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Studies on the Ecological Impact of Evaporation Retardation Monolayers

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By

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STUDIES ON THE ECOLOGICAL IMPACT OF EVAPORATION RETARDATION MONOLAYERS

A Dissertation

By

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LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I

INTRODUCTION

Clean fresh water is the most precious natural resource available to mankind. People must have water for personal, municipal, industrial and recreational use. At the present time, most of the available fresh water in the United States is used in some way or another, treated and returned to streams, rivers, lakes and reservoirs for reuse. Terrain, geographic location, climate and economics dictate that most of our usable fresh water be retained in lakes and reservoirs. This type of water storage allows for the greatest loss of water by evaporation.

The increasing demand for municipal, industrial and recreational fresh water has set in motion a vast impoundment program in the United States that will accelerate water evaporation control measures in the immediate future. According to Smerdon,[1] water loss by evaporation in the United States actually exceeds by over 10 times the total amount of water needed for municipal and industrial usage. In the United States alone, five billion acre-feet of water falls as precipitation each year. Of this amount over 3.5 billion gallons of water is returned to the atmosphere by evaporation or transpiration.

Americans are now consuming 355 billion gallons of water per day and this amount is expected to increase to 600 billion gallons per day by 1980.[2] Home use of water represents less than 10% of the national consumption. Nearly half of the water is used for irrigation and the remaining 40% is used by industry.

Water conservation is a necessity in arid states that have scant rainfall and high evaporation losses. Eaton [3] reported that approximately 11.5 million acre-feet of water is lost due to evaporation each year in our eleven western states.

Scientists and engineers have considered many physical and chemical methods in an attempt to reduce water evaporation losses from lakes and reservoirs. One of the new and most promising techniques is the application of a thin chemical film on the surface of the water to retard evaporation.

An array of evaporation reduction chemicals has been utilized on reservoirs and lakes in different manners by Mansfield,[4] Cruse and Harbeck,[5] Timblin, Florey and Garstka,[6] and Meinke and Waldrip [7] to suppress evaporation and conserve water. One of the most promising of the current evaporation retardant chemicals is a blend of hexadecanol and octadecanol (Durham and McArthur).[8] These long-chain fatty alcohols form a monomolecular film on the water surface that is self-healing at wind speeds of up to eight miles per hour (Gilby and Heymann) [9] and is capable of reducing water evaporation by 30 to 50% under ideal conditions. According to Ludzack and Ettinger, [10] and Chang, et al. [11] the monolayer is biodegradable and can be assimilated by bacteria in the water as food.

According to Wiltzius, [12] hexadecanol and octadecanol are non-toxic and do not present a health hazard in potable water. However, research indicates that monolayers change some of the physical and

chemical characteristics in the treated aquatic environments. A monolayer will calm the water surface and form a slight diffusion barrier to the transfer of gases into and out of the water environment. The film will also decrease the surface tension of the water surface by 50% or more from a normal 60 to 72 dynes per centimeter to less than 40 dynes per centimeter. Furthermore, the film causes a slight temperature increase in the water immediately below the film. All of these factors may significantly affect the ecology of ponds, lakes and reservoirs.

While field studies have shown hexadecanol and octadecanol films to be successful in suppressing water evaporation, the ecological studies of such treated water have not been adequate. A comparative evaluation of the biologic effects due to complete coverage of water by an evaporation retardant monolayer has not been possible under field conditions. The day-to-day environmental conditions of rapid temperature changes, wind, dust, rain, light fluctuations and other unpredictable factors do not allow a realistic evaluation of the ecological changes that may be caused by a continuous water-saving film.

The small laboratory ecosystem has long been a fundamental tool in the development of comparative ecology. These systems have also been called microcosms by Odum and Hoskins [13] and laboratory microecosystems by Beyers.[14] These small ecosystems may be used to study changes in water quality and population characteristics under controlled conditions obtained only in the laboratory. With the microcosm, one

does not experience the complexity, environmental variation, difficulty of replication, and handicap of sheer size presented by natural ecosystems. However, unnatural environmental conditions must be recognized when small laboratory ecosystems are used. Laboratory studies in experimental microcosms can not duplicate the complex ecosystem present in lakes and reservoirs.

An intensive literature survey has revealed no prior attempt to evaluate the ecological impact of a continuously applied evaporation reduction film on a laboratory experimental microcosm.

The objectives of this research have been to evaluate, under laboratory controlled conditions, the ecological changes caused by the continuous application of a hexadecanol and octadecanol evaporation-suppression film on experimental ecosystems. The effects of a monolayer on algal populations will provide information not currently available.

CHAPTER II

HISTORY AND REVIEW OF LITERATURE ON WATER EVAPORATION SUPPRESSION

Throughout recorded history man has utilized various types of oils to form a film on the surface of rivers, lakes and oceans for many different reasons. The ancient Greeks and Romans spread oil upon the surface of the Mediterranean Sea, allowed enemy vessels to sail into the coated water, and set the oil aflame to function as a deadly effective weapon of war. Sailors have often applied Sperm Whale Oil on storm driven surf to slick and calm the waters temporarily to allow their ships to ease through a treacherous reef opening to safety.

According to Davies and Rideal, [15] Benjamin Franklin experimented with oil on water as early as 1765. Franklin estimated that the oil film would spread into a layer approximately the thickness of the oil particles. During service as an American statesman in England, Franklin heard many stories from ship captains concerning the various world-wide uses of oils. One story was concerned with the natives of Bermuda who used oil to calm the ripples on the water surface in order to allow better spearfishing. In 1773 Franklin used this particular information when he applied a film of oil on Derwent Lake, England, to calm waves and astonish unbelieving friends.

Early experimental work led to the recognition of the existence of monomolecular films. Agnes Pockels [16] discovered the formation of

monomolecular surface films from spreading oils. She was able to vary the oil covered area by confining the film between movable barriers placed across a shallow water filled tray. Lord Rayleigh [17] followed Miss Pockel's work and also concluded that oil spreads to form a film one molecule in thickness. Devaux [18] studied not only the oil film on water but was concerned with the limit of film expansion. He found that oil would extend to a single layer of molecules at maximum extension and disappear if one tried to stretch the film thinner. Devaux also advocated using an inert powder on the treated water surface to render the film visible and show the monolayer's spreading ability.

According to Abbe, [19] Onofrio made the first use of oils to prevent fog formation and retard evaporation by applying a monolayer of oil to inland rivers and lakes in France.

Early water evaporation retardation studies started during the 1920's using different oils as a monolayer film agent. Sir William Hardy [20, 21] suggested that monolayers were formed from polar molecules consisting of hydrophobic and hydrophillic parts. He proposed that the hydrophillic carboxyl, or alcohol parts were buried in the water surface. The hydrophobic parts consisting of paraffinic chains would then be pointing away from the water and oriented toward the air in a gaseous phase.

Irving Langmuir [22] followed up Hardy's work and furnished conclusive support for the molecular water-air orientation hypothesis.

He also found that pure hydrocarbon oils (without the functional groups) did not spread on water but formed lenses. With these findings water evaporation retardation with monomolecular films appeared practical and research studies were initiated.

Water evaporation retardation experiments by Devaux [23] using oil mixtures and Hedestrand [24] using palmitic acid were disappointing. The theory was correct, but experimental procedures introduced a thick layer of air into the oil film. The air layer affected the diffusional resistance of the monolayer by not allowing proper paraffin chain orientation, therefore, the monolayer did not retard water evaporation.

Rideal [25] improved experimental techniques by using an inverted U-tube apparatus to show positive evaporation reduction by a fatty acid monolayer. In his experiments one arm of the inverted U-tube contained water at room temperature. The other arm contained water which was cooled by an ice bath. The air was then evacuated from the system and a comparison of the condensation rates in the cold arm was made both with and without a surface film of fatty acid on the water in the warm arm. From this comparison it was found that a monolayer of fatty alcohol could reduce evaporation rates by 50%.

Langmuir and Langmuir [26] extended Rideal's experimental procedure and studied monolayers of different substances. They found that cetyl alcohol was superior to oleic, stearic, and palmitic acids in respect to water evaporation reduction effects. Instead of measuring the rate of evaporation in grams per square centimeter per second, they used the reciprocal of this quantity. They termed the reciprocal

as R for evaporation resistance and expressed it in square-centimeter-second per gram. Using this measurement the thickness of the film through which diffusion must occur could be calculated by the equation $R = h/CD$ where h = thickness of the film, D = diffusion coefficient, and C = concentration of the diffusing substance when equilibrium was attained.

In 1932, Irving Langmuir [27] received the Nobel Prize for demonstrating that the various paraffinic chains of acids and alcohols all conform to the same limiting area when monolayers of the saturated series are compressed. This area occupied by a molecule on the water surface was found to be constant and characteristic of the paraffinic chain. It was also determined that these paraffinic chains formed a film thickness on the order of 21 Å per molecular unit.

Monolayer evaporation retardation studies were made in Russia by Baranaev, [28] Sklyarenko and Baranaev, [29] and Kheinman.[30] Evaporation studies in the United States were pursued by Docking, et al.[31] All of these investigators reported the superiority of cetyl alcohol (hexadecanol) $C_{16}H_{33}OH$ and stearyl alcohol (octadecanol) $C_{18}H_{37}OH$ over other tested filming substances.

Further laboratory studies of different evaporation control chemicals by Sebba and Briscoe [32] and Langmuir and Schaefer [33] demonstrated that hexadecanol was one of the most efficient chemicals for reducing water evaporation.

Despite successful laboratory results using monomolecular films, emphasis on chemical evaporation methods changed and increased

attention was given to the use of multimolecular films of oil mixtures. During the 1940's Heymann and Yoffe [34, 35] reported that multimolecular paraffin films 5 microns thick might reduce water evaporation up to 15%. However, field tests of multimolecular films were not successful according to Docking, et al.[31] and Heymann and Yoffe.[35] Wind, rain, and dust damaged the many-layered films in field use. Once the films were broken they would not heal or re-form. Gilby and Heymann [9] also found that duplex films more than 10 microns in thickness decreased water evaporation rates with increased wind speed. However, the film was not persistent and was very difficult to maintain.

When Mansfield [36] showed that monomolecular films were self-healing and more durable under normal field conditions, the evaporation reduction studies again shifted back to single-layered films. In one of the first successful field applications of a monomolecular film of hexadecanol, Mansfield [4] was able to reduce the water evaporation loss from an Australian reservoir by more than approximately 30%.

Archer and LaMer [37, 38] found that liquid monolayers of long-chain fatty acids gave good resistance to evaporation and were independent of film pressure over a wide range of atmospheric pressure.

At the Southwest Research Institute, Dressler, [39, 40] Dressler and Johnson, [41] and Freese [42] carried out research on evaporation reduction monolayers. The U. S. Bureau of Reclamation, [43, 44, 45, 46] Harbeck,[47] and Harbeck and Koberg [48] of the U. S. Geological Survey also studied the effects of evaporation suppression films on reservoirs.

According to Timblin, et al., [49] the need for a departmental

study of evaporation suppression monolayers was realized by interested U. S. Government agencies. The U. S. Bureau of Reclamation and the U. S. Public Health Service therefore joined forces with other agencies to study evaporation suppression on Kids Lake, Oklahoma, in 1956. From this combined study the decision was reached to film Lake Hefner, Oklahoma, in an attempt to check large evaporation losses.[50]

Rosano and LaMer [51] continued prior work by Archer and LaMer [38] and found that, in general, compressible films were poor evaporation retardants. Films with high resistance to lateral compression were found to be the most effective evaporation retardants.

Mansfield [52] used pure hexadecanol and demonstrated that the long-chain alcohols were capable of forming efficient self-healing monolayers on reservoirs. He also mixed some octadecanol with hexadecanol to study the performance of monolayers of mixed alcohols,[53] but concluded that pure hexadecanol offered the best resistance to water evaporation.

Contrary to Mansfield's finding, McArthur and Durham [54] demonstrated that a commercial blend of hexadecanol and octadecanol exhibited greater evaporation resistance than pure hexadecanol. Durham and McArthur [8] also found that the efficiency of the hexadecanol and octadecanol film to reduce water evaporation increased with chemical dosage up to a maximum value, after which a constant film efficiency was maintained.

In 1960, Magin and Randall [55] compiled and reviewed the available literature on evaporation suppression for the U. S. Geological Survey.

They indicated that the long-chain waxy alcohols were the best evaporation retardant materials at that time.

The influence of chemical dose rate on film performance was studied by Hellstrom and Janson, [56] and Genet and Rohmer.[57] Their work supported prior research on dose rates and pointed out that exceeding the maximum effective chemical application did not increase the film strength and was uneconomical.

Meinke, et al.[58, 59] performed research with evaporation retardation films in Texas. They used "Aquasave," a commercial mixture of equal parts of hexadecanol and octadecanol, which produced a good persistent monolayer on natural fresh water.

A symposium edited by LaMer [60] in 1962 presented papers concerned with the application of monolayers for water conservation and the problems of gaseous transport through such evaporation retarding films. The papers considered the properties of various monolayers, the physical processes that caused evaporation retardation, and the interrelationship between such processes.

Vines [61] treated large water reservoirs in Australia with finely ground cetyl alcohol. His spreading technique was useful for the rapid formation of an effective evaporation reduction monolayer over large water surface areas. Mansfield [62] continued his research on evaporation suppression methods and was concerned with the film spreading problems encountered in using solid hexadecanol.

Evaporation reduction investigations on small reservoirs in the United States were carried out in 1963 by Resnick and Cluff. [63]

In India, similar studies were performed by Shukla, et al.[64]

Meinke and Waldrip [7] summarized recent evaporation retardation research in Texas through 1962. The main emphasis was on economics, different types of film material and application, and the need for continuing research.

During 1964, Katti, et al.[65] and Deo, et al.[66] studied the use of long-chain alcohols to retard water evaporation in India. The ability of monolayers to reduce water evaporation was also studied in Russia by Trapeznikov, et al.[67, 68]

Dressler [69] continued water evaporation studies in the United States and developed an improved suspension process for film application on reservoirs. In 1965, LaMer, Healy and Aylmore [70] reported on improved monolayers that would last for a longer duration of time.

Many different methods of applying water evaporation suppressant films have been studied. Differing from the solid or solvent method, Myers [71] suggested applying the film material in a water soluble base material. According to Florey, et al.,[72] a long-chain alcohol film applied as a powder sprayed from a Robertson grinder-duster resulted in good water savings during the Sahuaro Lake Study. According to Mihara,[73] the emulsion application has become the standard method in Japan for dispersing selected films. Even aerial application of an evaporation-retardant monolayer was reported by Newkirk, [74, 75] but many problems were encountered necessitating additional tests to refine this technique.

Frenkiel [76] reviewed current literature dealing with the chemical and physical means of reducing water evaporation. In a report presented to UNESCO, he supported previous studies and recommended that evaporation-retardant films be applied continuously for maximum water savings. He also pointed out that further research for improved techniques and new ideas was needed.

Discrepancies Appearing in Literature Concerning Evaporation Reduction Monolayers

Evaporimeter experiments. Timblin, Florey and Garstka [6] have cited the use of class A evaporimeter pans as a reliable, straightforward, and simple means to determine the ability of a monolayer to reduce evaporation under limited field conditions. However, LaMer [60] opposed this view, based on his observations that a moving current of air over class A pans in the open influences the rate of evaporation in such studies. However, the main issue was the effect of impurities and the change in lateral film pressure needed for an effective monolayer. Therefore, screening tests for effectiveness of chemical retardants should be carried out in laboratories instead of evaporator pans in the open.

Large scale experiments. According to Frenkiel [76] the Lake Hefner investigations have been cited as the most comprehensive and carefully documented study on water evaporation reduction up to the present time. The results reported from this field test suggested that an evaporation-reducing monolayer of hexadecanol could be applied

with no apparent toxic effects or interference with normal recreational uses of a treated lake. The filming agent used in the Lake Hefner study was reported to be a commercially available high quality hexadecanol. Based on the results from the Lake Hefner study, Successful Farming [77] and other agricultural papers reported that hexadecanol had no adverse biological effects when used for evaporation suppression.

Hayes [78] described the Lake Hefner study as being "poorly controlled and badly misinterpreted." He questioned the existence of an effective monolayer since most biological and chemical changes noted during the tests were dismissed as being unimportant. Furthermore, LaMer [60] pointed out that the disappointing results obtained with the commercial evaporation retardant used in the Lake Hefner study might be blamed on the quality of the filming material. He presented the data obtained by Dr. G. T. Barnes, the Columbia University Laboratories, which show that the commercial alcohol employed in the Lake Hefner study exhibited no resistance to evaporation until a lateral compression of 32 dynes/cm was reached. This is a value seldom found in the field. Therefore, the behavior of the retardant was much below that of a pure hexadecanol which was supposed to be present. This unsatisfactory commercial filming agent was cited by LaMer as at least one of the valid reasons why only a 9% water evaporation reduction was obtained in the Lake Hefner study.

Biological effects. Mansfield [4] noted the importance of biological considerations in the use of evaporation suppressant films. He later reversed his opinion and implied that monolayers would have

no noticeable effect on "marine life," but he presented no supporting data indicating what kind of "marine life" was tested.

In Timblin's [79] work the initial toxicity experiments with hexadecanol were intended to be only perliminary in nature and were reported as such. These tests were simple and not intended to be definitive. Unfortunately the results have been misquoted and presented by many writers as "biological facts." Timblin's toxicity experiment based on two domestic white ducks, one control and one treated duck, might have detected some toxic effects of hexadecanol. However, the control duck died five days prior to the completion of the two month experiment and the test was terminated early. This somewhat inconclusive experiment using two domestic ducks should not warrant the conclusion by Eaton,[3] Dressler,[41] Western Water News [80] and numerous newspaper articles that hexadecanol has no adverse effect on "wild fowl."

In the same report, the toxicity effects of hexadecanol on algae and aquatic plants were evaluated. Conclusions were based on visual appearance and apparent new plant growth in 55 gallon drums under greenhouse conditions. Algae were not identified and quantitative data or biological changes were not presented.

CHAPTER III

MATERIALS AND METHODS

The ecological effects of a continuous monolayer of hexadecanol and octadecanol on laboratory aquatic ecosystems were studied in the Environmental Engineering Laboratory at Texas A&M University. Three consecutive thirty-day tests were run during the period May-September 1966. Chemical, physical and biological analyses were made on untreated and treated ecosystems. General limnological methods followed the procedures given by Welch [81] and Lagler.[82] Algae were identified following the keys in Ward and Whipple,[83] Needham and Needham,[84] and Palmer.[85]

Preparation of Laboratory Experimental Ecosystems

The following three types of experimental ecosystems were used:

1. Two 20-gallon glass aquaria with a surface area (air-water interface) of 2.08 square feet (11.3" X 26.5"). Radiant energy was supplied by vertically adjustable banks of Sylvania "Gro-Lux" fluorescent lamps. Air and water temperature were identical. Water and algae collected in the field at the beginning of each experiment were innoculated into aerated tap water in both tanks at the ratio of one part mixed innoculum to twenty parts aerated tap water. One 20-gallon aquarium was filmed with a monolayer of "Aquasave" and the other untreated 20-gallon glass aquarium served as a control. Figure 1 shows the arrangement of the two 20-gallon glass aquaria and the

fluorescent lamps in the Environmental Engineering Laboratory.

2. Eighteen 1-gallon wide mouth glass jars with a surface area (air-water interface) of 0.19 square feet for each jar. Radiant energy was supplied by vertically adjustable banks of Sylvania "Gro-Lux" fluorescent lamps. The jars were immersed to the jar neck in a large lucite water bath to maintain identical air and water temperature. Aerated tap water in all of the jars was innoculated with water and algae collected in the field at the beginning of each experiment. Nine of the jars were filmed with "Aquasave" and the other nine untreated jars served as controls. Thus, eighteen 1-gallon jars were used for the first two of the three experimental series.

3. A large rectangular transparent lucite tank which was divided into two 20-gallon areas for the third series of experiments. Each side of the lucite aquarium has a surface area (air-water interface) of 3.55 square feet (16" X 32"). Vertically adjustable banks of Sylvania "Gro-Lux" fluorescent lamps supplied radiant energy. Aerated tap water in both tanks was innoculated with water and algae collected in the field at the beginning of the third test series. One side of the lucite tank was filmed with "Aquasave" while the other side served as a control. Figure 2 shows the arrangement of the untreated and treated experimental plastic aquaria and the fluorescent lamps in the laboratory.

Air and microcosm water temperature were maintained at a constant $22^{o} \pm 2^{o}$ C. A large thermostatically controlled refrigerated air conditioner held the laboratory temperature at the temperature

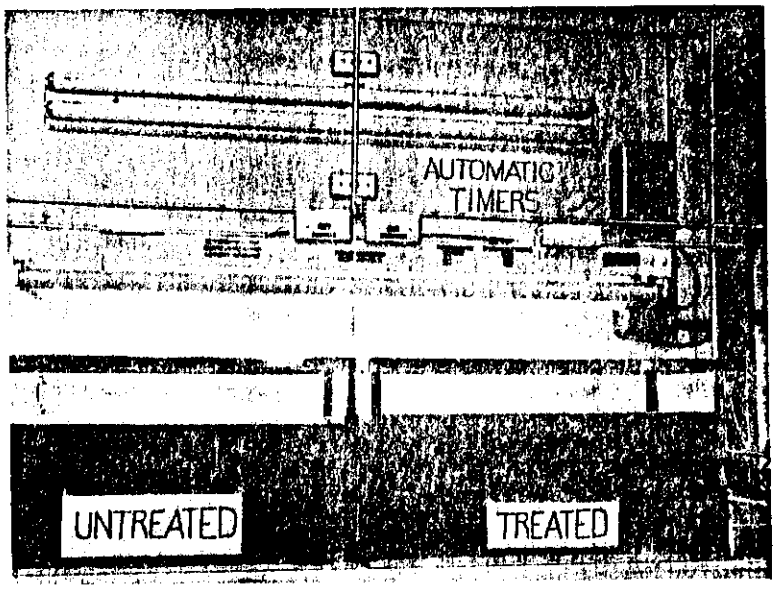


FIGURE 1. UNTREATED AND TREATED EXPERIMENTAL GLASS AQUARIA.

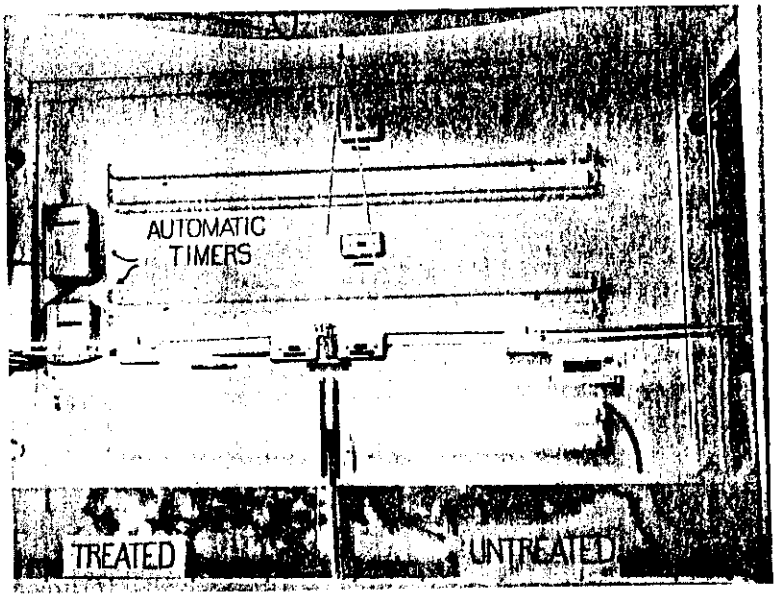


FIGURE 2. UNTREATED AND TREATED EXPERIMENTAL PLASTIC AQUARIA.

suggested by Timblin, et al.[5] to maintain maximum hexadecanol and octadecanol film efficiency.

Radiant energy intensity at the water surface was adjusted to 5,000 microwatts per square centimeter (approximately 520 foot-candles).

The "Gro-Lux" light banks were automatically controlled by electronic timers to give 12-hour photoperiods (8 AM to 8 PM).

Monolayer Materials and Methods

Application and dosage. All treated aquatic systems were filmed daily with "Aquasave," a 1:1 mixture of hexadecanol and octadecanol manufactured by Arista Industries, New York. The "Aquasave" was first applied as a solution in isopropanol for the first two series of tests. Isopropanol is widely used for a film spreading agent but it is infinitely soluble in water and will not evaporate from the water surface like hexane or other solvents. Since isopropanol is soluble, some carbon would be added to the system and be reflected as part of the five day BOD. Five day BOD tests showed that if the isopropanol carrier exceeded 3 milligrams per liter concentration it could exert an oxygen demand on the treated system. This limit was not exceeded by the isopropanol solvent during any of the tests. The "Aquasave" was applied in liquid emulsion form for the third series of tests. No difference between the solvent and liquid emulsion form was noted in physical, chemical or biological effects.

During all three series of tests, a continuous monolayer was maintained for each 30-day period at the dose rate equivalent to 0.05 pounds of "Aquasave" per day for each acre of treated water surface. The dose rate used was based on the work by Meinke and Waldrip [6] for maximum film coverage in the absence of wind. They also found that the surface film pressure attained with "Aquasave" was around 40 dynes per centimeter.

Test for film coverage. Complete film coverage of the treated microcosms in this study was determined with the "talc test" developed by Timblin.[86] In this test powdered "Aquasave" and mineral talc were mixed in a 1:1 portion. A small amount of the mixture was applied to the water surface for observation. Absence of a surface film resulted in a rapid spreading of the mixture. However, if the water surface was covered with a monolayer whose film pressure was near 40 dynes per centimeter (the spreading pressure of "Aquasave") the material would not spread but float on the surface where it was applied.

The surface film was replenished every 24 hours to replace losses due to physical and biological breakdown. This resulted in a continuous monolayer throughout each thirty-day experiment. No attempt was made to evaluate the evaporation control qualities of the "Aquasave."

Experimental Procedure

All experimental ecosystems were innoculated at the beginning of each thirty-day experiment with mixed algal and water samples collected from three local ponds located in Brazos County. Innoculation was at

the ratio of one part mixed innoculum to twenty parts aerated tap water. The three ponds sampled were: (a) Fish Lake, located south of Easterwood Airport, (b) "reactor pond" (location name only since pond does not receive any reactor effluent), located east of the entrance gate to the Texas A&M University nuclear reactor, and (c) Bryan Municipal Lake, in Bryan, Texas. Algae and water samples were collected by containers, dip nets and plankton nets from near-shore and off-shore locations. These mixed samples provided the laboratory microcosms with approximately the same initial algal populations as those present in the sampled ponds during the summer months when an "Aquasave" monolayer would normally be applied to prevent excessive water evaporation losses.

Mats of the filamentous algae Cladophora and Chara were collected by dip net from the three sampled ponds, identified and cut into equal diameter plugs with a laboratory cork boring apparatus. Four grams each of Cladophora and Chara were added to all of the experimental systems at the beginning of all three series of experiments. Additionally, during the third test, four grams of Anabaena were added to all systems at the beginning of the test period.

The aquatic weed Anacharis (Elodea) was collected from the three sampled ponds. The plant was identified following the key of Eyles.[93] Twenty grams of Anacharis were added to each 20-gallon microcosm at the beginning of each experiment.

Two local species of fish, Gambusia affinis (Baird and Girard) and Fundulus notatus (Rafinesque) were collected with dip nets from

the three sampled ponds. Identification followed the key in Moore.[94] Six G. affinis (mosquitofish) and six F. notatus (blackstripe topminnow) were introduced into each 20-gallon system by random sampling. All fish were held in the laboratory for three days prior to introduction into the microcosms. Additionally, the fish were allowed three days in the microcosms prior to starting the tests. In no case were the same fish used for more than one experiment.

Chemical Methods

Most of the chemical analyses in this study were conducted using the methods and techniques outlined in Standard Methods for the Examination of Water and Wastewater.[87] The following chemical tests were made:

a. Hydrogen ion concentration. The hydrogen ion concentration was measured with a Sargent Model DR research pH meter.

b. Hardness. Water hardness was determined by the EDTA titrametric method and expressed as mg/L of $CaCO_3$.

c. Carbonate and bicarbonate alkalinity. Phenolphthalein alkalinity was determined by titration with .025 N H_2SO_4 to the phenolphthalein end point. Total alkalinity was determined by the mixed bromcresol green-methyl red indicator method by titration with .025 N H_2SO_4 to the proper equivalence point. Carbonate and bicarbonate alkalinity were then calculated by means of the stoichiometric classification of the three principal forms of alkalinity present. In this study, however, hydroxide alkalinity was not present during any of the tests.

d. Turbidity. Turbidity was determined with a Jackson candle turbidimeter for turbidity measurements above 25 mg/L. Turbidity measurements in the 5 to 25 mg/L range were made with a Hellige turbidimeter.

e. Oxygen diffusion rate studies. Determinations of the oxygen diffusion rate through the "Aquasave" monolayer were made with a Gilson Differential Respirometer following the manometric techniques outlined by Umbreit.[88] A 50 ml sample of mixed water and algae was drawn from the untreated microcosm. The cells were centrifuged at 1,000 rpm for 3 minutes and then resuspended in 50 ml of aerated tap water. This was repeated until a total of six water and algal samples had been placed in the 125 ml reaction flasks.

The "direct method" of Warburg was used to absorb the CO_2 continuously. Folded "KOH papers" were inserted into alkali placed in the center well of the reaction flask. The center well was greased at the top to prevent alkali creep. Twenty percent KOH was used to provide sufficient CO_2 uptake.

Three of the flasks containing algae were filmed with "Aquasave" at the dose rate of 0.05 pounds per acre of water surface to compare with an equal number of untreated samples. Each reaction flask containing 50 ml of liquid had a surface area of four square inches. The flasks were immersed in the Gilson water bath at 20°C without shaking. The light bank in the bottom of the respirometer was left on for 60 minutes during the first test. Release of oxygen was measured in micro-liters of oxygen pressure increase. During the second test, the

flasks were not exposed to light. Reduction in oxygen pressure due to algal respiration was measured in micro-liters of oxygen. Measurements were made every five minutes for one hour.

The mean of the three untreated samples was compared with the mean of the three treated samples by plotting each as a single point in order to observe the difference in oxygen pressure between the untreated and treated samples.

f. Dissolved oxygen. The amount of dissolved oxygen in the microcosms was determined by the Winkler-azide method modified for microsamples by Rabinovich and Sherman.[89] This microtechnique makes use of a 10-ml syringe to withdraw the water sample. This permits accurate analyses of a small sample of water. Reagents were injected into the water sample in the larger syringe with 1-ml syringes. The microtechnique was calibrated against a standard 300-ml Winkler-azide oxygen analysis of laboratory water once a week.

g. Ohle test. Ohle's [90] test was used to determine if any oxidizing or reducing impurities were present that might interfer with the standard Winkler-azide reactions.

h. Diurnal oxygen measurements and primary productivity. During all three series of experiments, the diurnal oxygen techniques of Odum [13] were used to study the community productivity and metabolism of the untreated and "Aquasave" treated aquaria. Four diurnal oxygen studies were conducted during each of the three experimental series. Dissolved oxygen in the untreated and treated microcosms was determined every two hours for a 24-hour period using the microsample

oxygen technique.

A percent oxygen saturation curve was plotted from the dissolved oxygen values. When the curve was above the 100% line, oxygen was diffusing into the atmosphere; when the curve was below 100%, oxygen was diffusing into the water from the atmosphere.

The diffusion rate was adjusted with a correction factor of 1.01 as given by Lagler [82] for oxygen saturation at the College Station altitude (367 feet). The rate of change, in milligrams of oxygen per liter per hour, was calculated for the 24-hour period. The 24-hour total productivity was recorded as gain or loss of oxygen in mg/L.

Physical Factors

Temperature. Air and microcosm water temperature were checked with laboratory and immersion thermometers.

Light. Radiant energy intensity at the water surface was measured with an ISCO (Instrumentation Specialities Company, Lincoln, Nebraska) Model-SR Spectro Radiometer in microwatts per square centimeter. According to Mpelkas,[91] light energy measured in microwatts per square centimeter is a more realistic measurement of energy received by green plants in the red and blue bands of the visible spectrum.

Biological Factors

Bacteria. Water samples from the untreated and treated systems

were innoculated into Difco Plate Count Agar (Standard Methods agar) in sterile petri dishes. The petri plates were incubated at 35° ± 0.5°C. for 24 ± 2 hours. The number of bacterial colonies were then determined using a Quebec colony counter. All counts were recorded as bacterial colonies per ml of water according to Standard Methods.[87] Bacteria were not identified and only numbers of bacterial colonies per ml of water were determined to compare differences between untreated and treated microcosms.

Nonfilamentous algae. One hundred-ml water samples from the untreated and treated systems were filtered through 0.45 micron hard surfaced millipore filters. The filtered water was retained and used for chemical tests without interference from the algal cells. The algal cells were washed from the filter with deionized water sprayed from a polyethelene wash bottle. The millipore filters were back-washed by inverting and flushing with deionized water to check on the effectiveness of the squeeze bottle washing. The algal cells were then centrifuged at 2,000 rpm for five minutes to break the cell clotting caused by excessive bacterial growth. The concentrate was transferred to a small test tube and brought to 1-ml volume with deionized water. This gave a concentration of 100 to 1 which could be further diluted for study when the nonfilamentous algae were found to be too numerous to count under the microscope.

A Spencer hemacytometer was used for counting algae. Individual sub-samples from the algal sample concentrate were placed in the hemacytometer chamber for counting. This was repeated until a total

of four sub-samples had been counted. The four counts were then averaged and recorded.

The Spencer hemacytometer has a counting grid consisting of 9 square millimeters in a three by three square. The central square millimeter is ruled into 25 groups of 16 small squares, with each group of 16 bordered by a triple ruled line. The corner square millimeters are each ruled into 16 squares.

Counts of the larger algae were made by counting all the cells in the four corner 1 millimeter squares and in the central millimeter (5 square millimeters total) using a 10X objective and 10X eye piece.

The smaller algae in the concentrate were counted under the 43X objective by counting the cells in five out of the 25 groups of 16 small squares from the central square millimeter. The five groups counted were the four corner groups and one group in the center. Counts were averaged and calculated as total algae per milliliter of water. Colonies were counted for colonial forms, and single cells for the rest.

Filamentous algae. At the conclusion of each 30-day experiment all of the filamentous Cladophora and Chara were harvested, washed to remove excessive bacteria and reweighed. The filamentous algal growth was also examined microscopically throughout each 30-day study and photomicrographs taken to note unusual growth characteristics.

Chlorophyll analysis. Following the procedure in Barnes [92] the chlorophyll content method was used as an index to compare primary productivity in untreated and treated microcosms. Chlorophylls A, B,

and C were determined by taking a 50-ml sample from the untreated and treated systems and filtering it through a 0.45 micron millipore filter. The millipore filter with the chlorophyll was then placed in a centrifuge tube. Magnesium carbonate (0.1 gm) was added to prevent acidity followed by 5-ml of 90% acetone. The tube was shaken well and allowed to stand in the dark for 18 hours. At the end of this time the tube with the filter pad still in place was centrifuged for three minutes and the liquid decanted into a spectrophotometer cell. The pigments were measured for absorbancy and transmittance at 665, 645 and 630 millimicron wave lengths in a Hitachi Model EPS-3T Spectrophotometer for the first two experimental series. A Beckman Model DB Spectrophotometer was used for the third series. Chlorophyll values were then calculated from the spectrophotometer readings.

Aquatic plants. At the conclusion of each thirty-day test, all of the Anacharis (Elodea) was harvested and washed to determine the amount of growth.

Fish. The activity and mortality of the Gambusia affinis and Fundulus notatus in the untreated and treated experimental ecosystems were observed and recorded.

CHAPTER IV

RESULTS AND CONCLUSIONS

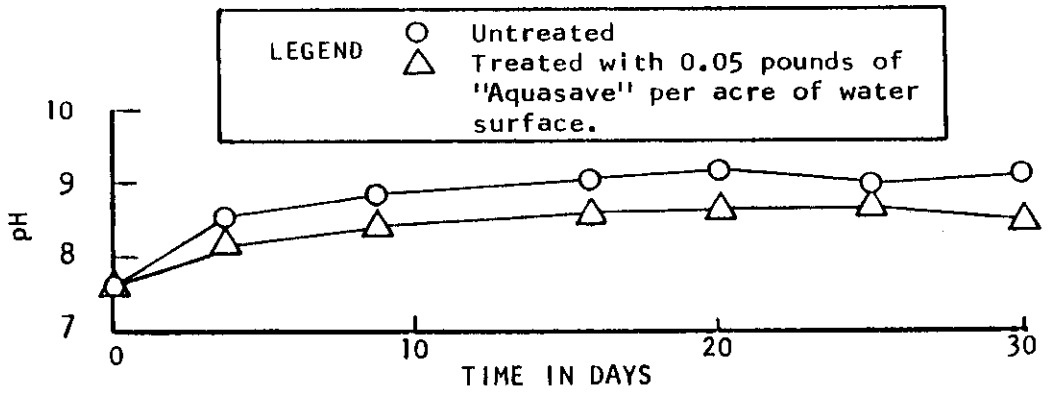
Water Chemistry

Hydrogen ion concentration. The pH concentration in the "Aquasave" treated systems, generally, was lower than the pH in the untreated systems. Figure 3 compares the hydrogen ion concentration in the untreated and treated aquatic systems for the three separate series of thirty-day experiments. In all three experiments the hydrogen ion concentration at the beginning of the experiment was influenced by the mixed innoculum of water and algae samples from the three lakes in Brazos County. The pH measurements used for the comparison in Figure 3 were taken at 1600 hours each day.

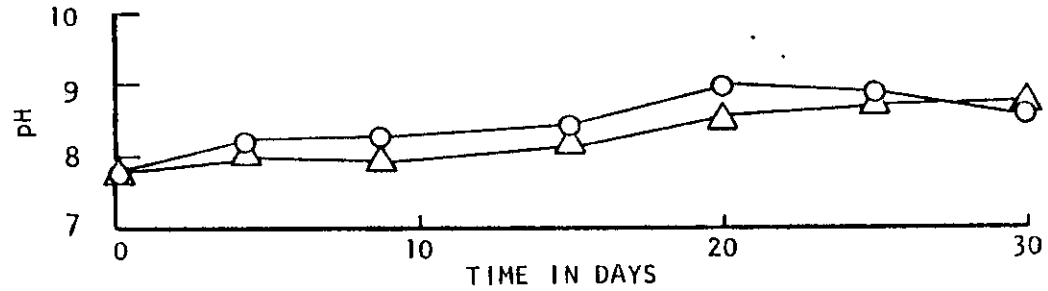
During the first experimental period, the hydrogen ion concentration in the treated microcosms varied from 0.1 to 0.4 below that found in the untreated microcosms. At no time during the first thirty-day period did the pH in the treated system exceed the pH in the untreated system.

In the second thirty-day test the hydrogen ion concentration in the treated microcosm remained slightly lower than that found in the untreated system until the 27th day of the test. At this time the pH in the treated system was slightly above the pH in the untreated system.

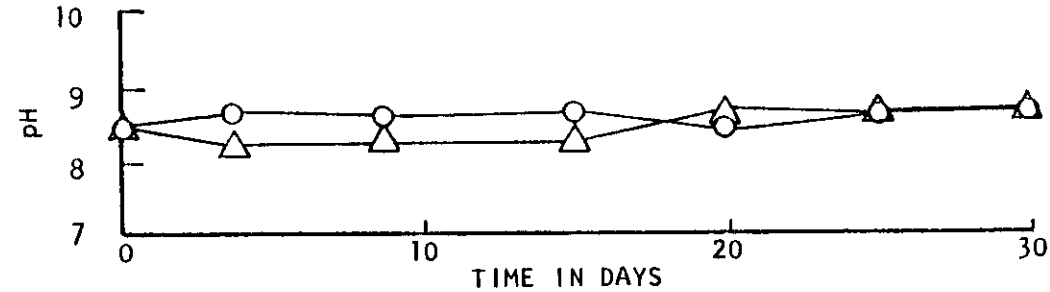
During the third experiment the pH in the treated systems was lower until the 18th day, when a reversal of values occurred between



EXPERIMENTAL SERIES A. 26 MAY TO 24 JUNE 1966.



EXPERIMENTAL SERIES B. 20 JULY TO 18 AUGUST 1966.



EXPERIMENTAL SERIES C. 24 AUGUST TO 22 SEPTEMBER 1966.

FIGURE 3. A COMPARISON OF THE HYDROGEN ION CONCENTRATION IN UNTREATED AND TREATED AQUATIC MICROCOSMS FOR THREE SEPARATE SERIES OF EXPERIMENTS. ALL DETERMINATIONS MADE AT 1600 HOURS.

the untreated and treated water. However, the pH in both the untreated and treated systems remained the same from the 25th day until the conclusion of the test.

It would appear from the data gathered during these experiments that a continuous film of "Aquasave" would not detrimentally affect the hydrogen ion concentration of fresh water within the basic pH range of seven to ten.

Hardness. Variations in water hardness were attributed to innoculum difference and algal or aquatic plant growth in both untreated and treated microcosms. As shown in Table I, a considerable amount of fluctuation occurred in both untreated and treated systems. Palmer [85] found that good algal growths were able to reduce the $CaCO_3$ in hard water by as much as one-third. He attributed this to photosynthetic or respiration activity of the algae that would respectively increase or decrease pH and water hardness. He believed the removal of the CO_2 from the water by the algae during photosynthesis caused an alteration in the relative amounts of soluble carbonic acid, bicarbonates and monocarbonates.

The aerated tap water used in this study contained 8-10 mg/L of $CaCO_3$ and therefore is classified as very "soft" water. Therefore, it would seem that the "soft" water in the experimental ecosystems would not be subjected to the decrease in hardness caused in "hard" water by good algal growths.

The higher beginning hardness values shown in series B of Table I were caused by the mixed water and algal innoculum collected from

TABLE I

CHEMICAL ANALYSES OF WATER FROM UNTREATED AND TREATED EXPERIMENTAL ECOSYSTEMS DURING THE TIME INDICATED. ALL DATA GIVEN AS MILLIGRAMS PER LITER.

LEGEND

o Untreated

Δ Treated with 0.05 pounds of "Aquasave" per acre of water

Test Series	Time in Days	Hardness as $CaCO_3$		Carbonate Alkalinity		Bicarbonate Alkalinity	
		o	Δ	o	Δ	o	Δ
"A" 26 May to 24 June 1966.	1	11	11	16	16	218	218
	6	12	16	52	24	210	224
	12	12	18	52	26	210	208
	18	12	16	60	40	226	238
	24	10	13	54	62	233	244
	30	9	12	40	76	266	262
"B" 20 July to 18 Aug. 1966.	1	26	26	56	56	272	272
	6	28	32	28	36	306	306
	12	34	26	56	40	286	334
	18	16	22	42	64	342	342
	24	12	10	48	52	346	380
	30	14	14	36	40	426	440
"C" 24 August to 22 Sept. 1966.	1	8	8	16	16	266	266
	6	12	10	18	12	254	246
	12	12	10	24	20	256	270
	18	10	16	40	32	270	258
	24	10	12	52	44	260	258
	30	11	12	60	52	288	274

the sampled ponds the day after a heavy rainfall. The higher values for series B are also reflected in the carbonate and bicarbonate alkalinity.

No significant difference in water hardness was noted between the treated and untreated systems. It would appear from these measurements that a continuous monolayer of "Aquasave" would not affect water hardness when applied to "soft" waters.

Carbonate and bicarbonate alkalinity. No constant trend was established for either carbonate or bicarbonate alkalinity in untreated and "Aquasave" treated systems. Table I shows the fluctuations in alkalinity measurements during all three experiments.

During series A, the carbonate alkalinity in the untreated systems reached the maximum value of 60 mg/L on the 18th day and dropped to 40 mg/L at the conclusion of the test. However, the carbonate alkalinity in the treated systems increased to a value of 76 mg/L at the end of the thirty-day series.

The beginning values for both carbonate and bicarbonate alkalinity during series B were considerably higher than series A and C due to heavy rain on the ponds in Brazos County just prior to the collection of water and algae samples used for innoculum in the tests. In series B the bicarbonate alkalinity in both untreated and treated systems was considerably higher at the conclusion of the test than the final values found for series A and C.

During series C the carbonate alkalinity in nontreated and treated systems showed a gradual increase throughout the thirty-day period.

No pattern was established for the bicarbonate alkalinity.

Turbidity. In field studies using Secchi disk measurements, Hayes [78] could not determine any difference between water turbidity in ponds filmed with a monolayer of hexadecanol and untreated ponds.

The data accumulated by the three replicate thirty-day tests reported here indicate that a continuous monolayer of "Aquasave" will increase turbidity in treated waters after 10 to 20 days. The water turbidity in both untreated and treated ecosystems was increased or decreased by the increase or decrease in the number of organisms. In all three of the experiments, the turbidity in the "Aquasave" filmed systems was lower than the values found in the untreated systems for the first half of the thirty-day period. However, the turbidity values reversed during the latter half of the thirty-day experiments resulting in high turbidity in the treated aquaria at the conclusion of the experiment.

In all experiments the water turbidity in the systems treated with "Aquasave" was higher at the end of thirty days.

Figure 4 compares the water turbidity measurements between untreated and treated systems during the first experiment. An increase in the turbidity of the untreated system during the first twelve days was shown. After day twelve, the turbidity in the untreated system decreased rapidly while the turbidity in the treated system increased to reach a maximum value higher than that recorded for the control. At the conclusion of the test a significant difference (24 parts per million SiO_2) in water turbidity existed with

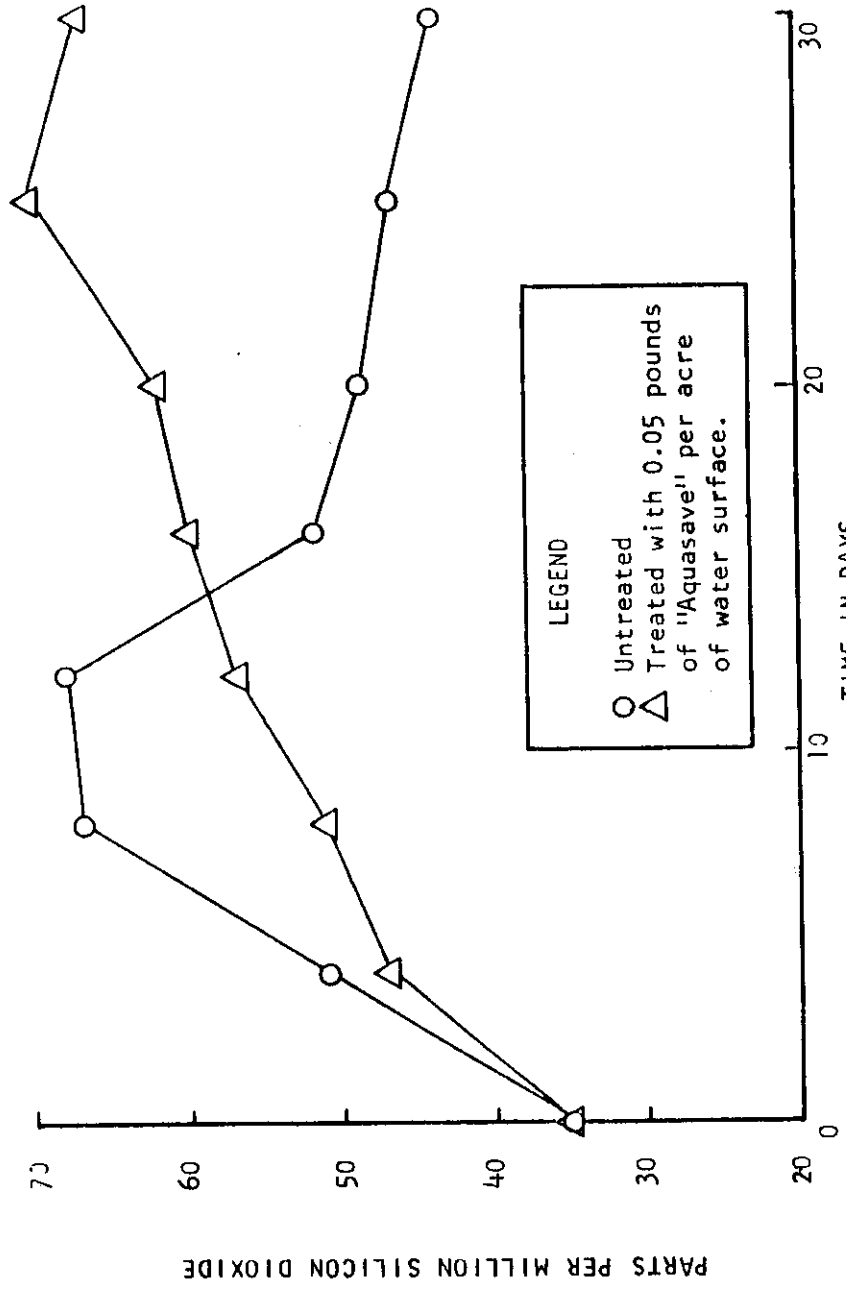


FIGURE 4. A COMPARISON OF TURBIDITY MEASURED IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 26 MAY TO 24 JUNE 1966.

the treated system having the higher values.

During the second series of experiments this same trend was noted. However, as shown in Figure 5, both the untreated and treated microcosms contained a large number of the colonial algal Volvox which was present in the innoculum at the beginning of the test. The population of Volvox in the untreated and treated systems had decreased by the end of the 8th day and the cell growth of Chlorella again became the dominant factor controlling water turbidity. The maximum turbidity increase in the control was reached on the 18th day and then turbidity in the untreated system decreased for the remainder of the test. At the conclusion of series B the water turbidity in the microcosms treated with "Aquasave" was considerably higher than the values measured in the untreated system.

The third experiment also displayed increased turbidity in the treated microcosms. These data are shown in Figure 6 on page 38. This series of experiments did not have the high initial turbidity values found in the first two experiments. At the conclusion of the test the microcosms treated with "Aquasave" had higher turbidity than the untreated microcosms.

The turbidity data show that, under the laboratory conditions used in this study, a continuous monolayer of "Aquasave" may increase the growth of nonfilamentous algae and other micro-organisms and thereby increase the water turbidity in treated systems.

Surface clarity. Based on the visual observations of the water surface in aluminum field tanks Meinke [7] reported that "Aquasave"

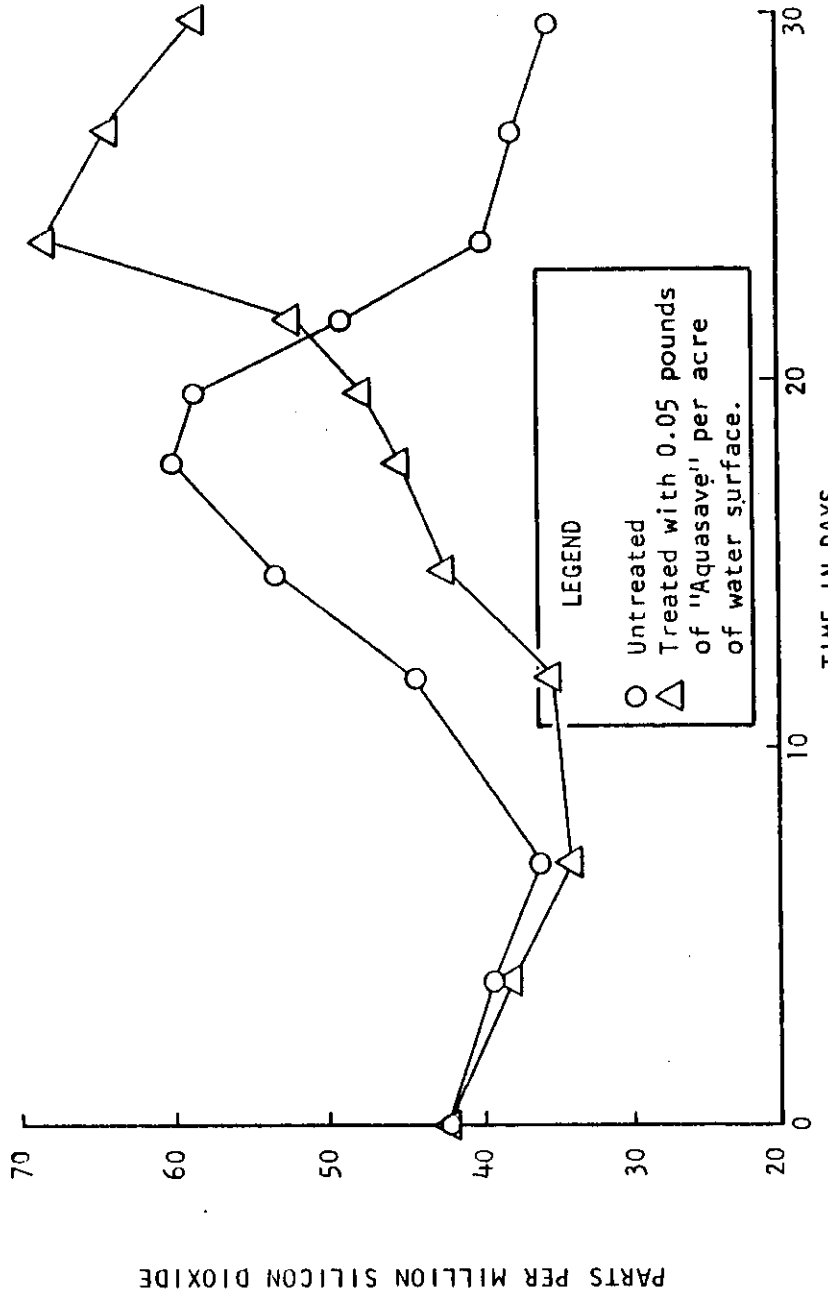


FIGURE 5. A COMPARISON OF TURBIDITY MEASURED IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 20 JULY TO 18 AUGUST 1966.

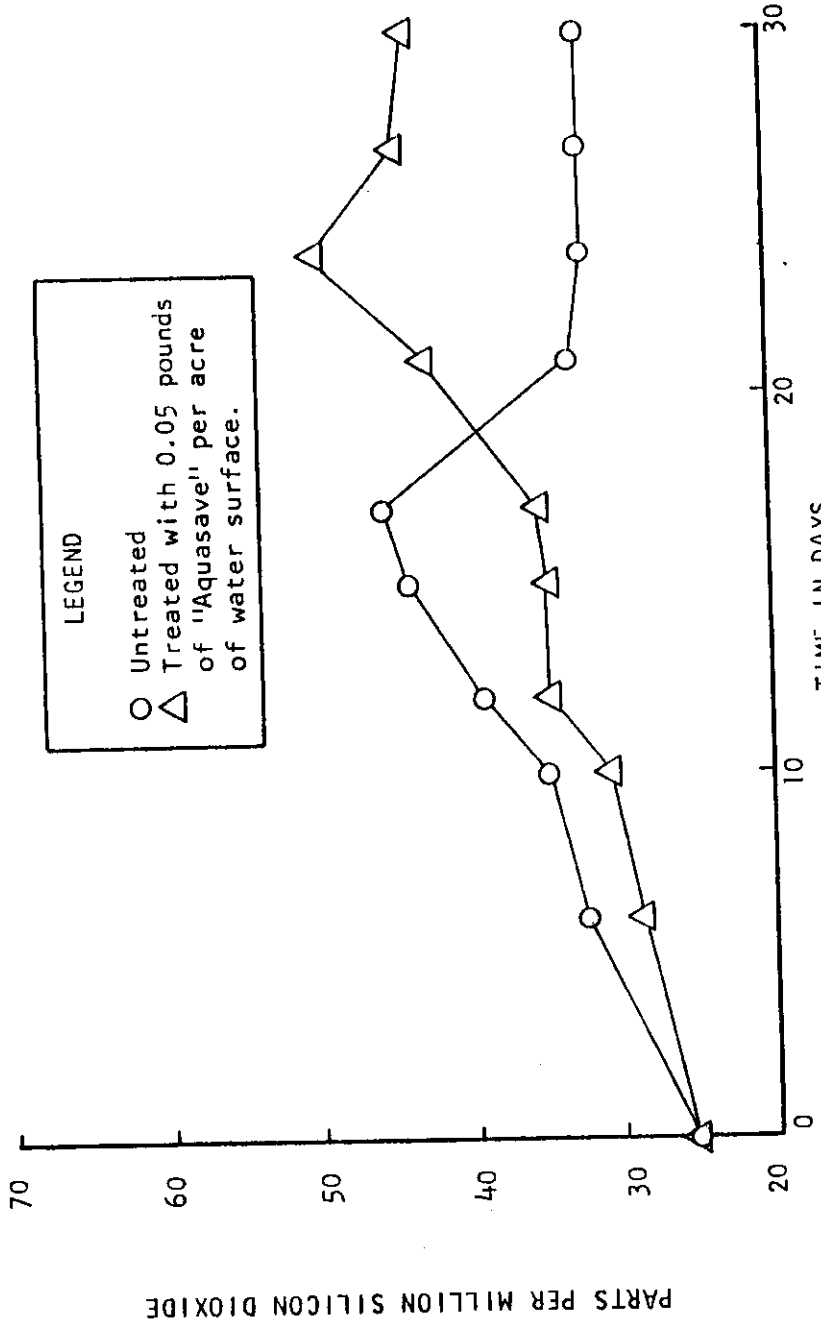


FIGURE 6. A COMPARISON OF TURBIDITY MEASURED IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 24 AUGUST TO 22 SEPTEMBER 1966.

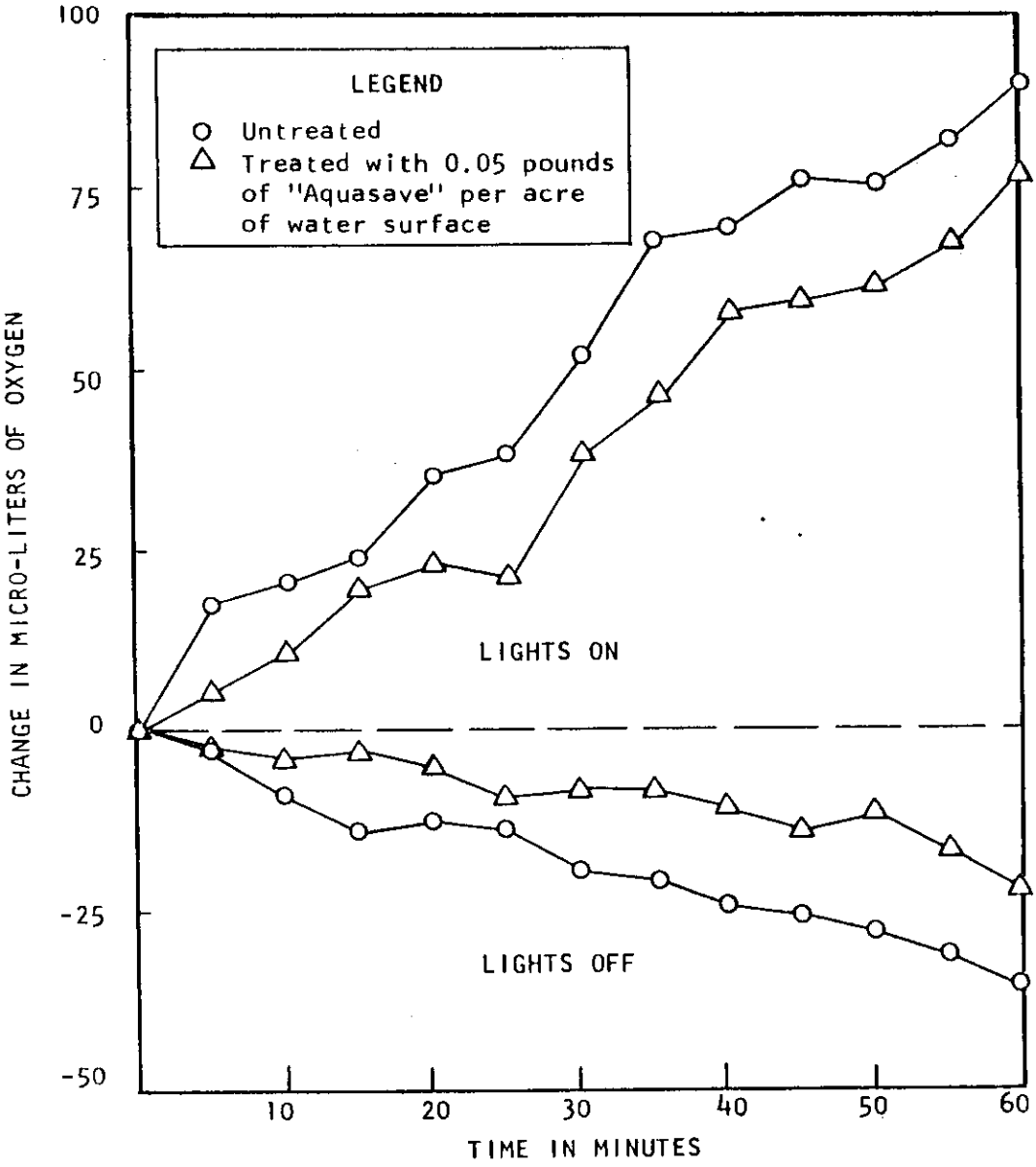


FIGURE 7. A COMPARISON OF OXYGEN PRESSURE IN UNTREATED AND TREATED 50 ML WATER AND ALGAL SAMPLES. EACH POINT REPRESENTS THE MEAN OF THREE SAMPLES.

by as much as 30%.

Certain limitations must be recognized for the direct method used in this study. The conditions that must be valid are:

a. the gases exchanged must be only O_2 and CO_2.

b. The atmosphere in the flask must be free from CO_2. (Sufficient alkali must be present to absorb all the CO_2).

c. The rate of oxygen uptake and the rate of CO_2 reduction must be within such a range that the fluid is always saturated with oxygen and the pressure of the CO_2 in the gas phase is at zero.

The reduced oxygen transfer rate has been ignored in past studies because it was considered to be unaffected by the presence of the monolayers. However, it should be pointed out that field tests have not been successful in maintaining a continuous monolayer because of wind and other problems. Development of a monolayer that can be maintained continuously will intensify the oxygen diffusion rate problem. As better evaporation reduction monolayers are developed it will be necessary to pay more attention to the transfer of oxygen through the film.

Primary Productivity

The ecological impact of a continuous evaporation retardation monolayer may be evaluated at the ecosystem level by measuring the primary productivity. According to Odum,[13] biological primary productivity is the amount of energy fixed during a given time.

Unfortunately, no method for direct measurement of productivity or energy flow through a complex ecosystem has been found. However, measurements of some indirect quantities, such as the amount of material used or by-products released, will provide an estimate of the productivity of an ecosystem.

The measurement of productivity may be the measurement of the rate at which organic matter and oxygen are produced. Using the calculations cited by Odum [13] the following equation would apply:

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6\ O_2\ .$$

This means, in general, that for each weight of organic matter formed from carbon dioxide, very nearly the same weight of oxygen will be liberated into the system. Therefore, by measuring oxygen production one makes a close estimate of the organic matter production.

The ratio of atoms of oxygen produced to atoms of carbon dioxide assimilated is called the photosynthetic quotient. The photosynthetic quotient in the preceeding equation is unity. In nature, with proteins and other substances being formed, Odum states the quotient is often higher, up to approximately 1.25.

An evaluation of the effects caused by a continuous monolayer of "Aquasave" was determined in this study by measurement of primary productivity. In these experimental ecosystems, approximately the same physical, chemical, and biological factors were maintained in both the treated and untreated systems in order to use the diurnal

oxygen method and the chlorophyll analysis method for measuring and comparing primary productivity.

Diurnal oxygen method. The results of the diurnal oxygen and primary productivity study are presented for each of the three series. The Ohle test [90] for interfering substances was found to be negative for all dissolved oxygen determinations.

Series A. During the first series of experiments, diurnal oxygen measurements were conducted on the second day. The initial dissolved oxygen measurements were conducted to establish any initial variation in the two systems to validate later tests. Figure 8 shows that the dissolved oxygen and primary productivity in both the untreated and treated microcosms were nearly equal. A slight amount of productivity occurred in both systems. Oxygen curves were plotted by connecting each two hour oxygen measurement for the entire 24-hour cycle. The diurnal oxygen curves for the two systems were very much alike.

The diurnal study conducted on the 12th day of the series resulted in different measurements. As shown in Figure 9, the microcosm treated with "Aquasave" had a significant oxygen deficiency as compared to the dissolved oxygen present in the untreated microcosm. A difference in primary productivity also occurred. The untreated aquaria had a gain of 0.97 mg/O_2/L while the treated aquaria had a loss of 0.89 mg/O_2/L. for the 24-hour period.

On the 19th day of the experiment a reversal of the dissolved oxygen values was noted. Figure 10 shows that the film treated system contained more dissolved oxygen than the untreated system. This

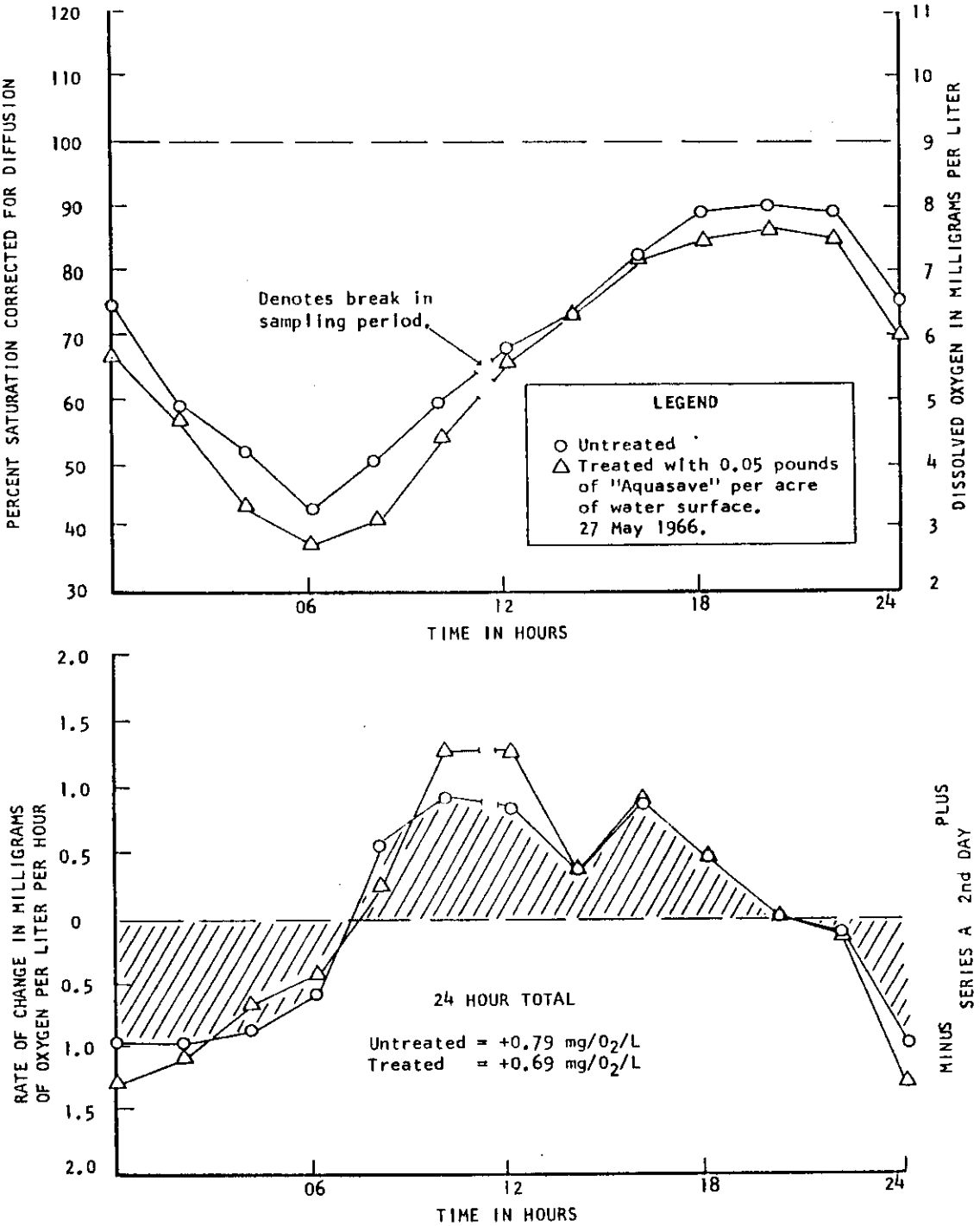


FIGURE 8. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, A-2

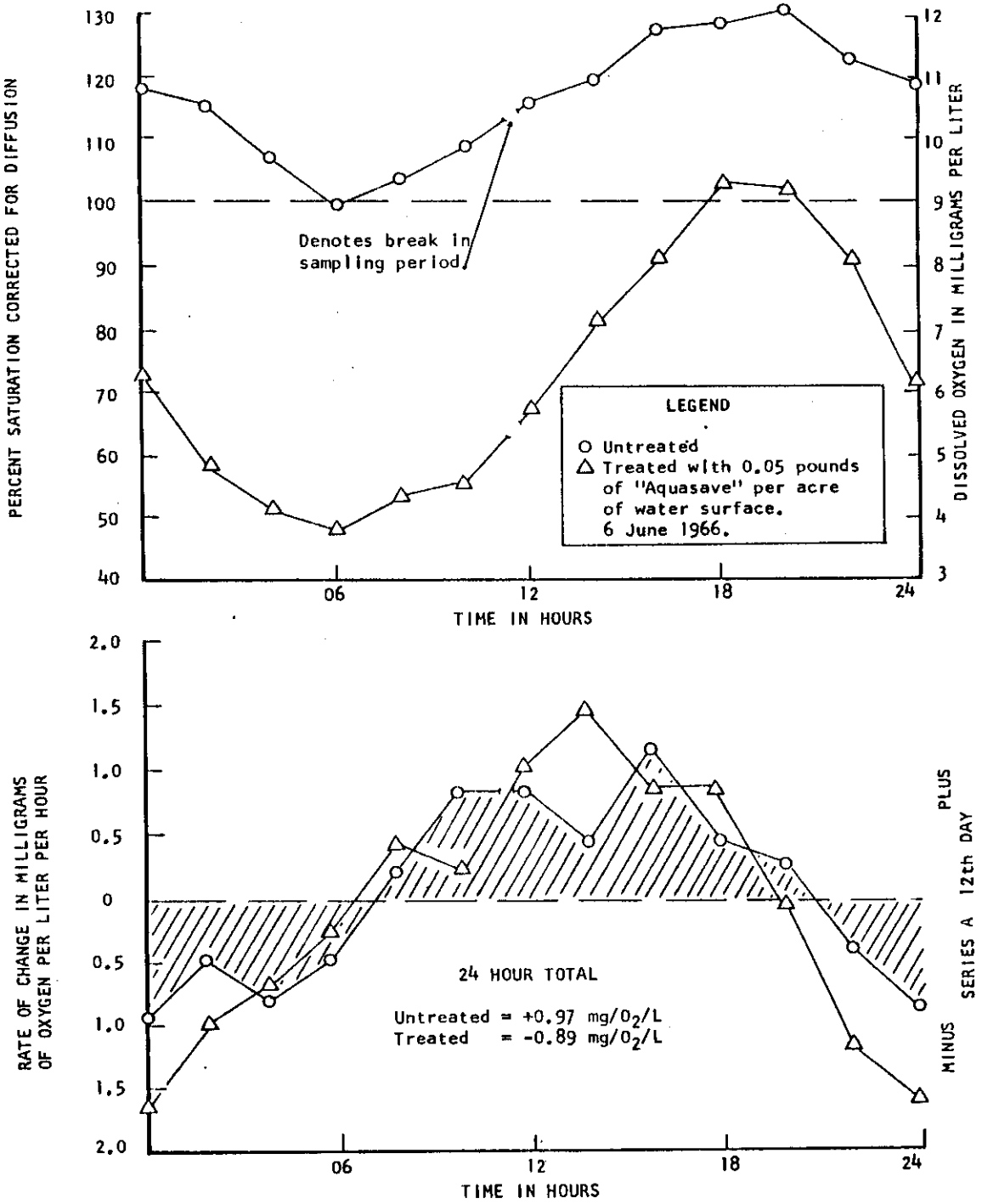


FIGURE 9. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, A-12

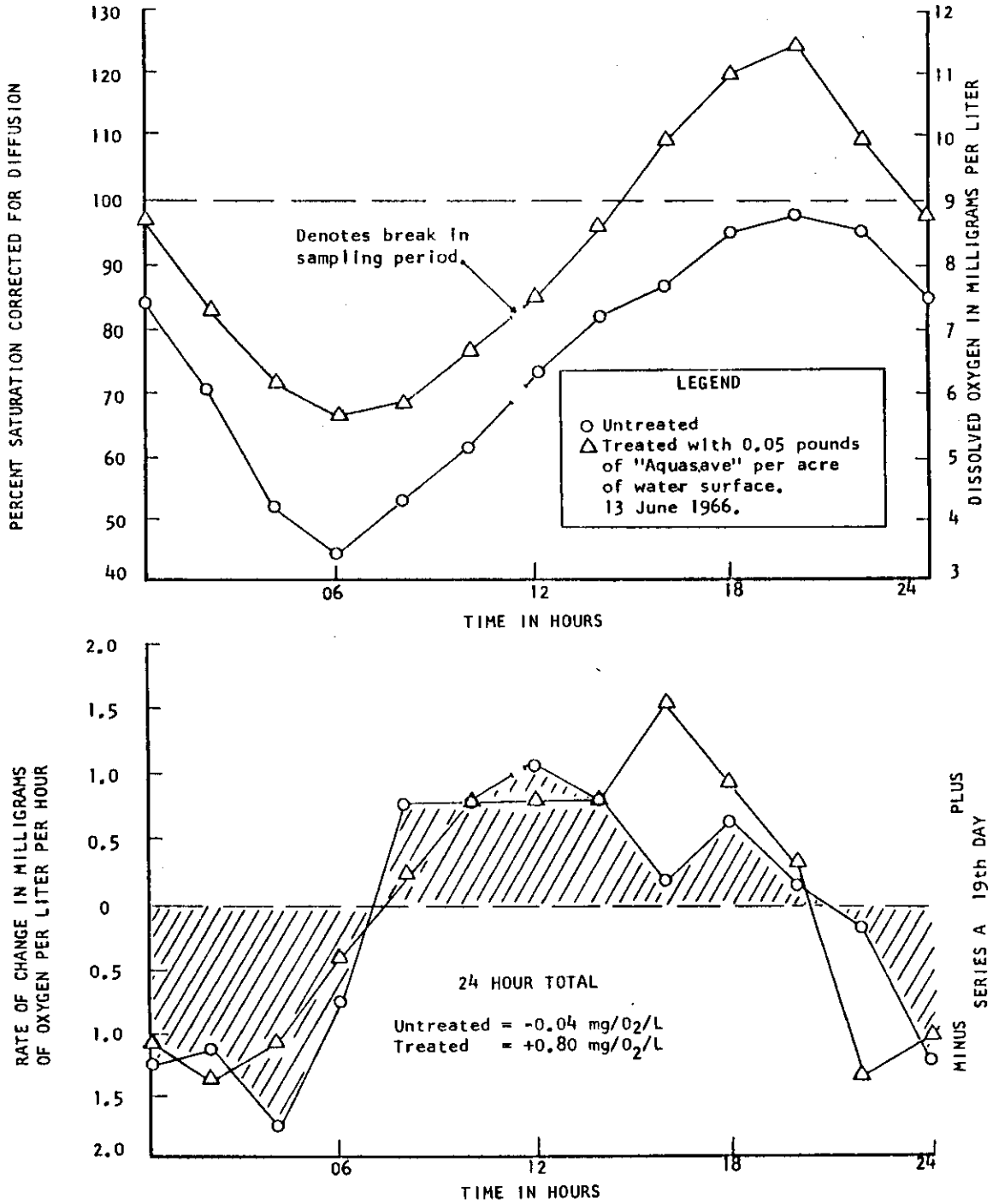


FIGURE 10. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TRTATED MICROCOSMS. A-19

change from that in earlier tests was also reflected in the primary productivity.

The final diurnal oxygen analyses for the first series indicate that the dissolved oxygen in the treated system was much higher than that found in the untreated system. Figure 11 shows the data recorded for the 25th day of series A. The comparison of primary productivity on the 25th day of the first series indicated that the system treated with "Aquasave" was showing an increase of 1.37 $mg/O_2/L$ while the other showed a loss. A comparison of Figure 9 and Figure 11 clearly illustrates the reversal of dissolved oxygen values in the thirty-day test.

The sequence of the changes in the dissolved oxygen cycles and primary productivity may be correlated with the algal population growth during the experiment. Figure 4 on page 35 shows the turbidity data for series A.

Series B. Diurnal oxygen measurements were made on the second day of the test to establish any initial variation between the systems. Little difference was found (see Figure 12). However, series B had a higher dissolved oxygen content at the beginning of the test due to the mixed water and algal innoculum which was collected and used after a rain on the sampled ponds. A large population of Volvox was also present in the innoculum and reflected in the data from the systems at the start of the test (500 colonies per ml).

Figure 13 indicates the diurnal oxygen measurements for the 13th day of the test. The dissolved oxygen in the untreated system was

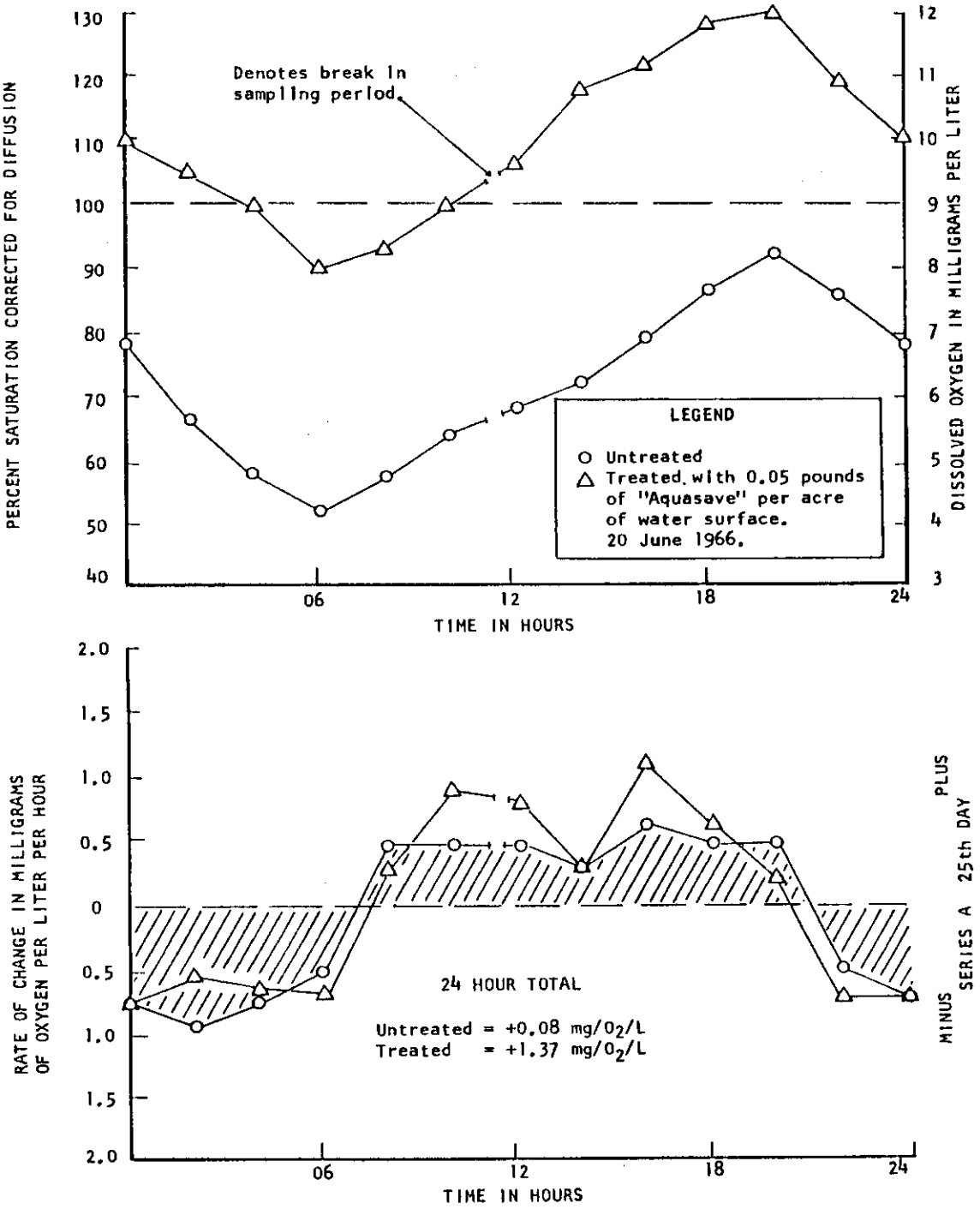


FIGURE 11. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, A-25

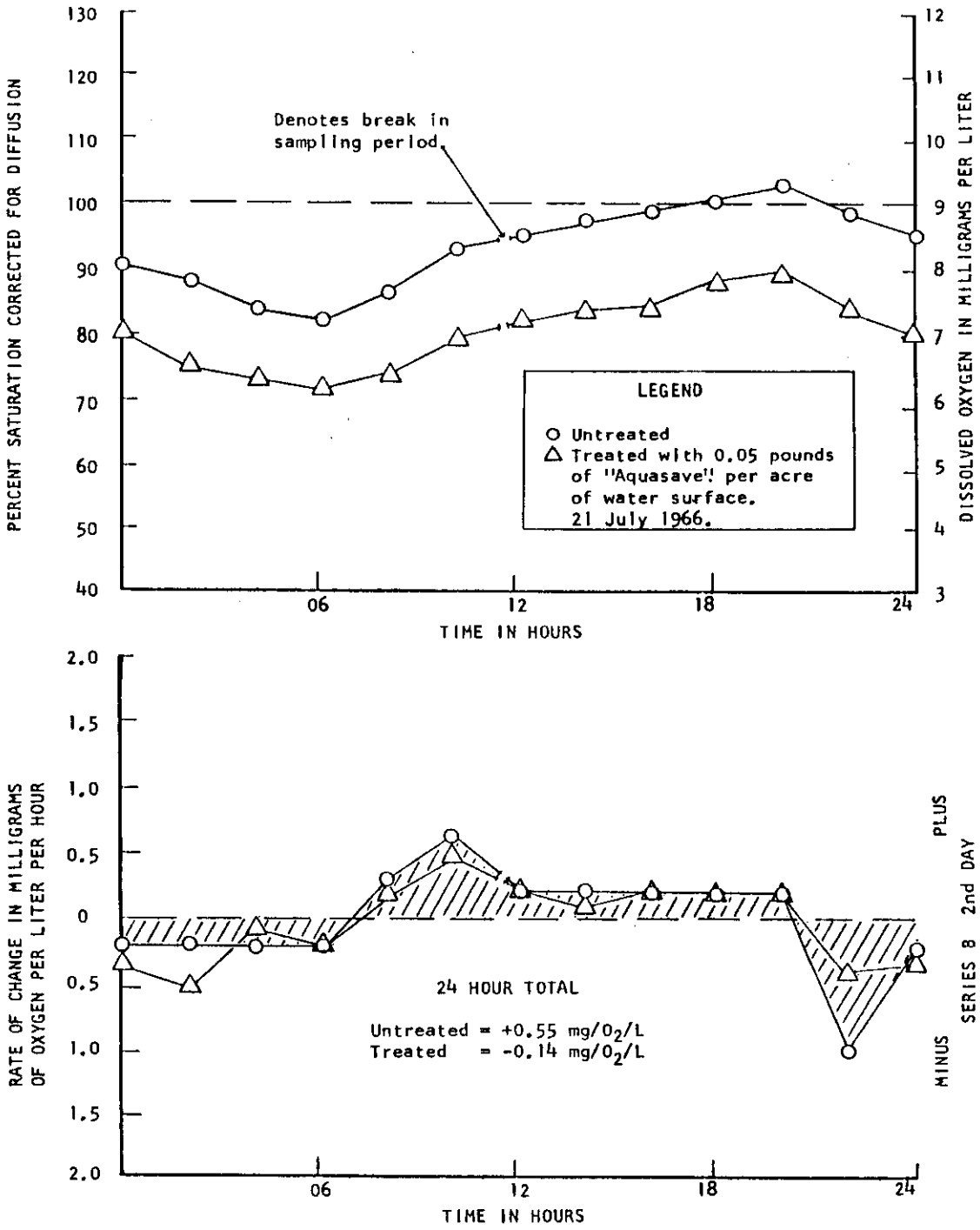


FIGURE 12. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, B-2

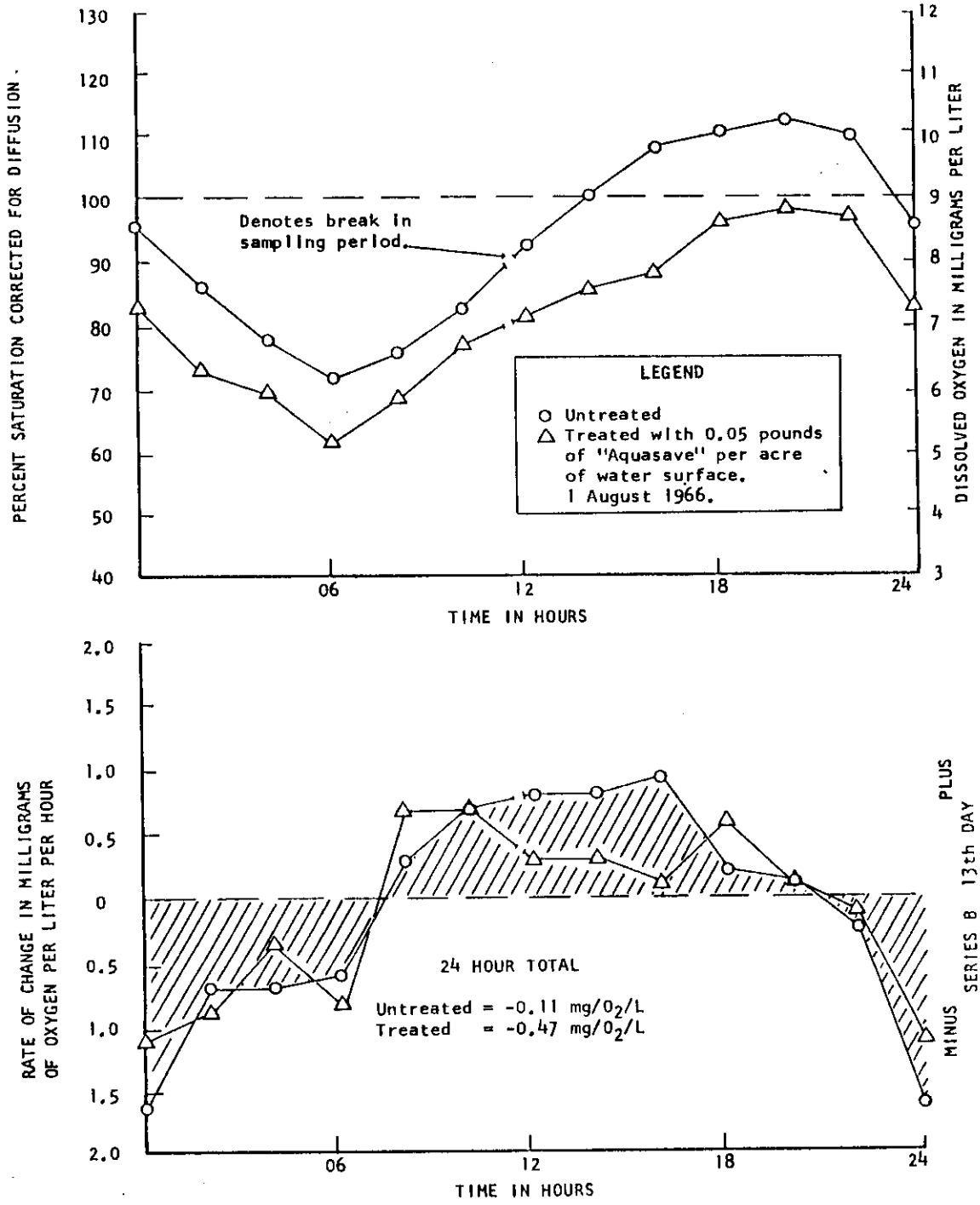


FIGURE 13. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, B-13

higher than that in the treated system. However, differences in primary productivity were very small. The Volvox colonies had decreased in numbers while the numbers of Chlorella were starting to increase.

Figure 14 compares the diurnal oxygen and primary productivity on the 20th day of the test. The untreated system still had higher oxygen values as well as higher primary production.

The diurnal oxygen and primary productivity measurements on the 27th day of the test are shown in Figure 15. A reversal of the oxygen and primary production occurred with the system treated with "Aquasave" having the higher values. A difference of more than three milligrams per liter of dissolved oxygen was found with the treated system having the higher value. Primary production in the treated system had a value of plus 0.72 mg/O_2/L while the untreated system showed a loss during this time.

Series C. Diurnal oxygen measurements were made on the third day of this series to establish any initial variation between the systems. Figure 16 compares the oxygen in the untreated and treated microcosms. The untreated system had a slight gain over the treated system both in oxygen values and in primary productivity.

Figure 17 shows the data recorded for the 14th day of the test. Oxygen values of the untreated system had good saturation values as compared with the treated system. Values for productivity parallels those found during the third day of the test. The untreated system had a small productivity gain while the treated system showed a loss of oxygen.

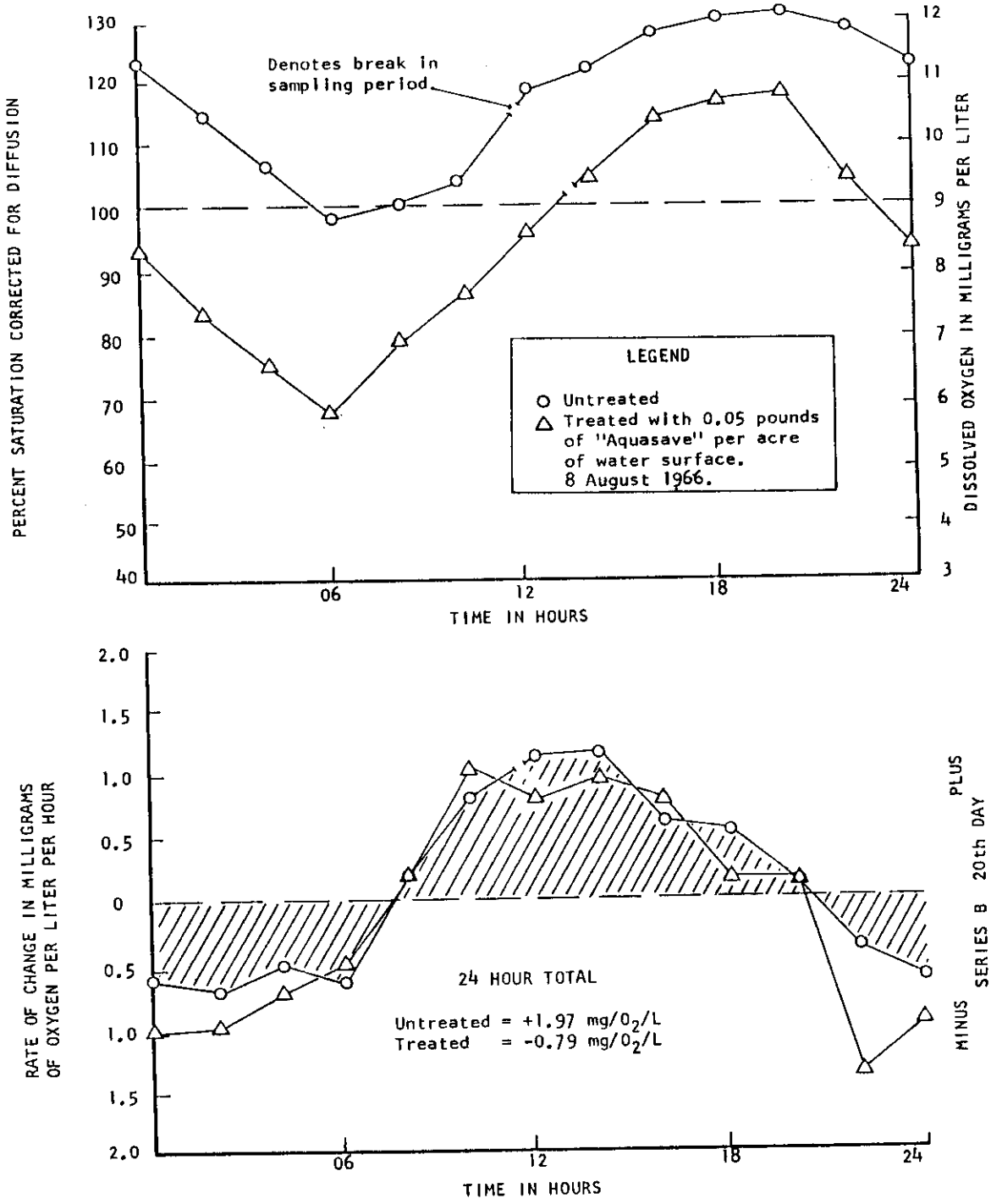


FIGURE 14. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, B-20

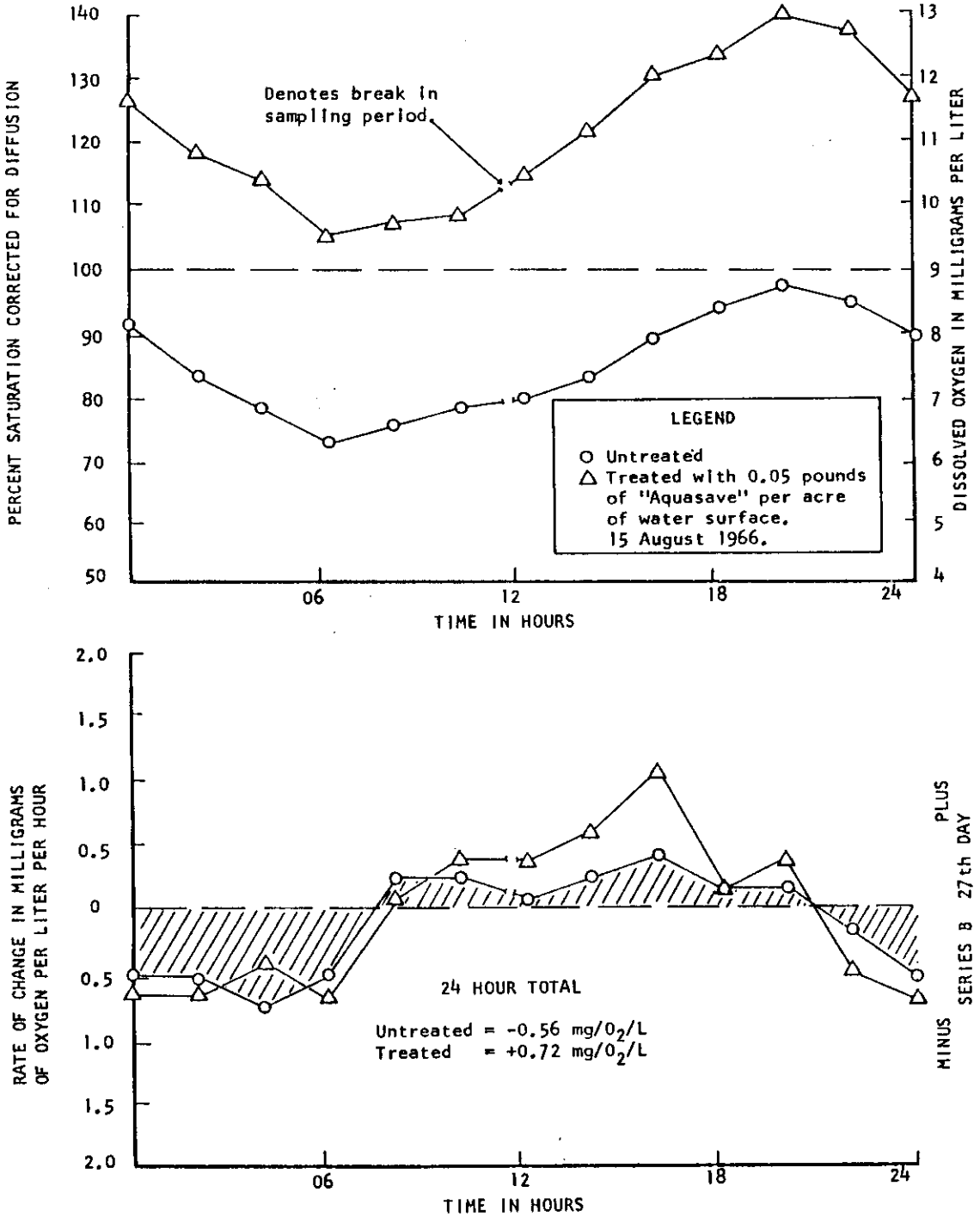


FIGURE 15. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS. B-27

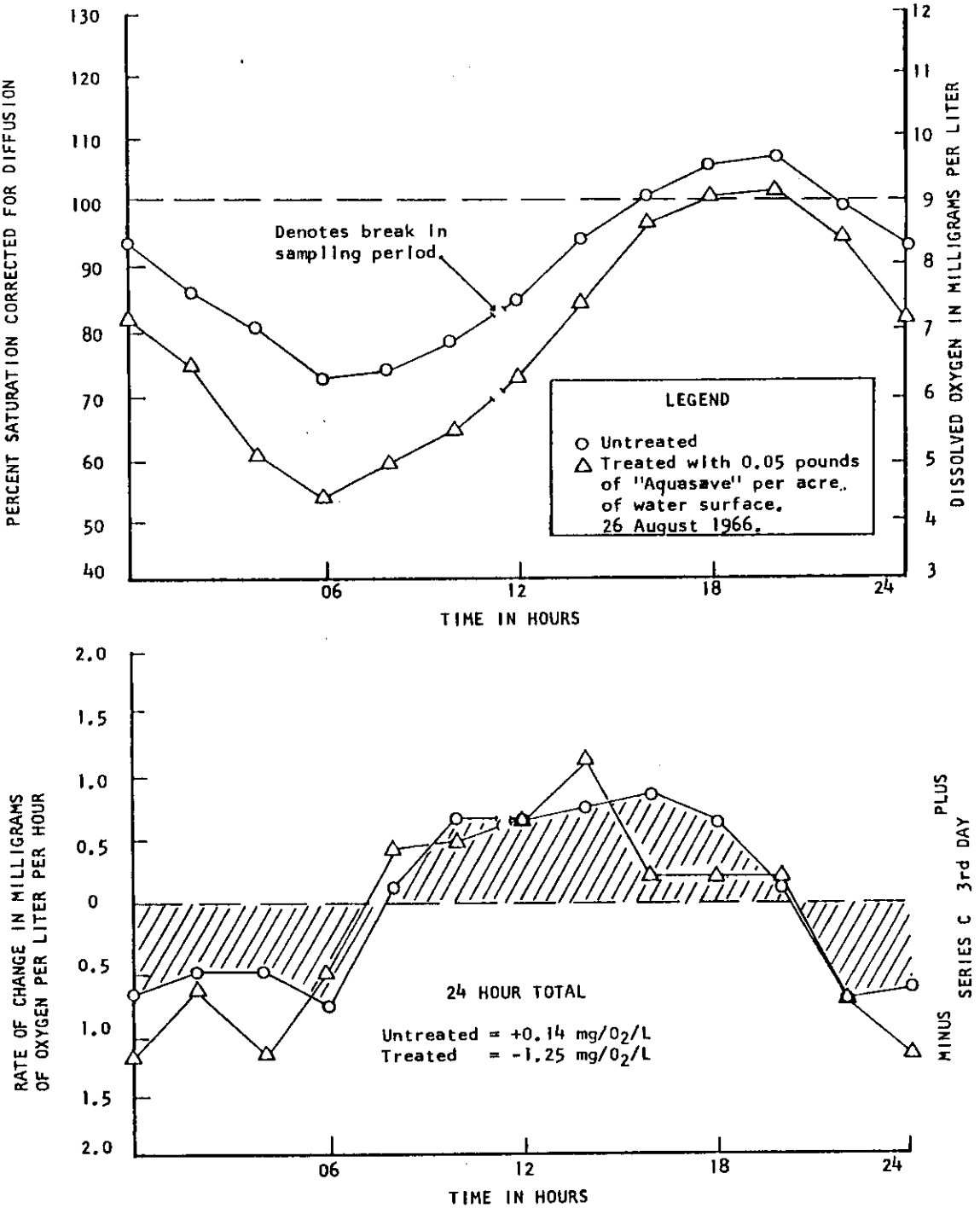


FIGURE 16. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS. C-3

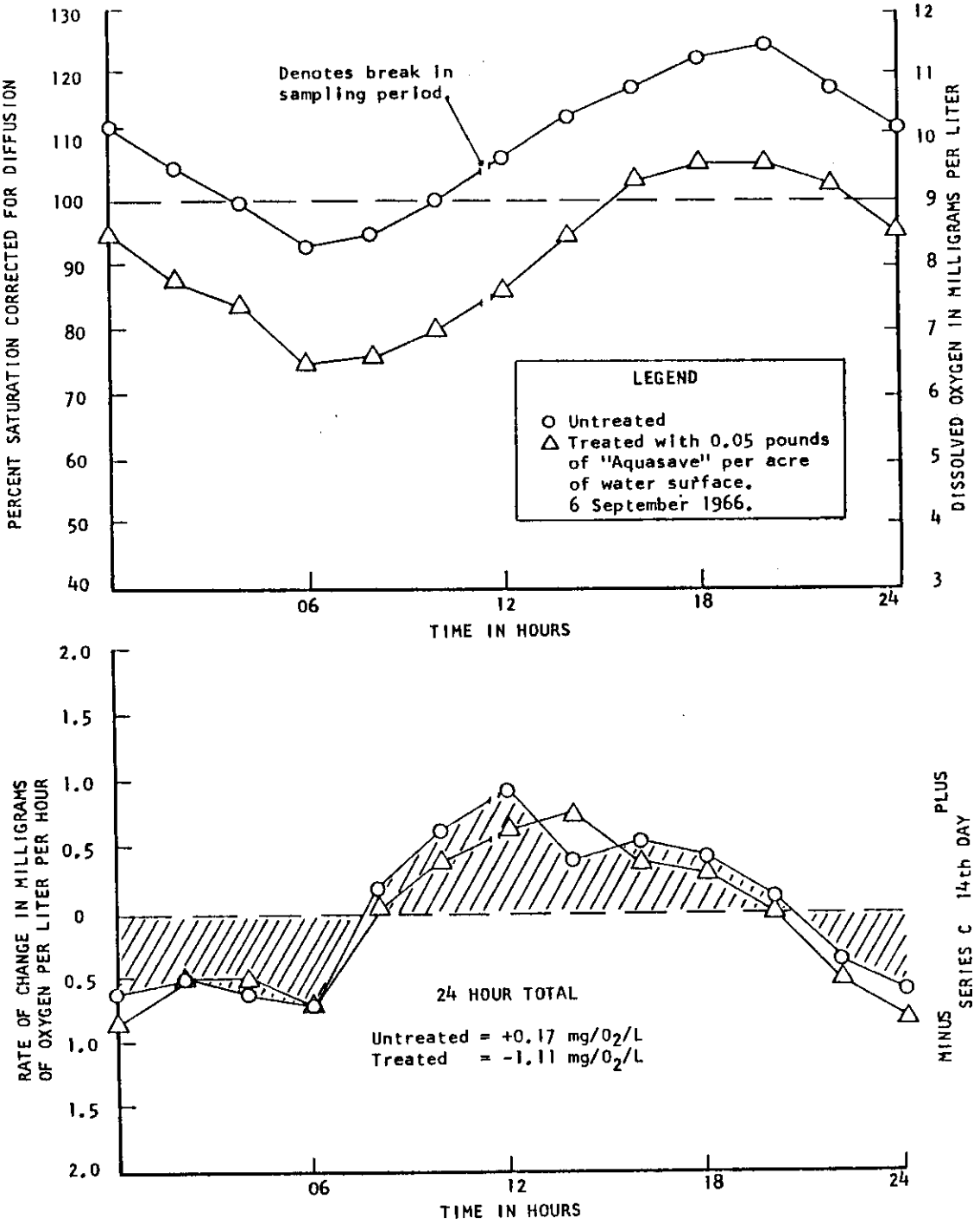


FIGURE 17. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS, C-14

A comparison of the diurnal oxygen measurements on the 20th day indicated that oxygen in the treated system had increased above the oxygen values found in the untreated systems (see Figure 18). The reversal of values is also reflected in the primary productivity measurements.

Figure 19 compares oxygen and primary productivity in the untreated and treated systems during the 27th day. A significant difference in dissolved oxygen existed between the systems. The system treated with "Aquasave" had the highest amount of dissolved oxygen. Measurements of primary productivity followed the trend established in prior experiments. The treated systems gained oxygen while the untreated systems lost oxygen.

In prior field and laboratory investigations, Hayes [78] found that a monolayer of hexadecanol would cause a small decrease in the rate of oxygen diffusion into film treated waters. However, Wiltzius [12] has reported an increase of the dissolved oxygen in film-treated waters. He attributed this increase to the oxygen being trapped under the film during periods of supersaturation.

The results of all three series of experiments indicate that a significant oxygen change may occur in microcosms treated with "Aquasave" under the conditions used in this study. The amount of dissolved oxygen and primary productivity was found to vary with the time of measurement as well as with the biological populations present in the experimental ecosystems. Measurements during the first half of the experiments have shown dissolved oxygen to be higher

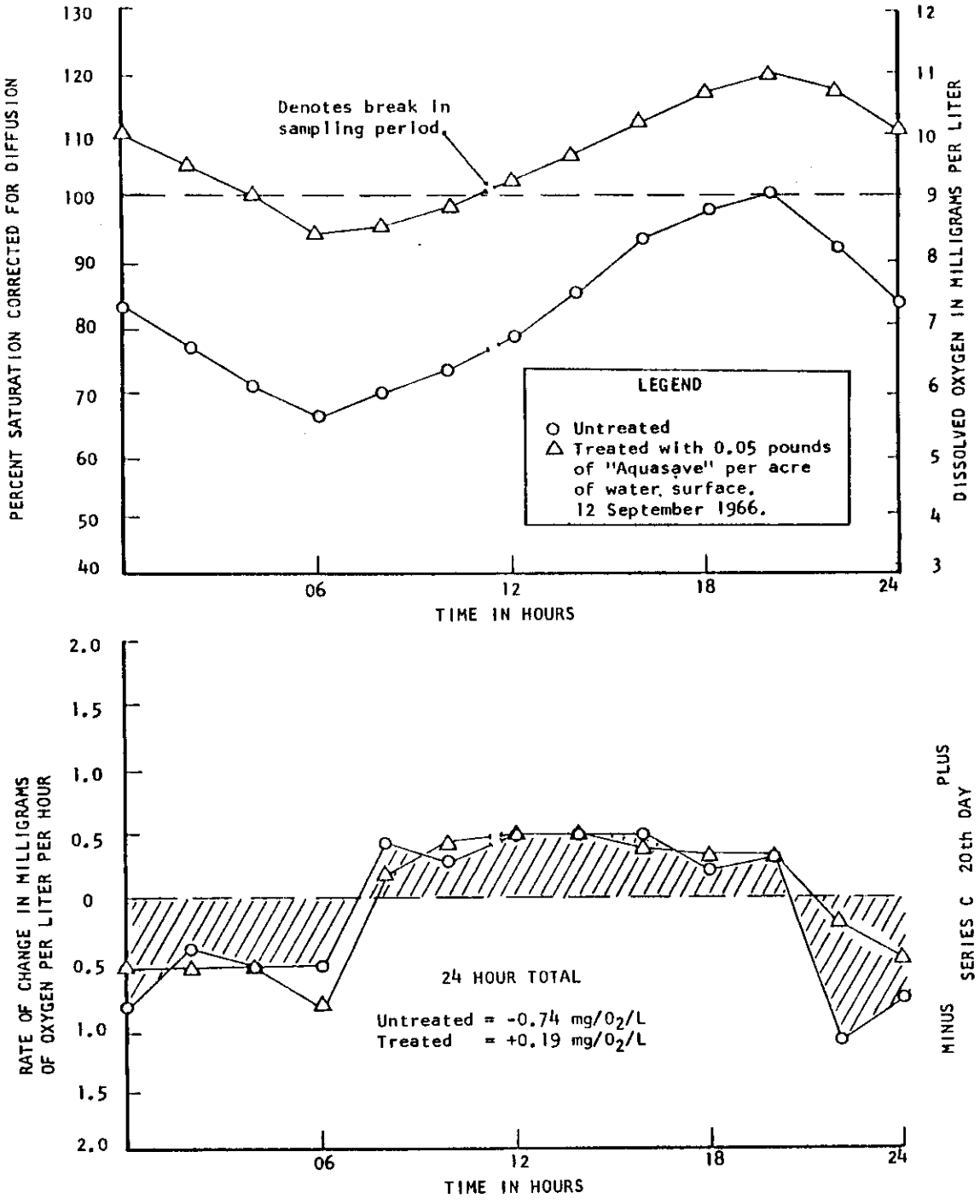


FIGURE 18. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS. C-20

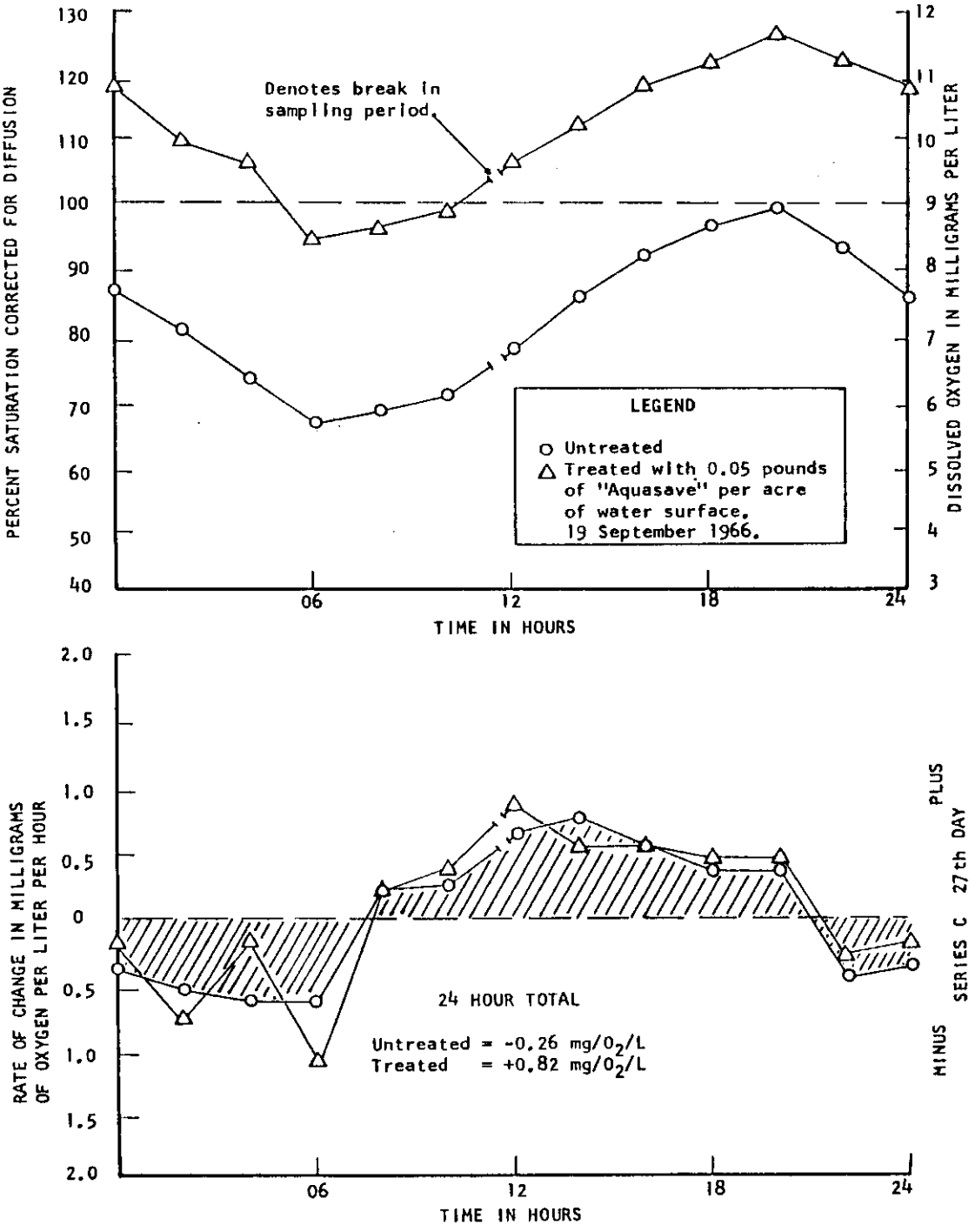


FIGURE 19. A COMPARISON OF DIURNAL OXYGEN AND PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED MICROCOSMS. C-27

in the untreated systems. Measurements at the conclusion of thirty days show that dissolved oxygen and primary production are higher in the systems filmed with "Aquasave."

Chlorophyll analysis. Following the procedure of Barnes,[92] the chlorophyll content method was used as an index to compare primary productivity in the untreated and treated systems. Chlorophylls A, B, and C were determined. However, the analyses cannot correct for the age of the chlorophylls producing growth or the respiratory losses occurring in natural aquatic communities.

Figure 20 compares the net primary productivity in the untreated and treated systems. The treated systems was found to have more of chlorophylls A, B, and C than that found in the untreated system. The chlorophyll analyses were made at the conclusion of the first thirty-day test and were not indicative of the maximum productivity occurring during the series.

Figure 21 compares the amounts of chlorophylls A, B, and C found at the conclusion of the second series. While the samples from the treated system contained more of all three chlorophyll types, the final differences were less than the values found during the series A test.

Figure 22 shows the chlorophyll contents found in the untreated and treated systems at the conclusion of the last experiment. A much lower chlorophyll content was found in both the untreated and treated system when compared with series A and series B. Correlation with turbidity and diurnal oxygen values show that series C had the

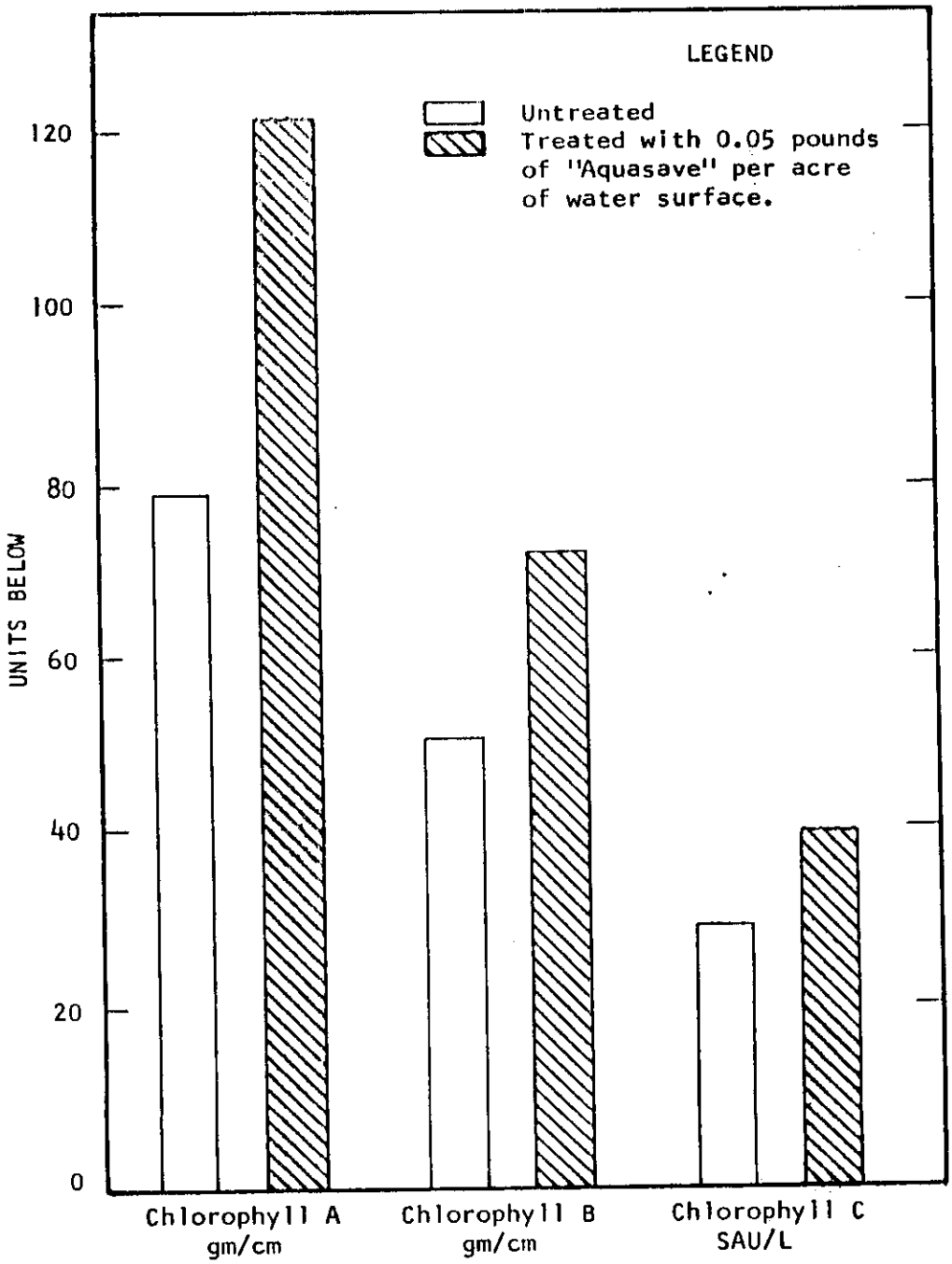


FIGURE 20. A CHLOROPHYLL ANALYSIS COMPARING NET PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS ON 24 JUNE 1966.

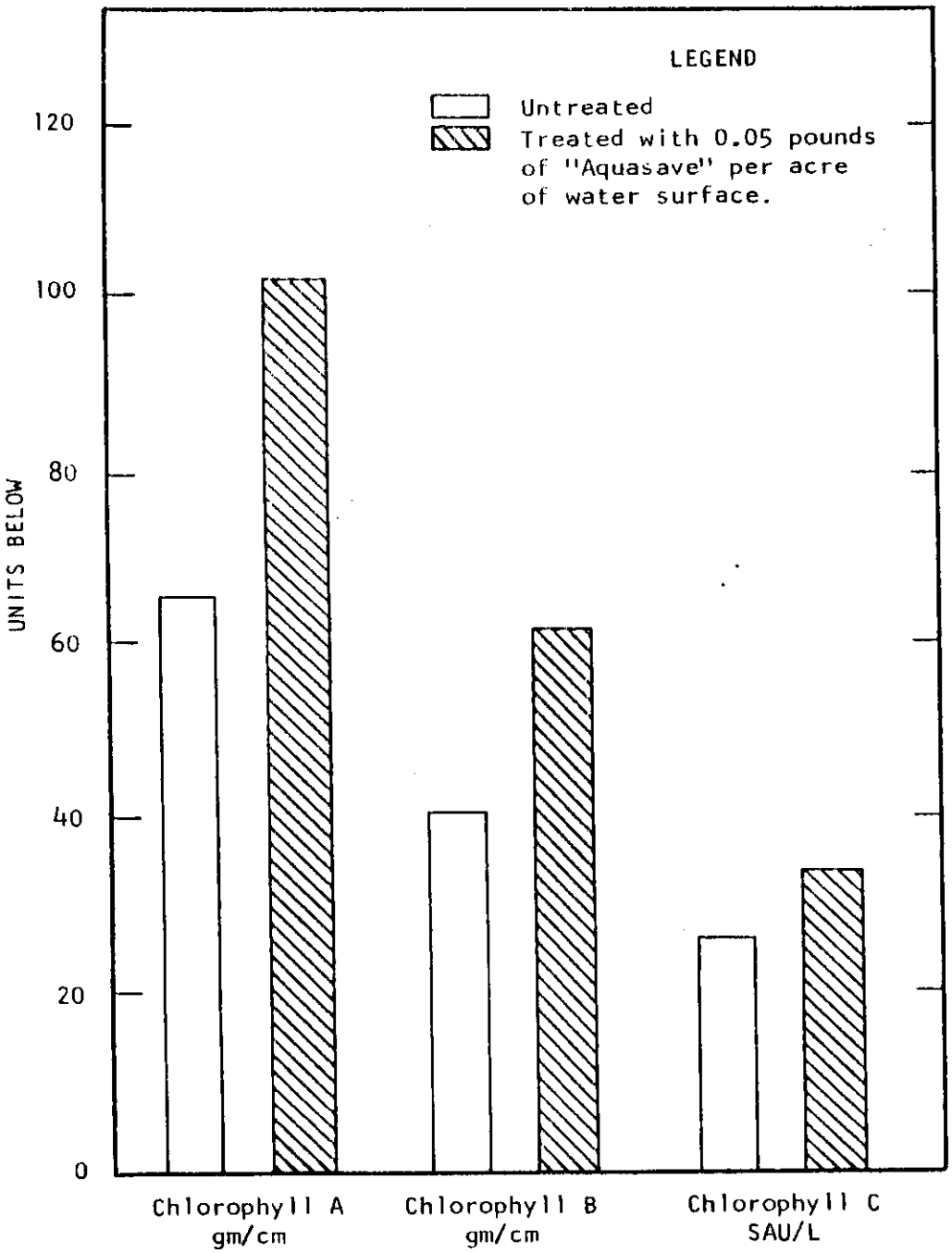


FIGURE 21. A CHLOROPHYLL ANALYSIS COMPARING NET PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS 18 AUGUST 1966.

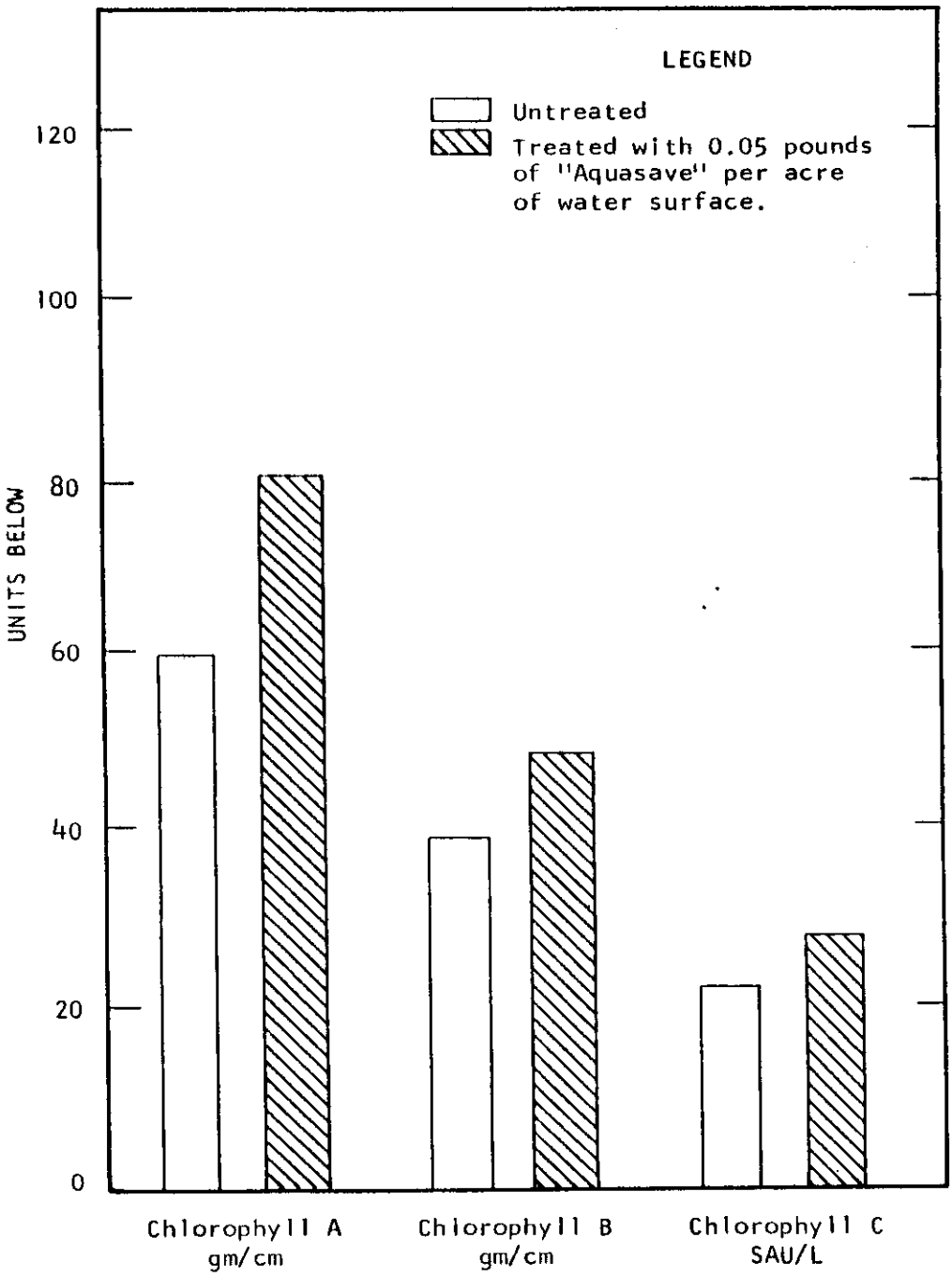


FIGURE 22. A CHLOROPHYLL ANALYSIS COMPARING NET PRIMARY PRODUCTIVITY IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS ON 22 SEPTEMBER 1966.

least amount of biological and primary productivity of the three series. However, the treated system again showed a slight gain over the untreated system.

The results of the chlorophyll analyses indicate that a significant primary productivity increase occurred in the systems treated with "Aquasave." It appears that the biological population in the treated systems benefited indirectly from the film application.

Biological Factors

Effects of "Aquasave" on bacteria. The data collected for three thirty-day replicate tests show that bacteria increase in microcosms treated with a continuous monolayer of "Aquasave." The major bacterial growth was immediately under the "Aquasave" film.

Figure 23 compares the number of bacterial colonies per ml of water in untreated and treated aquatic ecosystems during the first experiment. After the 6th day, the water in the microcosm treated with "Aquasave" had a notable increase in the number of bacteria. At the termination of the experiment, a concentration difference of 5,800 bacterial colonies per ml of water existed.

The second experiment (Figure 24) and the third experiment (Figure 25) also resulted in increased numbers of bacteria in the microcosms treated with a monolayer of "Aquasave."

The oxygen demand exerted by the increased bacteria population may be significant. However, the concentration of bacteria colonies found in this study were not of sufficient numbers to cause a

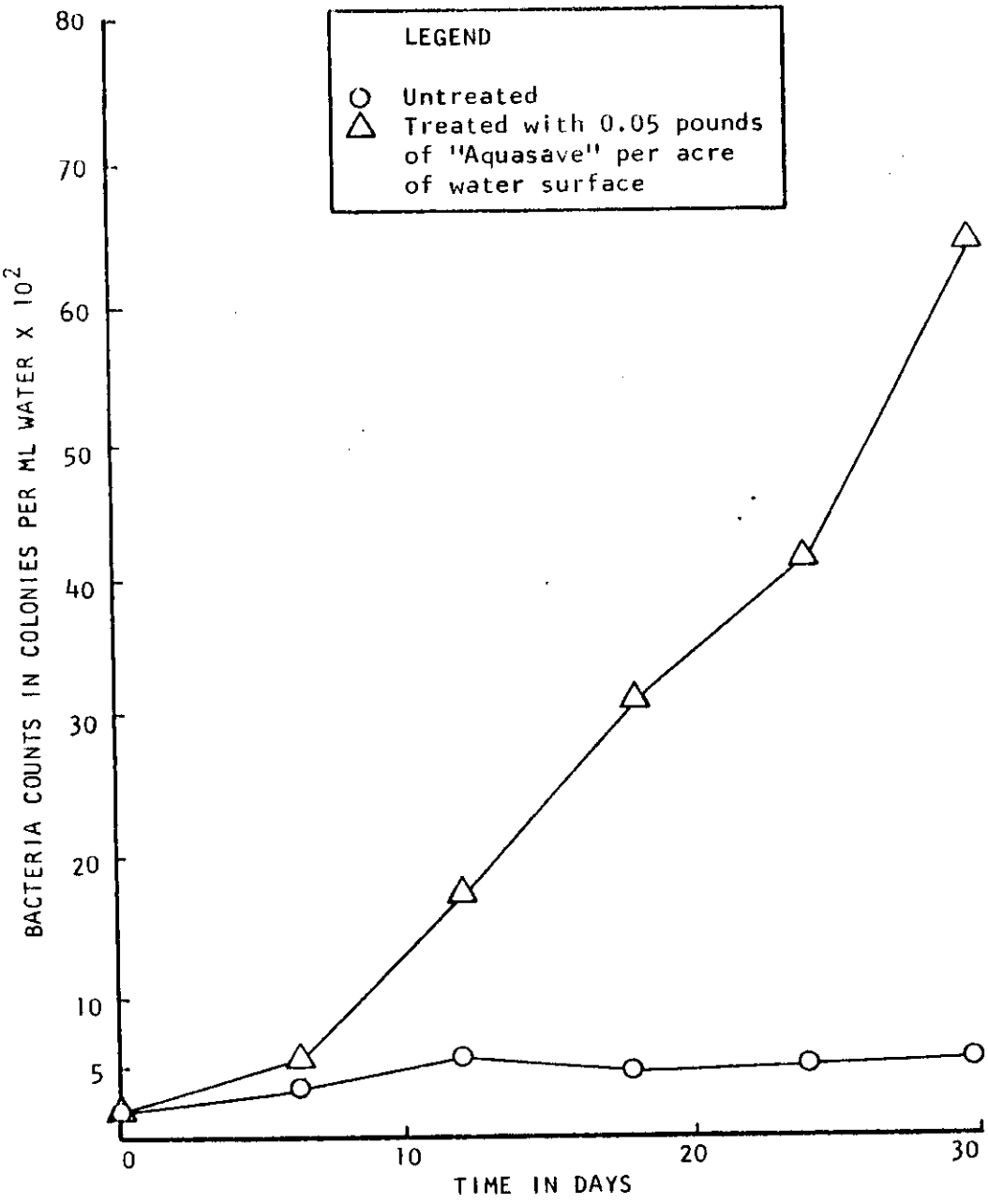


FIGURE 23. A COMPARISON OF BACTERIA COLONIES PER ML OF WATER IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 26 MAY TO 24 JUNE 1966.

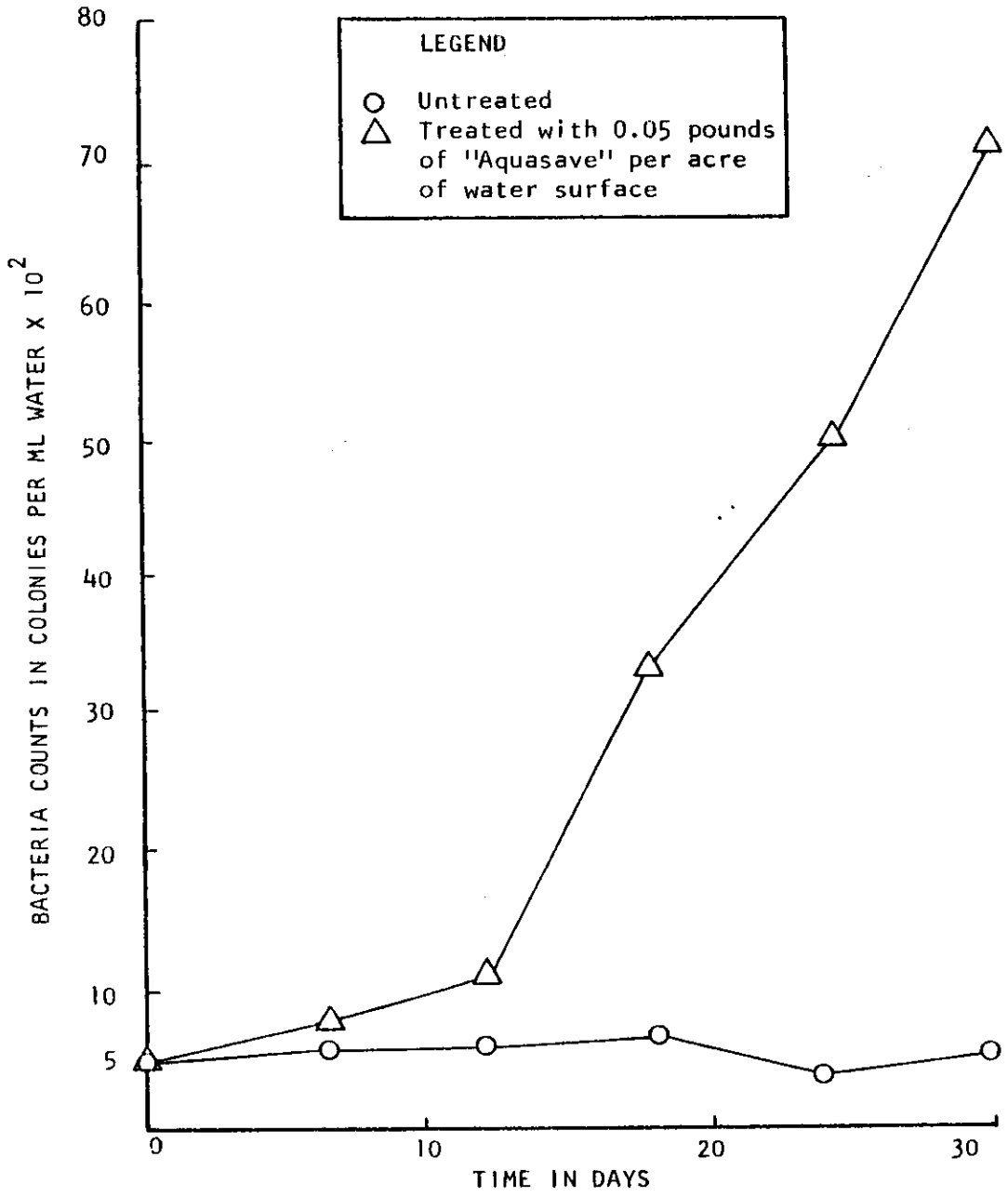


FIGURE 24. A COMPARISON OF BACTERIA COLONIES PER ML OF WATER IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 20 JULY TO 18 AUGUST 1966.

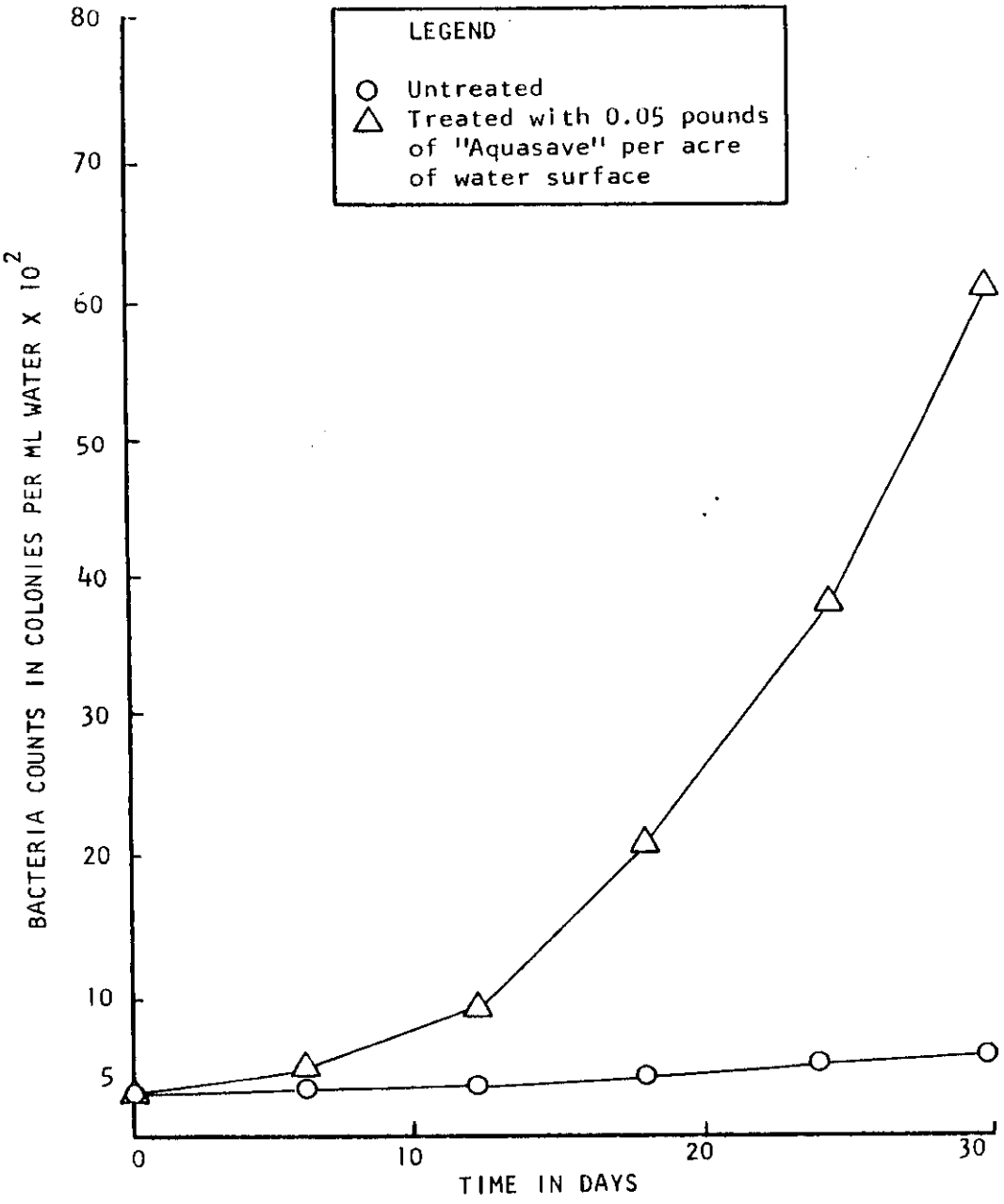


FIGURE 25. A COMPARISON OF BACTERIA COLONIES PER ML OF WATER IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 24 AUGUST TO 22 SEPTEMBER 1966.

significant oxygen demand as shown by diurnal oxygen variation and primary productivity. These parameters have been discussed in a previous section. In light of various investigators comments concerning the variation in results with hexadecanol additional comments seem warranted.

Silvey [96] observed large increases in bacteria during the Lake Hefner studies. He reported that some bacteria counts were as high as 15,000,000 per ml of water for certain locations on the lake. It was also found that when the hexadecanol (food source) was removed, the bacteria population decreased to a normal concentration in about three weeks.

Ludzack and Ettinger [10] reported similar rapid bacterial increases in their study on the biological oxidation of hexadecanol under laboratory conditions. They also pointed out that a good algal growth could result from the bacterial waste products derived during the oxidation of the hexadecanol.

Chang, et al.[11] pointed out the economic difficulties of maintaining a film and the possible hazards to health that might be caused by the bacterial decomposition of hexadecanol and octadecanol in monolayers.

Hinckley [97] found that bacteria feeding on hexadecanol films usually reached the maximum growth concentration after approximately six weeks. He recommended that hexadecanol be applied for a period of six weeks followed by a three-week lapse prior to another filming period of six weeks.

Results from this study indicate that the increased bacterial concentration in waters filmed with a continuous monolayer of "Aquasave" might have a critical effect on oxygen concentration if the oxygen were already limited and if the algal production did not compensate.

Results of this study are inconclusive, however, and the effects of bacterial growth and its relation to algae are not well understood. On the basis of the evidence presented herein, it does not appear that bacteria growth will have any affect on the oxygen resources. Additional research should be extended before the effects of bacterial growth can be delineated.

Effects of "Aquasave" on algae. Biological changes that affect water quality are usually caused or initiated by changes in the primary producers, i.e., the algae. Rapid algal growth can detrimentally affect water quality by causing water taste and odor directly or indirectly.

In the Lake Hefner study, Silvey [96] felt that a monolayer of hexadecanol was neither directly nor extensively utilized by the normal plankton biota. However, the data gathered indicate that a continuous monolayer of "Aquasave" will increase both nonfilamentous and filamentous algal growth over a "long term" thirty-day period.

Nonfilamentous algae. Throughout all three experimental replications, the green algae Chlorella was found to be the dominant nonfilamentous algae.

Figure 26 shows the increase in cell numbers of Chlorella in untreated and treated systems for the period 26 May to 24 June 1966. The population of Chlorella in the treated microcosm may also be correlated with the water turbidity shown in Figure 4. The maximum number of Chlorella cells in the untreated system was attained around the 10th day. During the same experiment, the algae population in the treated system contained fewer cells than the untreated system until the 13th day of the test. The number of Chlorella in the treated system then increased during the last half of the test to a count of 100 more cells per ml than the highest count found in the untreated system. Counts of Chlorella (and water turbidity) in the microcosms treated with "Aquasave" were found to be considerably higher than those in the untreated systems at the conclusion of the thirty-day experiment.

Counts of other algae studied during the first experiment are indicated in Table 2. Individual cell and colony counts of diatoms, Ankistrodesmus and Scenedesmus did not indicate any notable difference in the algal populations between the untreated and treated microcosms.

During the second experiment from 20 July to 18 August 1966 (series B) the increase in the numbers of Chlorella in the treated microcosms again was higher than the untreated at the conclusion of the thirty-day test (see Figure 27). Also a noticeable population of Volvox colonies (500 per ml) was present at the start of the second experiment which may have influenced oxygen, primary productivity and water turbidity for the first eight days. The increase of Chlorella

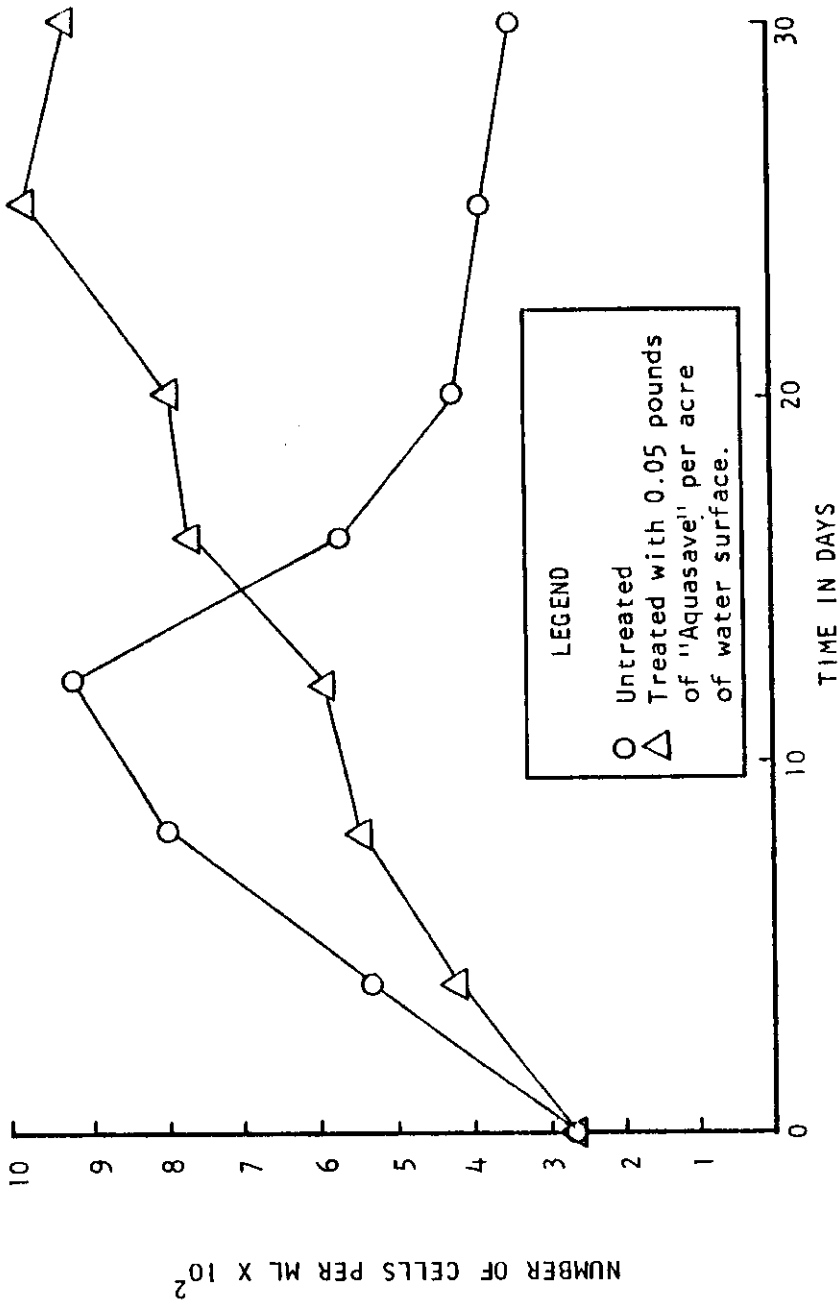


FIGURE 26. CHLORELLA CELL COUNTS IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 26 MAY TO 24 JUNE 1966.

TABLE 2

COUNTS OF DIFFERENT ALGAE FOUND IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 26 MAY TO 24 JUNE 1966. DIATOMS AND ANKISTRODESMUS COUNTED AS CELLS PER ML. SCENEDESMUS COUNTED AS COLONIES PER ML.

LEGEND

O Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Time in Days	Diatoms		Ankistrodesmus		Scenedesmus	
	O	△	O	△	O	△
1	20	20	10	10	5	5
4	20	20	15	15	10	5
8	20	20	25	15	15	5
12	20	20	30	15	25	15
16	25	20	20	20	15	15
20	25	15	20	25	10	20
25	15	10	10	35	10	30
30	10	10	10	15	5	20

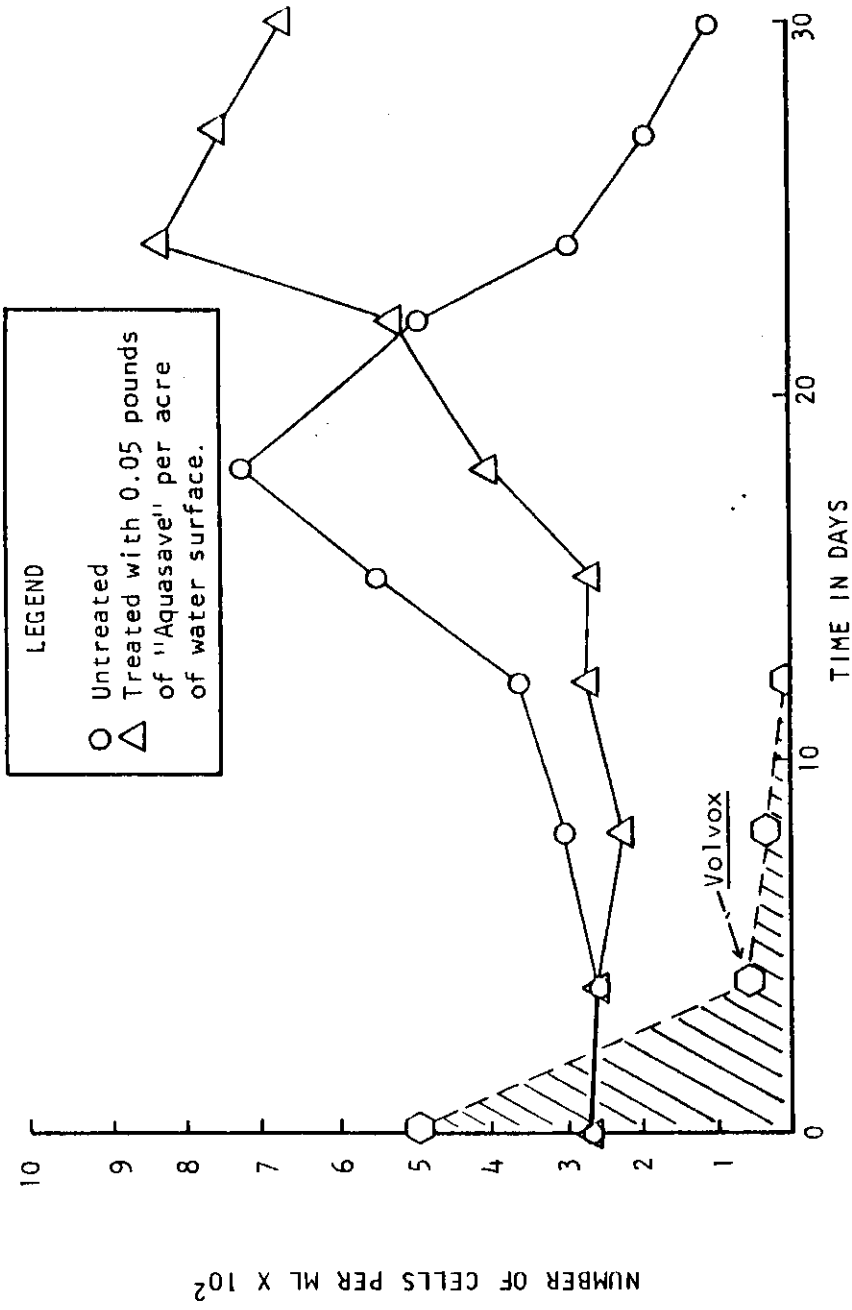


FIGURE 27. CHLORELLA CELL COUNTS IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 20 JULY TO 18 AUGUST 1966. VOLVOX COLONY COUNTS INDICATED BY LINED AREA AT LEFT OF GRAPH. NO DIFFERENCE WAS NOTED BETWEEN THE VOLVOX COUNTS IN THE UNTREATED AND TREATED ECOSYSTEMS.

in the treated system was again inhibited for the first 18 days. The Chlorella population in the treated system then increased to attain the maximum number on about the 24th day of the series. The algal populations in the untreated system reached the maximum numbers on the 18th day and then decreased in numbers for the rest of the test.

Table 3 compares the individual cell counts of diatoms and Ankistrodesmus and the colony counts of Volvox and Scenedesmus in the untreated and treated systems during experimental series B. The cell counts of Ankistrodesmus showed an increase of over 100 cells per ml in the treated microcosm during the latter part of the experiment. Volvox colonies were noticeable at the beginning of the series, but were not found after the 20th day. The colony counts of Scenedesmus did not seem to indicate any difference between the untreated and treated systems.

The third series of experiments during the period of 24 August to 22 September 1966 also showed an increase in the numbers of Chlorella. However, Figure 28 shows that the number of cells counted were below the counts found in prior experiments. The growth of Chlorella in the untreated system again peaked early in the experiment (around the 18th day) and then decreased to around 60 cells per ml for the remainder of the test. The increase of the Chlorella in the treated system seemed to be suppressed until around the 15th day. At the conclusion of the third experiment, the microcosms treated with "Aquasave" contained the greater number of Chlorella cells.

TABLE 3

COUNTS OF DIFFERENT ALGAE FOUND IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 20 JULY TO 18 AUGUST 1966. DIATOMS AND ANKISTRODESMUS COUNTED AS CELLS PER ML. VOLVOX AND SCENEDESMUS COUNTED AS COLONIES PER ML.

LEGEND

○ Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Time in Days	Diatoms		Ankistrodesmus		Volvox		Scenedesmus	
	○	△	○	△	○	△	○	△
1	20	20	5	5	500	500	10	10
4	20	20	0	5	20	11	10	5
8	15	10	10	10	10	5	5	5
12	10	10	10	5	5	0	10	5
15	20	10	20	10	5	0	10	5
18	20	20	30	20	5	0	30	5
20	20	30	30	30	---	---	20	10
22	20	30	20	30	---	---	20	20
24	20	21	15	110	---	---	10	20
27	10	20	20	150	---	---	10	10
30	10	20	10	100	---	---	10	10

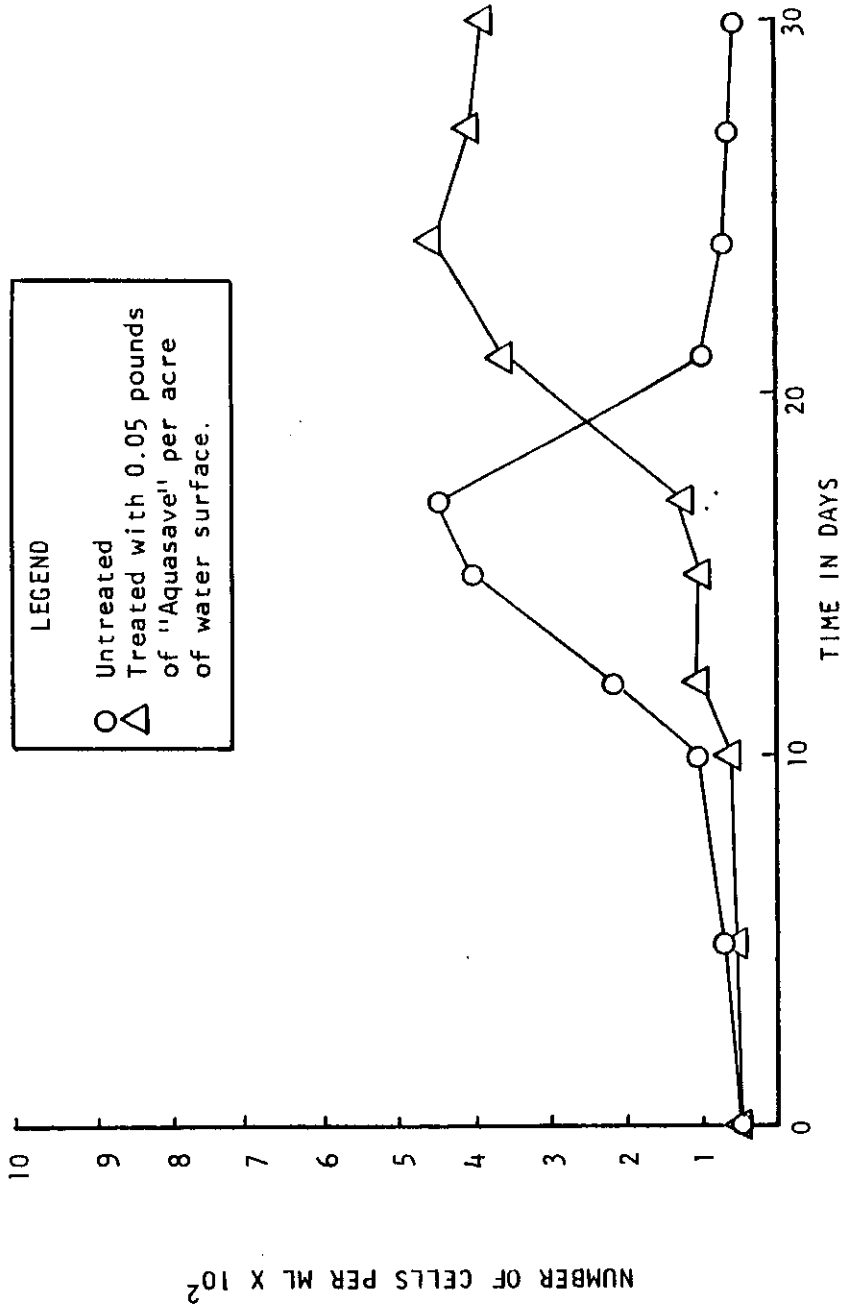


FIGURE 28. CHLORELLA CELL COUNTS IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 24 AUGUST TO 22 SEPTEMBER 1966.

Table 4 gives the count and comparison of different algae studied during the last experiment. The diatoms in the treated microcosms increased over the number found in the untreated microcosms. The growth of Ankistrodesmus in the treated aquaria was greater than that in the untreated aquaria. About midway through the experiment, a population of Phytoconis developed, but no difference in cell numbers was detected between systems. Some minor insignificant growth variations occurred in the population of Scenedesmus.

Algal counts and growth curves indicated that the number of nonfilamentous algae in the untreated systems increased during the first half of all three series of experiments. From the 12th to the 18th day the nonfilamentous algae in the untreated system would decrease in numbers while the algae in the systems treated with "Aquasave" increased in numbers.

The number of nonfilamentous algae in the untreated systems seemed to decline rapidly once the nutrient supply was depleted. The earlier growth peak in nonfilamentous algae in the untreated microcosms occurred during all three series of tests.

The "short term" effect of "Aquasave" was to inhibit the growth of Chlorella in the treated systems as compared with the early increase in the number of Chlorella in the untreated systems. The mechanism causing the "short term" effect is not understood. However, the "long term" effect of "Aquasave" may be understood. The biodegradation of the monolayer by bacteria adds nutrients to

TABLE 4

COUNTS OF DIFFERENT ALGAE FOUND IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS FROM 24 AUGUST TO 22 SEPTEMBER 1966. DIATOMS AND ANKISTRODESMUS COUNTED AS CELLS PER ML. PHYTOCONIS AND SCENEDESMUS COUNTED AS COLONIES PER ML.

LEGEND

○ Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Time in Days	Diatoms		Ankistrodesmus		Phytoconis		Scenedesmus	
	○	△	○	△	○	△	○	△
1	30	30	--	---	--	--	10	10
6	20	15	10	10	--	--	10	5
10	20	20	35	15	--	--	20	10
12	20	20	40	40	--	10	20	15
13	30	20	45	60	15	--	15	15
15	60	20	100	60	20	--	20	20
17	80	25	140	70	25	--	20	20
21	55	90	30	123	15	15	25	20
24	25	100	35	200	20	30	30	40
27	20	100	40	188	20	20	20	35
30	20	100	40	160	20	25	20	30

the treated systems that may encourage the growth of Chlorella. It appears that only certain nonfilamentous algae may utilize the bacterial waste products derived from the oxidation of the "Aquasave" film. In this study Chlorella was selective for the "Aquasave" film. Other nonfilamentous algae in the treated systems did not show a significant growth.

Filamentous algae. The filamentous algae in the untreated systems and the systems treated with "Aquasave" were compared. Growth was measured by weight loss or gain by using the complete harvest method at the conclusion of each experiment (Table 5). Cladophora in the treated microcosms had a significant growth increase when compared with the untreated microcosms.

Chara growth as measured by weight, was found to be twice as much in the treated systems as in the untreated systems. The filamentous algae were washed to remove as much of the bacterial growth as possible prior to weighing.

Anabaena were innoculated into the systems for the last experiment and no apparent growth difference was noted.

For all three series of experiments the filamentous algae in the treated systems had a higher weight gain than the filamentous algae in the untreated systems. This suggests filamentous algae may indirectly benefit from the application of a monolayer of "Aquasave."

Effects of "Aquasave" on Anacharis (Elodea). Growth of Anacharis in the untreated and treated aquaria for all three experimental periods is compared in Table 6. The Anacharis in the systems treated

TABLE 5

A COMPARISON OF FILAMENTOUS ALGAE GROWTH IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS AT THE CONCLUSION OF THE INDICATED THIRTY-DAY TEST. GROWTH IS EXPRESSED IN GRAMS.

LEGEND

○ Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Test Series	Cladophora		Chara		Anabaena	
	○	△	○	△	○	△
"A" 26 May to 24 June 1966.	22	55	6	9	-	-
"B" 20 July to 18 August 1966.	8	22	4	13	-	-
"C" 24 August to 22 Sept. 1966.	5	10	3	9	4	5

TABLE 6

A COMPARISON OF THE ANACHARIS (ELODEA) GROWTH GAIN OR LOSS IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS AT THE CONCLUSION OF THE INDICATED THIRTY-DAY TEST.

LEGEND

○ Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Test Series	"A" 26 May to 24 June 1966	"B" 20 July to 18 August 1966	"C" 24 August to 22 Sept. 1966
○ Untreated	+ 10 grams	+ 7 grams	+ 3 grams
△ Treated	+ 5 grams	+ 1 gram	- 4 grams

with "Aquasave" tended to sink to the bottom of the aquaria. A larger bacterial growth was also noted on the leaves and stems of Anacharis in the treated aquaria.

The waterweed in the treated systems did not show the growth gain that was found in the untreated systems. During the last of the three experiments (series C) Anacharis in the treated microcosms had a weight loss and did not show new growth.

Microscopic observations and weight relationships seem to indicate that a continuous monolayer of "Aquasave" is not beneficial to the growth of Anacharis under the conditions used in this study. The reduction of the water surface tension and the large bacterial concentrations found at the water surface seemed to inhibit growth.

The possibility of selective light penetration because of filtration by the monolayer should not be overlooked. A preliminary study of selective filtration using isopropanol as a solvent and "Aquasave" indicated that some fluctuation of light transmission through the "Aquasave" and solvent occurred as presented in the Appendix. Further work with specialized equipment is needed.

Effects of "Aquasave" on fish. Prior work by Hayes [78] and Wiltzius [12] indicated that a diet including hexadecanol would not affect Lepomis cyanellus (Rafinesque) (Green sunfish). They also reported no noticeable adverse effect on redear sunfish, frogs, turtles or other aquatic vertebrates observed in and around ponds treated with hexadecanol. However, these studies were conducted in

Colorado where the average water temperature is much lower and less susceptible to change than in Texas lakes and reservoirs.

Mortality records for Gambusia affinis and Fundulus notatus used in three replicate experiments are shown in Table 7. No significant difference was found between the death rate of the fish in the untreated and treated systems.

Certain observations made during the study should be noted. The mosquitofish and blackstripe topminnows schooled just below the monolayer during all three experiments. This pattern did not occur in the untreated aquaria.

When the dissolved oxygen in the treated aquaria was around 3 to 4 milligrams per liter concentration, the fish would persistently break through the surface film. It appeared that they were attempting to gain more oxygen by this procedure. Similar observations were made for the second and third series of experiments.

While hexadecanol and octadecanol may be incorporated in the diet of fish without harm, the indirect effect of the film on dissolved oxygen may present the greatest potential danger to fish.

The application of a monolayer of "Aquasave" or similar films to slightly polluted or warm shallow lakes or reservoirs may result in oxygen deficiencies sufficient to give fish kills.

TABLE 7

THE NUMBER OF DEATHS RECORDED FOR TWO SPECIES OF FISHES IN UNTREATED AND TREATED AQUATIC EXPERIMENTAL ECOSYSTEMS AT THE CONCLUSION OF THE INDICATED THIRTY-DAY EXPERIMENT.

LEGEND

○ Untreated

△ Treated with 0.05 pounds of "Aquasave" per acre of water

Note. Six Gambusia affinis and six Fundulus notatus were introduced into each 20-gallon aquarium prior to the beginning of each experiment.

Test Series	"A" 26 May to 24 June 1966		"B" 20 July to 18 August 1966		"C" 24 August to 22 Sept. 1966	
	○	△	○	△	○	△
Gambusia affinis	0	3	1	2	2	1
Fundulus notatus	1	1	0	0	2	0

CHAPTER V

SUMMARY AND RECOMMENDATIONS FOR FURTHER STUDIES

Summary. The effects of a continuously applied evaporation retardation monolayer of hexadecanol and octadecanol ("Aquasave") were evaluated using aquatic microcosms. The studies were carried out in the Texas A&M University Environmental Engineering Laboratory. No significant water temperature, pH, hardness or alkalinity changes occurred in the experimental ecosystems.

The growth of organisms in untreated and treated microcosms influenced water turbidity. At the conclusion of all thirty-day experiments the turbidity was found to be higher in the systems treated with a monolayer of "Aquasave." The long-chain alcohol film also reduced the water surface tension and caused some filamentous algae and Anacharis to sink rather than float normally at the surface.

At 20°C the evaporation retardation monolayer reduced the oxygen diffusion rate approximately 10 to 15%. Diffusion rate studies indicated that under certain conditions serious oxygen deficiencies might occur in systems treated with "Aquasave."

A continuous monolayer of "Aquasave" was evaluated at the ecosystem level by measurements of the effects on the primary producers, i.e., the algae. A film of "Aquasave" was found to decrease the oxygen transfer, inhibit algal growth and reduce primary productivity for a "short term" effect (1 to 15 days) when compared with

the algal growth (same forms) and primary productivity in the untreated systems. However, over a longer term (15 to 30 days) the systems treated with "Aquasave" displayed higher oxygen values, increased the growth of some algal species and increased primary productivity when compared to the controls. This shows that a monolayer will inhibit primary productivity on the "short term" basis and encourage algal growth and primary productivity over a "long term" under the conditions used in this study.

Biological degradation of the evaporation suppressant film resulted in increased growth in bacterial populations. A significant increase in the growth of bacteria was found for all three thirty-day experiments. Bacterial increase caused by a monolayer of hexadecanol and octadecanol could present a problem by demands on a limited oxygen supply.

Biotic changes in the experimental ecosystems were evaluated by comparison and enumeration of phytoplankton populations in untreated and treated systems. Nonfilamentous algae in the untreated systems were found to increase in numbers during the first half of all experiments. Nonfilamentous algae in the systems treated with "Aquasave" seemed to have inhibited growth for the first half of the thirty-day experiments but increased in numbers during the latter half of the experiments. At the conclusion of all experiments, the growth of the nonfilamentous algae in the systems treated with "Aquasave" were significantly higher than the numbers of the same algal forms found in the untreated systems.

Filamentous algae in the systems treated with a monolayer had better growth than the same algae in the untreated systems.

A monolayer of "Aquasave" was detrimental to the growth of the waterweed Anacharis. Less new growth was found for the Anacharis in the treated systems.

No significant effect of "Aquasave" was noted for two species of fishes Gambusia affinis and Fundulus notatus. Observations indicate that the indirect effect of dissolved oxygen deficiencies could prove dangerous to fish life.

Water quality studies and biological analyses should be conducted on reservoirs or lakes prior to filming with hexadecanol and octadecanol monolayers. The ecology of the aquatic environment to be filmed must be understood prior to indiscriminate application of an evaporation retardation monolayer.

Determination of dissolved oxygen, primary productivity and diurnal oxygen cycles are mostimportant. Identification and enumeration of bacteria, algae and aquatic plants will determine the impact of the "short term" and "long term" effects of a continuous monolayer of "Aquasave" upon the ecosystem.

Recommendations for further studies. Additional laboratory studies are needed to understand the effects caused by a continuous monolayer of hexadecanol and octadecanol. Larger experimental systems and greater temperature ranges would give additional knowledge on dissolved oxygen and primary productivity in filmed systems.

Additional studies are needed on the effects of a hexadecanol and octadecanol monolayer on a larger number of algal species. Chemical and physical conditions as well as biological populations and primary productivity may differ from locality to locality dependent upon the location of the individual reservoir or lake.

Patterns and trends established in laboratory studies should be correlated with simultaneous field measurements of the source materials. For example, the growth of Chlorella in the laboratory systems should be compared with that in the reservoir used as the source of the micro-organism.

Further investigations are needed on the effects of hexadecanol and octadecanol monolayers on bacteria. In light of the results of diurnal oxygen variation and primary productivity, growth of algae seems to offset any effect of the bacteria on the oxygen resources. Extended studies (6 to 12 months) to encourage the development of large bacterial concentrations, using a continuous film, should give additional information on the oxygen demand exerted by increased bacterial growth.

REFERENCES

1. Smerdon, E. T. "Water Development and the Agricultural Sciences." Agricultural Science Review, 4 (1966).

2. "Hydrology." Time, 86, 70 (Oct. 1, 1965).

3. Eaton, E. D. Control of Evaporation Losses. (Committee Print No. 6, 85th Congress, 2nd Session). U. S. Govt. Printing Office, Washington, D. C. (1958).

4. Mansfield, W. W. "Influence of Monolayers on the Evaporation of Water." Nature, 175, 247 (1955).

5. Cruse, R. R., and Harbeck, G. E. Evaporation Control Research, 1955-58. (Geo. Survey Water-supply Paper 1480). U. S. Govt. Printing Office, Washington, D. C. (1960).

6. Timblin, L. O., Florey, Q. L., and Garstka, W. U. "Laboratory and Field Reservoir Evaporation Reduction being Performed by The Bureau of Reclamation." In: LaMer, V. K. (ed.). Retardation of Evaporation by Monolayers: Transport Processes. Academic Press, New York, 177 (1962).

7. Meinke, W. W., and Waldrip, W. J. Research on Evaporation Retardation in Small Reservoirs, 1958-63. (Tex. Water Commission Bull. 6401)., (1964).

8. Durham, K., and McArthur, I. K. H. "The Influence of Dosage on the Efficiency of Films of Fatty Alcohols for Water Conservation." Research, 10, 291 (1957).

9. Gilby, A. R., and Heymann, E. "The Rate of Evaporation of Water through Duplex Films." Austral. Jour. Sci. Res., 1, 197 (1948).

10. Ludzack, F. J., and Ettinger, M. B. "Biological Oxidation of Hexadecanol under Laboratory Conditions." Jour. Amer. Water Works Assn., 49, 849 (1957).

11. Chang, S. L., McClanahan, M. A., and Kabler, P. W. "Effect of Bacterial Decomposition of Hexadecanol and Octadecanol in Monolayer Films on the Suppression of Evaporation Loss of Water." In: LaMer, V. K. (ed.). Retardation of Evaporation by Monolayers: Transport Processes. Academic Press, New York, 119 (1962).

12. Wiltzius, W. J. Evaluation of the Influences of Long-Chain Alcohol Monomolecular Films on Insects, Fish, and Wildlife. (Contract No. 14-06-D-3777). U. S. Bureau of Reclamation, (1965).

13. Odum, H. T., and Hoskins, C. M. "Metabolism of a Laboratory Stream Microcosm." Publ. Inst. Mar. Sci. Univ. Tex., 4, 115 (1957).

14. Beyers, R. J. "The Metabolism of Twelve Aquatic Laboratory Microecosystems." Ecol. Monogr., 33, 281 (1963).

15. Davies, J. T., and Rideal, E. K. Interfacial Phenomena. Academic Press, New York, 217 (1963).

16. Pockels, A. "Surface Tension." Nature, 43, 437 (1891).

17. Lord Rayleigh, Third Baron, John William Strutt. "Investigations in Capillarity." Phil. Mag., 48, 331 (1899).

18. Devaux, H. E. "Oil Films on Water and on Mercury." Summary of 1903-1913 Researches on Oil Films. In: Ann. Rep. Smithsonian Inst., 261 (1913).

19. Abbe, C. "Prevention of Fog." Monthly Weather Rev. (U. S. Weather Bureau), 42, 104 (1914).

20. Hardy, W. B. "The Tension of Composite Fluid Surfaces and the Mechanical Stability of Films of Fluid." Proc. Roy. Soc., Ser. A, 86, 610 (1912).

21. Hardy, W. B. "The Tension of Composite Fluid Surfaces-No. 2." Proc. Roy. Soc., Ser. A, 88, 303 (1913).

22. Langmuir, I. "The Shapes of Group Molecules Forming the Surfaces of Liquids." Proc. Nat. Acad. Sci., 3, 251 (1917).

23. Devaux, H. "La Permeabilite des Lames Minces. Etude de l'influence sur l'evaporation des Huiles et des Lames Minces Solides et Liquides." Conference, 20 mai 1921. Societe Francaise de Physique, 23 (1921).

24. Hedestrand, G. "The Influence of Thin Surface Films on the Evaporation of Water." Jour. Phys. Chem., 28, 1244 (1924).

25. Rideal, E. K. "On the Influence of Thin Surface Films on the Evaporation of Water." Jour. Phys. Chem., 29, 1585 (1925).

26. Langmuir, I., and Langmuir, D. B. "The Effect of Monomolecular Films on the Evaporation of Ether Solutions." Jour. Phys. Chem., 31, 1719 (1927).

27. Langmuir, I. "Oil Lenses on Water and the Nature of Monomolecular Expanded Films." Jour. Phys. Chem., 1, 756 (1933).

28. Baranaev, M. K. "The Effect of Surface Layers of Insoluble Substances on the Rate of Evaporation of Water." (In Russian) Zhurnal Fizicheskoi Khimii (Moskva), 9, 69 (1937).

29. Sklyarenko, S. I., and Baranaev, M. K. "Effect of Films of Surface-active Substances on the Rate of Evaporation of Solutions." (In Russian) Zhurnal Fizicheskoi Khimii (Moskva), 12, 271 (1938).

30. Kheinman, A. S. "Effect of Surface Films on the Rate of Evaporation of Water and Aqueous Solutions." (In Russian) Zhurnal Fizicheskoi Khimii (Moskva), 14, 118 (1940).

31. Docking, A. R., Heymann, E., Kerley, L. F., and Mortensen, K. N. "Evaporation of Water Through Multimolecular Films." Nature, 146, 265 (1940).

32. Sebba, F., and Briscoe, H. V. A. "The Evaporation of Water Through Unimolecular Films." Jour. Chem. Soc. (London), 106 (1940).

33. Langmuir, D. B., and Schaefer, V. J. "Rates of Evaporation of Water Through Compressed Monolayers on Water." Jour. Franklin Inst., 235, 119 (1943).

34. Heymann, E., and Yoffe, A. "The Stability of Multimolecular Films of Hydrocarbon Oils, Containing Spreaders, on Water Surfaces." Trans. Faraday Soc., 38, 408 (1942).

35. Heymann, E., and Yoffe, A. "The Equilibrium between Lens and Unilayer in the System Hydrocarbon Oil-oleic Acid-water in Relation to the Interfacial Film." Trans. Faraday Soc., 39, 217 (1943).

36. Mansfield, W. W. "The Effect of Surface Films on the Evaporation of Water." Nature, 172, 1101 (1953).

37. Archer, R. J., and LaMer, V. K. "The Effect of Monolayers on the Rate of Evaporation of Water." Ann. New York Acad. Sci., 58, 807 (1954).

38. Archer, R. J., and LaMer, V. K. "The Rate of Evaporation of Water Through Fatty Acid Monolayers." Jour. Phys. Chem., 59, 200 (1955).

39. Dressler, R. G. "The Southwest Cooperative Research Project on Reservoir Evaporation Control." In: Proc. First International Conf. on Reservoir Evaporation Control, San Antonio, Texas. Southwest Research Inst. (1956).

40. Dressler, R. G. "Fifth Monthly Report." Southwest Cooperative Committee on Reservoir Evaporation Control, (March 1956).

41. Dressler, R. G., and Johnson, A. G. "Water Reservoir Evaporation Control." Chem. Eng. Prog., 54, 66 (1958).

42. Freese, S. W. "Reservoir Evaporation Control by Other Techniques." In: Proc. First International Conf. on Reservoir Evaporation Control, San Antonio, Texas. Southwest Research Inst. (1956).

43. Chemical and Engineering News. "Chemical Shields to Prevent Water Evaporation." 35, 28 (1957).

44. U. S. Bureau of Reclamation. "The Effect of Surface Tension Upon the Flotation of Small Beads of Hexadecanol." Chem. Eng. Lab. Rept. SI-8, (1957).

45. U. S. Bureau of Reclamation. "Reservoir Evaporation Reduction with Monomolecular and Similar Films - Progress Report March 1955 Through March 1956." Chem. Eng. Lab. Rept. SI-9, (1957).

46. U. S. Bureau of Reclamation. "Reservoir Evaporation Control - Screening Tests on Monolayers and Duplex Films." Chem. Eng. Lab. Rept. SI-12, (1957).

47. Harbeck, G. E., Jr. "Evaporation Suppression in Laboratory and Field." Hydraulics Conf. Iowa Inst. for Hydraulics Research, 7th, Proc. (June 16-18, 1958).

48. Harbeck, G. E., Jr., and Koberg, E. G. "A Method of Evaluating the Effect of a Monomolecular Film in Suppressing Reservoir Evaporation." Jour. Geophys. Research, 64, 89 (1959).

49. Timblin, L. O., Jr., Moran, W. T., and Garstka, W. U. "Use of Unimolecular Layers for Reservoir Evaporation Reduction." Jour. Amer. Water Works Assn., 49, 841 (1957).

50. Committee of Collaborators. Water-Loss Investigations: Lake Hefner 1958 Evaporation Reduction Investigation. U. S. Bureau of Reclamation, Denver, Col., 93 (1958).

51. Rosano, H. L., and LaMer, V. K. "The Rate of Evaporation of Water Through Monolayers of Ethers, Acids and Alcohols." Jour. Phys. Chem., 60, 348 (1956).

52. Mansfield, W. W. "The Use of Hexadecanol for Reservoir Evaporation Control." In: Proc. 1st Int. Conf. on Reservoir Evaporation Control, San Antonio, Texas. Southwest Research Inst., 13 (1956).

53. Mansfield, W. W. "The Influence of Monolayers on Evaporation from Water Storages. I. The Potential Performance of Monolayers of Cetyl Alcohol." Austral. Jour. Appl. Sci., 9, 245 (1958).

54. McArthur, I. K. H., and Durham, K. "Fatty Alcohols for Water Conservation." In: Proc. 2nd. Int. Congr. of Surface Activity., Butterworths Sci. Publications, London, 1, 262 (1957).

55. Magin, G. B., and Randall, L. E. "Review of Literature on Evaporation Suppression. (Geol. Survey Prof. Paper 272-C), Washington, D. C. (1960).

56. Hellstrom, B., and Janson, L. E. Evaporation from Water Surfaces Coated with a Film of Stearyl Alcohol. Div. of Hydraulics, Royal Inst. of Technol. (Bull. No. 57), Stockholm, 18 (1959).

57. Genet, E., and Rohmer, R. "L'economie de l'ean: lutte contre l'evaporation des nappes d'eau libre." Terres et Eaux, 36, 1 (1961).

58. Meinke, W. W., Waldrip, W. J., Stiles, G. B., and Harris, W. D. "Reducing Reservoir Evaporation by the Use of Monomolecular Films." Water Works Eng'g., 115, Pt. 1:4, 274 (1962).

59. Meinke, W. W., Waldrip, W. J., Stiles, G. B., and Harris, W. D. "Reducing Reservoir Evaporation by the Use of Monomolecular Films." Water Works Eng'g., 115, Pt. 11:5, 344 (1962).

60. LaMer, V. K. (ed.). Retardation of Evaporation by Monolayers: Transport Processes. Academic Press, New York (1962).

61. Vines, R. G. "Evaporation Control: A Method of Treating Large Surfaces." In: LaMer, V. K. (ed.). Retardation of Evaporation by Monolayers: Transport Processes. Academic Press, New York, 137 (1962).

62. Mansfield, W. W. "Spreading from Solid Hexadecanol." Austral. Jour. Chem., 16, 78 (1963).

63. Resnick, S. D., and Cluff, C. B. Evaporation Reduction Investigations on Small Reservoirs. Paper Presented at ASCE Second Water Res. Eng. Conf., Milwaukee, Wisc. (1963).

64. Shukla, R. N., Deo, A. V., Gharpurey, M. K., and Biswas, A. B. "Some Observations on the Water Evaporation Reduction due to Monolayers of N-Fatty Alcohols, N-Alkoxy Ethanols and their Mixtures." Indian Jour. Technol., 1, 141 (1963).

65. Katti, S. S., George, K. V., Deo, A. V., Sanjana, N. R., and Gharpurey, M. K. "Evaporimeter Studies on Water Evaporation Reduction due to Monomolecular Films during Day and Night." Indian Jour. Technol., 2, 81 (1964).

66. Deo, A. V., et al. "Film Pressure-Area Isotherms of Monolayers of Normal Long-chain Alcohols and Normal Alkoxyethanols on WaterSurface." Indian Jour. Chem., 2, 45 (1964).

67. Trapeznikov, A. A., and Avestisyan, R. A. "Wave and Time Effect on the Ability of a Unimolecular Layer to Lower Water Evaporation." (In Russian) Dokl. Akad., Nank. SSSR., 158, 945 (1964).

68. Trapeznikov, A. A., and Ogarev, V. A. "Use of a Single Layer of Synthetic Alcohols for the Reduction of Evaporation of Water." (In Russian) Zh. Prikl. Khim., 37, 8 (1964).

69. Dressler, R. G. "The Suspension Process for Reservoir Evaporation Control." Ind. Chem. Eng., 56, 36 (1964).

70. LaMer, V. K., Healy, T. W., and Aylmore, L. A. G. "Evaporation of Water: Its Retardation by a Monolayer." Science, 148, 36 (1965).

71. Myers, L. E. "Evaporation Retardants: Application by Means of a Water Soluble Matrix." Science, 148, 70 (1965).

72. Florey, Q. L., Backstrom, T. E., and Ensign, T. F. "1959 Monolayer Behavior Studies at Lake Mead Arizona - Nevada, and 1959 Monolayer Behavior Studies at Sahuaro Lake, Arizona." In: 1960 Evaporation Reduction Studies at Sahuaro Lake, Arizona, and 1959 Monolayer Behavior Studies at Lake Mead, Arizona - Nevada and Sahuaro Lake, Arizona. (Chem. Eng. Lab. Rept. No. S1-32). U. S. Bureau of Reclamation, Denver, Colo., 20 (1961).

73. Mihara, Y. Practical Use of Mono- and Multi-molecular Films for Evaporation Control. Paper Presented at the UNESCO/CSIR Symposium on Water Evaporation Control, Poona, India, (1962).

74. Newkirk, H. D. "Film Application." In: Aerial Application-Technique Development and Monolayer Behavior Study: Elephant Butte Reservoir-1962. (Water Cons. Lab. Rept. No. WC-1). U. S. Bureau of Reclamation, Denver, Colo., 15 (1963).

75. Newkirk, H. D. "Strip Tests for Comparison of Spreading Rates." In: Aerial Application-Technique Development and Monolayer Behavior Study: Elephant Butte Reservoir-1962. (Water Cons. Lab. Rept. No. WC-1). U. S. Bureau of Reclamation, Denver, Colo., 59 (1963).

76. Frenkiel, J. Evaporation Reduction. UNESCO, Place de Fontenoy, Paris, France (1965).

77. "Cutting Evaporation Losses on Farm Ponds." Successful Farming, 55, 60 (1957).

78. Hayes, M. L. Biological Effects of Hexadecanol Used to Suppress Water Evaporation from Reservoirs. Colo. State Univ., Fort Collins, Colo. (1959).

79. Timblin, L. O. "Preliminary Toxicity Studies with Hexadecanol Reservoir Evaporation Reduction." (Chem. Eng. Lab. Rept. No. Sl-10). U. S. Bureau of Reclamation, Denver, Colo. (1957).

80. "Evaporation Control." Western Water News, 10, 4 (1958).

81. Welch, P. S. Limnological Methods. The Blakiston Co., Phila. (1948).

82. Lagler, K. F. Freshwater Fishery Biology. Wm. C. Brown Co., Dubuque, Iowa (1952).

83. Ward, H. B., and Whipple, G. C. Fresh-water Biology, John Wiley & Sons, New York (1945).

84. Needham, J. G., and Needham, P. R. A Guide to the Study of Fresh-water Biology. Holden-Day, Inc., San Francisco, Calif. (1962).

85. Palmer, C. M. Algae in Water Supplies. (Public Health Ser. Publ. No. 657). U. S. Govt. Printing Office, Washington, D. C., (1962).

86. Timblin, L. O., Jr. "Determination of the Presence and Degree of Compression of a Monolayer Layer Using Indicator Oils." (Lab. Rept. No. SI-13). U. S. Bureau of Reclamation, Denver Colo. (1957).

87. Standard Methods for the Examination of Water and Wastewater. 12th Ed., Amer. Pub. Health Assn., New York (1965).

88. Umbreit, W. W., et al. Manometric Techniques. Burgess Publ. Co., Minneapolis, Minn. (1964).

89. Rabinovich, V. A., and Sherman, E. E. "Modification of Winkler's Method for Determining Dissolved Oxygen in Small Liquid Samples." (In Russian) Rol Mikroorganizmov v Obrazov. Zhelezo-Mergant-Sevykh Ozern. Rud, Akad, Nauk SSSR, Lab. Gidrogeol., 81 (1964).

90. Ohle, W. "Die Chemische und Electrochemische Bestimmung des Molekular gelosten Sauerstoffes der Binnengewasser." Mitt. Int. Ver. Theor. u. Ang. Limnol., 3, 1 (1953).

91. Mpelkas, C. C. "Gro-Lux Wide Spectrum Fluorescent Lamp." (Eng. Bull. O-285). Sylvania Electric Products, Inc. (1965).

92. Barnes, H. Apparatus and Methods of Oceanography. Interscience Publishers, Inc., New York (1959)

93. Eyles, D. E. A Guide and Key to the Aquatic Plants of Southeastern United States. U. S. Public Health Service, Public Health Bull. No. 286, Washington, D. C. (1963).

94. Moore, G. A. "Fishes." In: Vertebrates of the United States. McGraw-Hill Book Co., Inc., New York (1957).

95. Mahmoud, T. "The Effects of Monomolecular Films on Oxygen Transfer Across an Air-Water Interface." Master's Thesis. Texas A&M University, College Station, Texas (1967).

96. Silvey, J. K. G. "Physical, Chemical and Biologic Effects on Hexadecanol on Lake Hefner, 1958." Jour. Amer. Water Works Assn., 52, 791 (1960).

97. Hinckley, N. "Bacterial Degradation of Hexadecanol." Master's Thesis. North Texas State College, Denton, Texas (1957).

APPENDIX

TABLE 8

KLETT-SUMMERSON PHOTOELECTRIC COLORIMETER MEASUREMENT OF PERCENT LIGHT TRANSMITTANCE THROUGH ISOPROPANOL AND "AQUASAVE" IN ISOPROPANOL.

SPECTRAL RANGE	PERCENT TRANSMITTANCE	
(Millimicrons)	Isopropanol Blank	"Aquasave" in Isopropanol
660 to 740	97.5%	100.0%
590 to 660	99.3%	100.0%
540 to 590	100.0%	99.7%
520 to 580	100.0%	99.5%
470 to 530	100.0%	98.6%
410 to 490	100.0%	98.2%
400 to 450	100.0%	97.0%
380 to 430	100.0%	96.6%

Note. All determinations given as percent light transmittance represent the mean of three separate measurements.