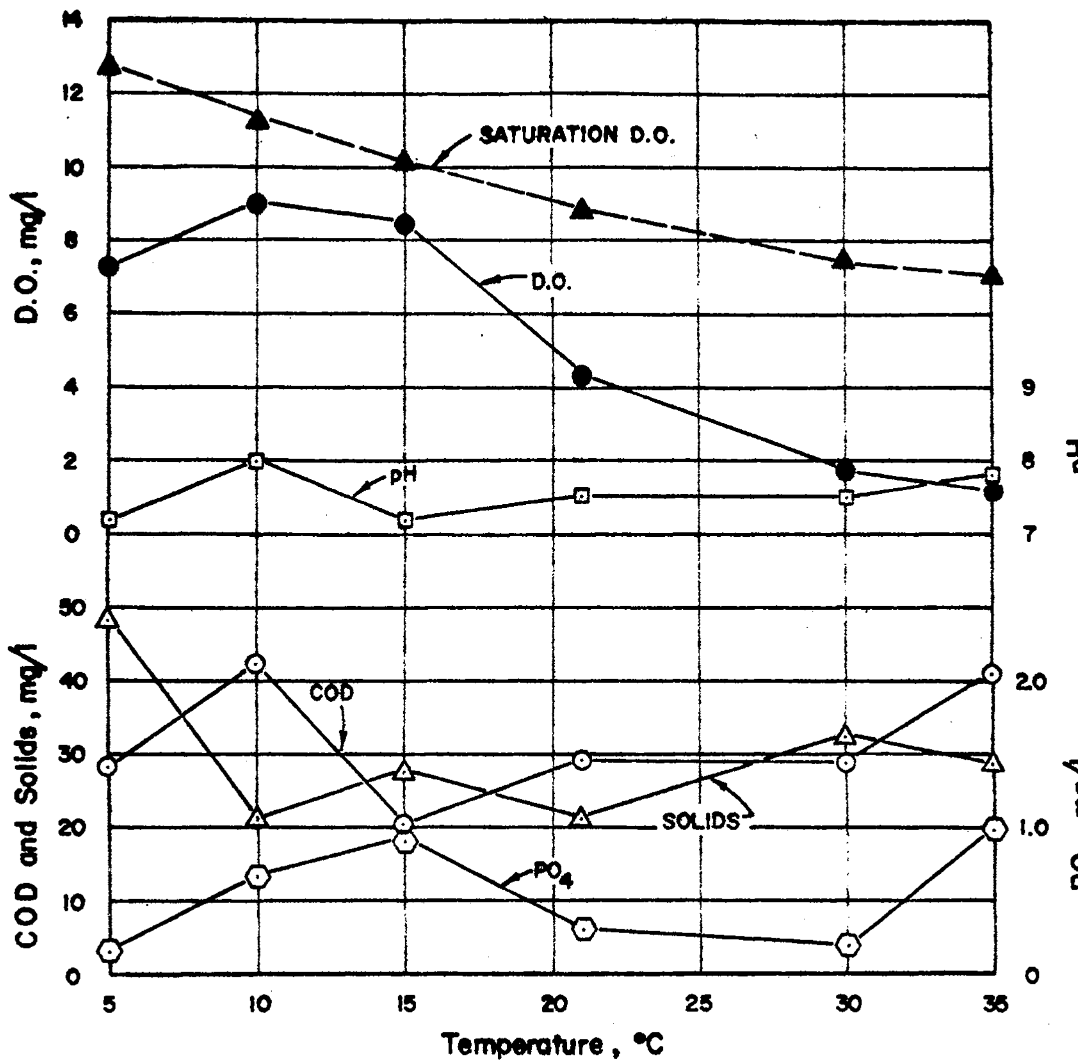

Figure 11. Response of the System to Various Temperatures for the Dark Cycle Only.

have greater concentrations of phosphates and soluble COD. This is not necessarily true for any one particular temperature but does hold true considering the differences in the parameter concentrations between the dark and light cycles.

Figure 12 is a plot of the DO difference that occurred at each temperature between the light and dark cycles. All of the data previously discussed indicated that of the six temperatures investigated the 5, 10, 30, and 35^{o} C temperatures produced the least amount of pond activity and that at 15 and 21^{o} C the pond showed the greatest activity. Figure 12 stresses these results further by showing that the greatest difference in the effluent DO occurred at 21^{o} C, which would in essence mean that more DO was produced. Another interesting point is that from all other indications the 15^{o} C temperature had appeared to be the most active (Figure 9), but from the plot in Figure 12 it is seen that the 21^{o} C temperature produced on the average far greater concentrations of DO in the effluent above that required for respiration.

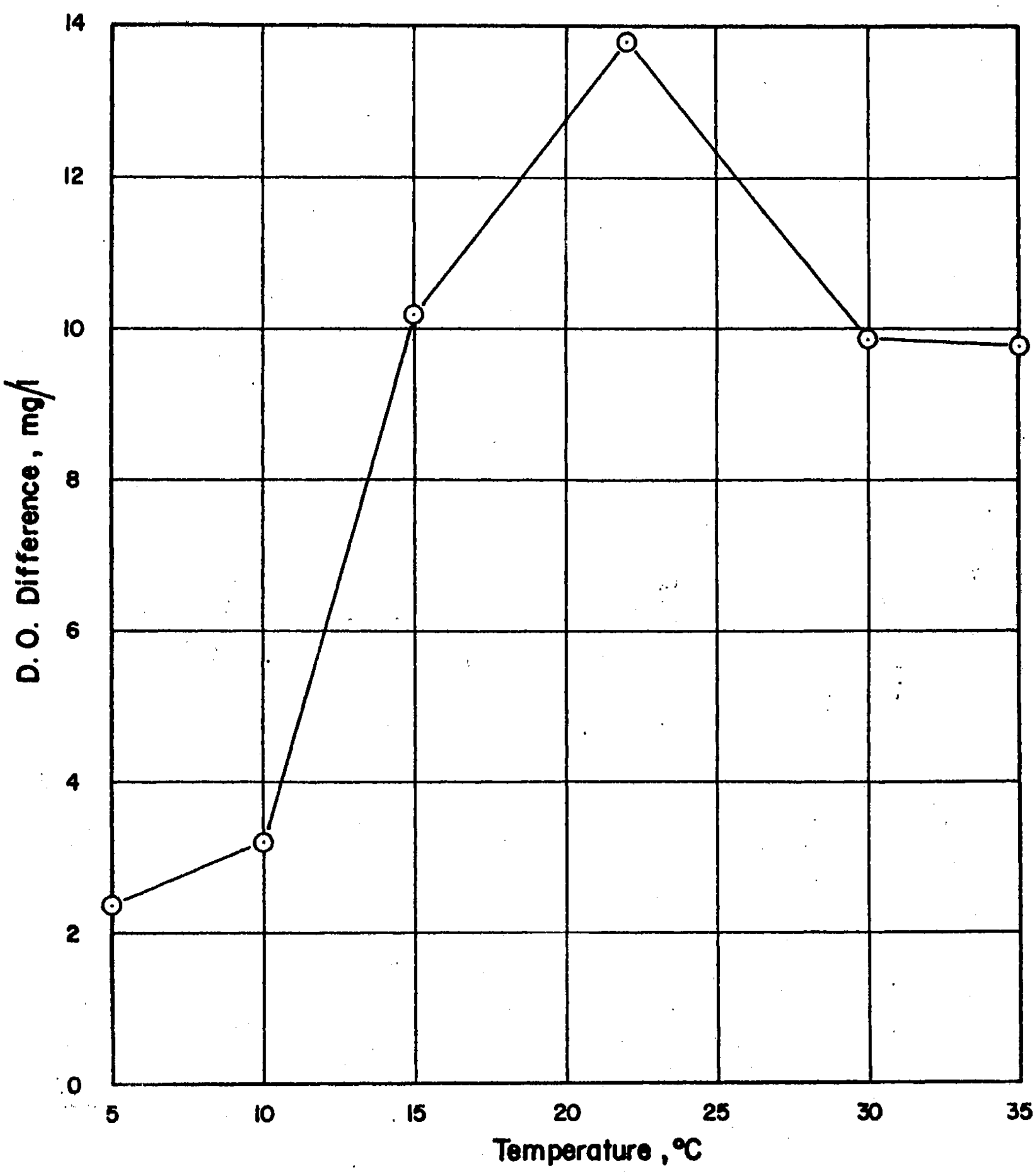

Figure 12. Relationship of the Dissolved Oxygen Difference Between the Light and Dark Cycles with the Various Temperatures.

CHAPTER V

DISCUSSION OF THE RESULTS

A. pH

The effects of pH on the results of this study were minimal. Most waste stabilization ponds are hampered by extremely high pH values which by the algal extraction of free CO_2 from the medium can raise the pH to values of 10-11 (39). It appears from the pH data summarized in Figure 9 that the inclusion of sodium bicarbonate in the feed solution was effective as a buffering system. The buffering system's effectiveness increased as the temperature increased, since the disassociation rate of the bicarbonate ion is increased as the temperature rises, which would in effect result in maintaining a certain amount of free CO_2 in the medium.

Substantiation of the bicarbonate suppression of high pH can be further emphasized from some preliminary work that was done prior to the actual experiments. The concentrations of all constituents in the synthetic waste were the same as those given in Chapter III, with the exception of glucose being 18 g/l (35 lbs BOD/acre/day) and the sodium bicarbonate was eliminated from the solution. Temperatures of 24 and 28^{o} C were used and the results yielded consistently high pH values (9-10.5). The pond grew well for a short period of time at both temperatures. After about three days of operation the algal population began to take on a yellow color indicating a loss of chlorophyll and quantities of slime-like material floated to the pond surface. The effluent COD showed a gradual increase and the DO in the effluent went practically to zero during the twelve-hour dark period. Initial thoughts were that the algal cells were being bleached by the high temperatures because similar results had been reported by Wolken (44). From the results shown in Figures 7 and 8 for the 30 and 35^{o} C temperatures, the DO never reached zero following the dark periods and the soluble COD did not increase at the 35^{o} C temperature but did increase slightly at the 30^{o} C temperature. Visual observations during these experiments did not reveal any apparent loss of algal chlorophyll. It would appear that the pH has more effect on algal chlorophyll synthesis than does temperature in an unbuffered system.

Wu (55), Solook (56), and Natarajan (6) concluded from their work with laboratory waste stabilization ponds that a concentration of 100 mg/l of sodium bicarbonate gave a greater algal cell yield. They also used a phosphate buffer system and experiments were performed at room temperature. No attempts were made to explain the bicarbonate effect on pH.

B. Nutrient Leakage

From the results of this study there appears to be no distinct relationship between increased or decreased phosphate leakage and the other parameters at any of the temperatures investigated. If the phosphate leakage is observed only on terms of the temperature and the initial phosphate concentration (1.68 mg/l) then it could be said that there was increased leakage from 5^{o} C to a maximum concentration at 15^{o} C which then decreases with increasing temperature up to 30^{o} C.

Above 30^{o} C the effluent concentration of phosphate again starts to increase. These trends of PO_4 leakage agree to some extent with those of Jewell and McCarty (43). They reported that at 20^{o} C the species Scenedesmus released 12 per cent of its phosphorus. At 25^{o} C this increased to 71 per cent, and at 35^{o} C 88 per cent of the phosphorus was released. At 4^{o} C they observed phosphorus releases often equal to zero. The main discrepancy lies at the higher temperatures between 20 and 35^{o} C where a decreasing rather than an increasing trend was observed.

The work by Azad (48) indicated little or no PO_4 leakage at 15^{o} C and a small detectable amount at 30^{o} C. He was studying pure cultures of algae and starving them of phosphorus, which might account for his results' varying from those already discussed.

Actual field data analyzed by Clare (49) and Assenzo (29) support the findings of this study, that maximum PO_4 leakage occurred between 5 and 20^{o} C.

C. DO Concentration

Temperature effects on oxygen production in a waste stabilization pond would be more or less a function of the total microbial activity within the pond and the level of dissolved oxygen DO saturation (magnitude of the DO deficit) at any particular temperature. From the data, as summarized in Figure 9, no trouble was encountered in maintaining DO levels above those of saturation at either the 15 or 21^{o} C temperatures. The data also serve to point out that as the temperature is increased or decreased on either side of the 15 to 21 degree range, the ability of the algae to produce quantities of DO up to and above DO saturation becomes more difficult. Then it can be seen that temperature extremes can have seasonal effects on pond operation. This seasonal effect would be greatest during the warmer months of the year when a pond is most active, resulting in the deposition of greater quantities of biological solids into the receiving stream. As long as it was light these algal cells would continue to produce favorable amounts of DO, but at night the DO could go to zero, creating anaerobic conditions. This problem becomes even more critical in sluggish streams, streams with high turbidity, or in those on which reservoirs have been developed.

Other evidence to support the effects of temperature extremes on a pond's effluent quality can be gained by a study of Figure 11. As compared to the 5, 10, and 15 degree temperatures the 21, 30, and 35 degree temperature experiments show DO concentrations below 5 mg/l. This concentration is below the minimum DO set by many regulatory agencies as a standard to be maintained in receiving waters.

D. Substrate Removal

From the experimental data it appears that a temperature of 15° C was optimum for substrate removal. Above 15° C the concentration of soluble COD in the effluent varied only slightly between those temperatures investigated as shown in Figure 9. Below 15° C the overall microbial activity within the pond was less; therefore, more of the applied organic substrate remained in solution. A point in question is the origin of the detected effluent COD, as to whether or not it was the original substrate or metabolic intermediates; this is a question which cannot be answered from the results of this study.

When considering the results of this experimental work as to the COD parameter it must be recognized that the COD of the total biomass (algae plus bacteria cells) was not considered. Had the total COD been considered there would have been increases in the effluent COD corresponding to the effluent solids concentration. Therefore, in terms of optimum temperature producing the most desirable effluent, the 15 degree might well prove to be the most undesirable. Tschortner (58) studied two ponds, one under normal loading conditions and the other under overloaded conditions, to determine what effect their effluents had on the receiving stream. His conclusions were that neither the solids content, nor the total or filtered COD alone, can give a reliable indication of the load in the ponds or the reduction of COD in the effluents. He further determined that 1 mg/l of dried algae was equivalent to 0.2 mg/l of COD. If for reasons of comparison the values for equivalent COD were applied to the data in Figure 9 for the 15^{o} C temperature, the total COD of the effluent would be approximately 30 mg/l, and for the 5^{o} C temperature the total COD would be 49 mg/l.

Rich (57) reported that, based on the BOD parameter, waste stabilization pond efficiencies may reach as high as 90 per cent, but due to the high algal content of the effluent, the COD parameter as a measure of efficiency may indicate only 50-60 per cent removal of organics.

E. Pond Activity

The effects of temperature on pond activity are best evaluated by the dissolved oxygen parameter. Effluent DO increases and decreases respectively between the light and dark periods, as shown in Figure 12, give a true picture of the overall microbial responses. The mechanisms of waste stabilization ponds are such that large amounts of DO are produced during the light hours. Oxygen production during the light hours is usually over and above that required for the aerobic bacteria. During the absence of light the DO is lost via respiration of the microbial population and the atmosphere. It would stand to reason that if the microbial population were less in number or inhibited by temperature, smaller amounts of DO would be available for respiration and anaerobic conditions could exist. The difference in the average DO production and respiration would readily indicate the status of the microbial population.

Pond activity has been postulated to follow the Q_{10} relationship and is normally estimated to have a value of 2, meaning that for every 10^{o} C rise in temperature the reaction rates within a pond double. The data from this study do not support this estimate. Although semi-continuous studies would be necessary for accurate evaluation of the Q_{10} relationship, it can be seen from Figure 9 as well as the tabulated summary in the appendix that the activity or reaction rates in this study do not depict a doubling rate for each 10^{o} rise in temperature.

CHAPTER VI

CONCLUSIONS

(1) In general phosphate leakage from waste stabilization ponds is greater at temperatures between 5° C and 15° C and above 30° C.

(2) No predictable trends for phosphate leakage were observed under the conditions of this study that could be reliably correlated with the other parameters investigated.

(3) At the temperatures which gave higher algal yields, the soluble (filtrate) COD in the effluent also showed marked increases.

(4) Sodium bicarbonate, at a concentration of 100 mg/l, is effective as a buffer against the high pH levels often attained during the light hours in waste stabilization ponds.

(5) pH is a critical factor in controlling the suppression of chlorophyll synthesis at high temperatures in unbuffered systems.

(6) Temperatures below 15° C and above 30° C have an inhibiting effect on the microbial population in waste stabilization ponds as measured by the dissolved oxygen parameter.

(7) The dissolved oxygen difference between the light and dark hours is the best indicator of pond activity, as influenced by temperature, when compared with the other parameters investigated.

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDY

From the results of this study, the following suggestions are made for future study on the effects of temperature on waste stabilization ponds.

(1) The effects of temperature on the performance of a laboratory scale waste stabilization pond should be studied under the conditions of various organic and hydraulic loadings, water depths, light intensities, and light duration periods.

(2) The response of a waste stabilization pond to other synthetic wastes, subject to varying temperatures, should be studied.

(3) Studies should be made to determine what effect temperature has on algal species predomination.

SELECTED BIBLIOGRAPHY

(1) Canter, Larry W., Englande, Andrew J., Jr., and Mauldin, Alfred F., Jr., "Loading Rates on Waste Stabilization Ponds." Jour. of the Sanitary Engineering Division, American Society of Civil Engineering, SA6, 95, 12, 1117-1129 (1969).

(2) Sawyer, C. N., "Biological Treatment of Sewage and Industrial Wastes." In Biological Treatment of Sewage and Industrial Wastes, 1, Ed. by McCabe and Eckenfelder, New York, Reinhold (1956).

(3) Gloyna, E. F., "Basis for Waste Stabilization Pond Designs." In "Advances in Water Quality Improvement." Water Resources Symposium No. 1, University of Texas Press, Austin, Tex. (1968).

(4) ________, Standard Methods for the Examination of Water and Wastewater, 12th Edition, APHA, AWWA, WPCF, New York (1965).

(5) Fiske, C. H., and Subbarow, Y., Jour. of Biological Chemistry, 81, 629-679 (1929).

(6) Natarajan, O. V., "Studies on Factors Affecting Dissolved Oxygen Concentrations in Aerobic Oxidation Ponds." Unpublished Ph.D. Thesis, Oklahoma State University, Stillwater, Okla. (1969).

(7) Yeoh, Joseph, "An Evaluation of the Oxidation Pond as a Waste Treatment Process." Unpublished Master's Report, Oklahoma State University, Stillwater, Okla. (1965).

(8) Fitzgerald, G. P., and Rohlich, G. A., "An Evaluation of Stabilization Pond Literature." Sewage and Industrial Wastes, 30, 10, 1213-1224 (1958).

(9) Porges, Ralph, "Industrial Waste Stabilization Ponds in the United States." Jour. Water Pollution Control Federation, 35, 4, 456-468 (1963).

(10) Oswald, W. J., "Quality Management by Engineered Ponds." In Engineering Management of Water Quality, Ed. by McGauhey, New York, McGraw-Hill, 210 (1968).

(11) Oswald, W. J., and Gotaas, H. B., "Photosynthesis in Sewage Treatment." Proceedings of the American Society of Civil Engineers, 81, 686, 1-25 (May, 1955).

(12) Lange, Willy, "Effect of Carbohydrates on the Symbiotic Growth of Planktonic Blue-Green Algae with Bacteria." Nature, 215, 1277 (September 16, 1967).

(13) Ludwig, H. F., Oswald, W. J., Gotaas, H. B., and Lynch, Victoria, "Algae Symbiosis in Oxidation Ponds I. Growth Characteristics of Euglena Gracilis Cultured in Sewage." Sewage and Industrial Waste, 23, 11, 1337-1355 (1951).

(14) El-Baroudi, H. M., and Moawad, S. K., "Rate of BOD Reduction by Oxidation Ponds." Jour. Water Pollution Control Federation, 39, 10, 1626-1652 (1967).

(15) Pipes, Wesley O., "Basic Biology of Stabilization Ponds." Water and Sewage Works, 108, 4, 131-136 (1961).

(16) Danforth, W. F., "Substrate Assimilation and Heterotrophy." In Physiology and Biochemistry of Algae, Ed. by Lewin, New York, Academic Press, 101 (1962).

(17) Varma, Man M., and Wilcomb, Maxwell, J., "Effect of Light Intensity on Photosynthesis." Water and Sewage Works, 110, 12, 426-429 (1963).

(18) Bartsch, A. F., and Allum, M. D., "Biological Factors in Treatment of Raw Sewage in Artificial Ponds." Limnology and Oceanography, 2, 77-84 (1957).

(19) Lubbers, Ralph H., and Parikh, Dilip N., "The Effect of Algal Concentration, Luminous Intensity, Temperature, and Diurnal Cycle or Periodicity upon Growth of Mixed Algal Cultures from Waste Stabilization Lagoons as Determined on the Warburg Apparatus." Proceedings of the 21st Purdue Industrial Waste Conference, Purdue University, Ext. Ser. 121, 348-367 (1966).

(20) Oswald, W. J., "Light Conversion Efficiency of Algae Growth in Sewage." Jour. Sanitary Engineering Division, American Society of Civil Engineers, 86, SA4, 71-95 (July 1960).

(21) Rohde, W., "Environmental Requirements of Fresh-Water Plankton Algae." Symb. Botan. Upsal. X,I Uppsala, 123 (1948).

(22) Kuentzel, L. E., "Bacteria, Carbon Dioxide and Algal Blooms." Jour. Water Pollution Control Federation, 41, 10, 1737-1747 (1969).

(23) ________, "General Features of Algal Growth in Sewage Oxidation Ponds." State Water Poll. Cont. Bd., Sacramento, Calif., Pub. No. 13.

(24) Meron, A., Rebhun, M., and Sless, B., "Quality Changes as a Function of Detention Time in Wastewater Stabilization Ponds." Jour. Water Pollution Control Federation, 37, 12, 1657-1670 (1965).

(25) Pipes, Wesley O., "pH Variation and BOD Removal in Stabilization Ponds." Jour. Water Pollution Control Federation, 34, 11, 1140-1150 (1962).

(26) Thimann, Kenneth V., The Life of the Bacteria, Second Edition, New York, Macmillan Company, 167 (1963).

(27) Keefer, C. E., and Meisel, Joseph, "Activated Sludge Studies III. Effect of pH of Sewage on the Activated Sludge Process." Sewage and Industrial Wastes, 23, 8, 982-991 (1951).

(28) Ferguson, F. Alan, "A Nonmyopic Approach to the Problem of Excess Algal Growths." Environmental Science and Technology, 2, 3, 188-195 (1968).

(29) Assenzo, Joseph R., and Reid, George W., "The Removal of Nitrogen and Phosphorus by Bio-oxidation Ponds in Central Oklahoma." National Symposium on Sanitary Engineering Research, Development and Design, Pennsylvania State University, University Park, Penn. (July 27-30, 1965).

(30) Dryden, F. D., and Stern, Gerald, "Renovated Wastewater Creates Recreational Lake." Environmental Science and Technology, 2, 4, 268-278 (1968).

(31) Neds, Charles, and Varma, Man M., "The Removal of Phosphate by Algae in River Water." Water and Sewage Works, 113, 456-460 (December, 1966).

(32) Karanik, J. M., and Nemerow, N. L., "Removal of Algal Nutrients from Domestic Wastewaters." Water and Sewage Works, 112, 460-463 (December, 1965).

(33) Kuhl, Adolf, "Inorganic Phosphorous Uptake and Metabolism." In Physiology and Biochemistry of Algae, Ed. by Lewin, Ralph A., New York, Academic Press, 211 (1962).

(34) Azad, H. S., and Borchardt, Jack A., "Phosphorous Uptake by P-Starved Algae." Proceedings of the 23rd Purdue Industrial Waste Conference, Purdue University, Ext. Ser. 132, 325-342 (May, 1968).

(35) Neel, Joe K., and Hopkins, Glen J., "Experimental Lagooning of Raw Sewage." Jour. Water Pollution Control Federation, 58, 11, 1326-1356 (1956).

(36) Bogan, R. H., Albertson, D. E., and Pluntze, J. C., "Use of Algae in Removing Phosphorous from Sewage." Jour. of Sanitary Engineering Division, American Society of Civil Engineers, 86, SA5 (September, 1960).

(37) Herman, E. R., and Gloyna, E. F., "Waste Stabilization Ponds I. Experimental Investigations." Sewage and Industrial Wastes, 30, 4, 511-538 (1958).

(38) Phelps, Earle B., Stream Sanitation, New York, John Wiley and Sons, Inc., 72 (1944).

(39) Sawyer, C. N., and McCarty, P. L., Chemistry for Sanitary Engineers, Second Edition, New York, McGraw-Hill, 204 (1967).

(40) Herman, E. R., and Gloyna, E. F., "Waste Stabilization Ponds III. Formulation of Design Equations." Sewage and Industrial Wastes, 30, 8, 963-975 (1958).

(41) Shih, Chia Shun, and Stack, Vernon T., Jr., "Temperature Effects on Energy Oxygen Requirements in Biological Oxidation." Jour. Water Pollution Control Federation, 41, 11, R461-R474 (1969).

(42) Kehrberger, G. J., Norman, J. D., Schroeder, E. D., and Busch, A. W., "BOD Progression in Soluble Substrates VII Temperature Effects." Proceedings of the 20th Purdue Industrial Waste Conference, Purdue University, Ext. Ser. 117, (1965).

(43) Jewell, W. J., and McCarty, P. L., "Aerobic Decomposition of Algae and Nutrient Regeneration." Report to the Federal Water Pollution Control Administration, Civil Engineering Department, Stanford University, Technical Report No. 91 (1968).

(44) Wolken, Jerome J., Euglena, Second Edition, New York, Appleton-Century-Crofts (1967).

(45) Marre, Erasmo, "Temperature." In Physiology and Biochemistry of Algae, Ed. by Lewin, R. A., New York, Academic Press, 541 (1962).

(46) Brock, Thomas D., Principles of Microbial Ecology, Englewood Cliffs, New Jersey, Prentice-Hall (1966).

(47) Varma, Man M., and Digiano, Francis, "Kinetics of Oxygen Uptake by Dead Algae." Jour. Water Pollution Control Federation, 40, 4, 613-626 (1968).

(48) Azad, H. S., "Phosphorous Uptake by Green Algae Under Different Environmental and Physiological Conditions." Doctoral Dissertation, University of Michigan, Ann Arbor, Mich. (1968).

(49) Clare, H. C., Neel, Joe K., and Monday, C. A., Jr., "Studies of Raw Sewage Lagoons at Fayette, Missouri." Proceedings of Symposium on Waste Stabilization Lagoons, Kansas City, Missouri, 19-40 (1960).

(50) Carpenter, W. L., Vamvakias, J. G., and Gellman, I., "Temperature Relationships in Aerobic Treatment and Disposal of Pulp and Paper Mill Wastes." Jour. Water Pollution Control Federation, 40, 5, 733-740 (1968).

(51) Oswald, W. J., Gotaas, H. B., Golueke, C. G., and Kellen, W. R., "Algae in Waste Treatment." Sewage and Industrial Wastes, 29, 4, 437-457 (1957).

(52) Parker, C. D., Jones, H. L., and Taylor, W. S., "Purification of Sewage in Lagoons." Sewage and Industrial Wastes, 22, 6, 760-775 (1950).

(53) Steffen, A. J., "Stabilization Ponds for Meat Packing Wastes." Jour. Water Pollution Control Federation, 35, 4, 440-444 (1963).

(54) Oswald, W. J., and Gloueke, C. G., "Biological Transformation of Solar Energy." Advances in Applied Microbiology, 2, 223-262 (1960).

(55) Wu, Y. C., "Effects of Organic Loading on Reaeration in Semi-Quiescent Waters." Unpublished Master's Thesis, Oklahoma State University, Stillwater, Okla. (1967).

(56) Solook, John T., "Light and Dark Microbial Response in Semi-Quiescent Waters." Unpublished Master's Thesis, Oklahoma State University, Stillwater, Okla. (1968).

(57) Rich, Linvil G., Unit Processes of Sanitary Engineering, New York, John Wiley and Sons, 69 (1963).

(58) Tschortner, U. S., "Biological Parameters for the Operation and Control of Oxidation Ponds II." Water Research, 2, 327-346 (1968).

(59) Myers, J., "Culture Conditions and the Development of the Photosynthetic Mechanism. IV. Influence of Light Intensity on Cellular Characteristics of Chlorella." Jour. General Physiology, 29, 429 (1946).

APPENDIX

AVERAGE VALUES FOR ALL PARAMETERS FOLLOWING THE LIGHT-DARK CYCLES

Temp. °C	DO, mg/l			pH			PO_4, mg/l			Solids, mg/l			COD, mg/l		
	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.
5	8.3	12.0	4.6	7.2	7.4	7.0	0.21	0.68	0	47.6	67.0	31.6	33.6	90.0	15.0
10	10.9	14.2	8.1	8.1	9.2	7.2	0.78	2.30	0	22.9	49.0	9.2	40.1	47.1	25.4
15	13.3	19.6	6.3	7.8	8.9	7.0	1.45	3.50	0	31.2	43.0	20.8	23.5	37.8	11.3
21	11.7	22.2	3.6	8.1	7.0	7.3	0.48	2.80	0	27.7	62.0	8.6	30.0	41.4	24.1
30	7.2	13.3	1.1	8.2	9.1	7.3	0.25	0.80	0	32.1	47.8	5.8	29.7	42.3	15.6
35	4.2	9.6	0.6	8.1	8.7	7.3	0.95	4.00	0	29.5	70.0	18.4	33.0	52.6	18.8

AVERAGE VALUES FOR ALL PARAMETERS FOLLOWING THE LIGHT CYCLES

Temp. °C	DO, mg/l			pH			PO_4, mg/l			Solids, mg/l			COD, mg/l		
	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.
5	9.4	12.0	6.0	7.3	7.4	7.2	0.25	0.68	0	46.9	66.4	31.6	37.2	90.0	22.6
10	12.2	14.2	11.1	8.2	9.2	7.2	0.84	1.68	0	24.0	49.0	9.2	38.7	47.1	25.4
15	18.7	23.6	16.1	8.6	8.9	8.2	2.01	3.50	1.43	34.3	43.0	27.0	22.2	26.5	15.1
21	18.1	22.2	15.9	8.6	9.0	7.8	0.62	2.76	0.71	33.5	62.0	14.8	30.4	41.4	24.1
30	11.6	13.3	9.3	8.6	9.1	8.1	0.28	0.83	0	31.8	47.8	5.8	30.3	42.3	19.5
35	10.9	9.6	4.0	8.3	8.7	8.1	0.64	2.70	0.42	29.5	42.2	18.4	30.1	41.4	18.8

AVERAGE VALUES FOR ALL PARAMETERS FOLLOWING THE DARK CYCLES

Temp. °C	DO, mg/l			pH			PO_4, mg/l			Solids, mg/l			COD, mg/l		
	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.
5	7.2	9.2	4.6	7.2	7.3	7.0	0.18	0.41	0	48.5	67.0	33.4	28.9	79.5	15.0
10	9.0	10.4	8.1	8.0	8.9	8.1	0.69	2.30	0	21.2	32.4	11.0	42.3	47.1	39.9
15	8.5	12.0	6.3	7.2	7.7	7.0	0.95	2.17	0	28.0	36.2	20.8	20.8	22.7	15.1
21	4.3	5.5	3.6	7.5	7.7	7.3	0.32	1.84	0	21.7	32.0	8.6	29.5	41.4	24.1
30	1.7	2.6	1.1	7.5	7.8	7.3	0.22	0.48	0	32.5	40.8	24.2	29.0	39.0	15.6
35	1.2	1.6	0.6	7.8	8.0	7.5	1.02	0.42	0	29.0	70.0	18.8	41.2	70.0	18.8

VITA

Charles Henry Glaus

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF TEMPERATURE ON THE RESPONSE OF A LABORATORY WASTE STABILIZATION POND

Major Field: Bioenvironmental Engineering

Biographical:

Personal Data: Born September 24, 1943, in Nashville, Tennessee, the son of Andy and Melissa Glaus.

Education: Graduated from Cumberland Senior High School, Nashville, Tennessee, in 1961; received the Degree of Bachelor of Science in Civil Engineering from Tennessee Technological University, Cookeville, Tennessee, in June 1967; received the Master of Science Degree from Oklahoma State University, Stillwater, Oklahoma, in May 1971.

Professional Experience: Sanitary Engineer with the Tennessee Department of Public Health, Division of Sanitary Engineering, Nashville, Tennessee, June 1967 - August 1970.

Membership in Professional Societies: American Society of Civil Engineers, American Water Works Association, Water Pollution Control Federation, Tennessee Public Health Association, Chi Epsilon.