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AN INTEGRATED SYSTEM FOR THE CONVERSION OF SOLAR ENERGY WITH SEWAGE GROWN MICROALGAE

Final Report for October 1, 1976—September 30, 1977

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ABSTRACT

An integrated system for wastewater treatment and microalgae biomass production for conversion to fuels is proposed. The economically limiting factor of such a system is the harvesting of the microalgae. Microstrainers, rotating fine mesh screens, can harvest the larger, filamentous or colonial microalgae at low cost. To apply microstraining to sewage treatment ponds requires establishment of environmental conditions that allow large microalgae types to predominate to the exclusion of the smaller, mostly unicellular, microalgae commonly found in such ponds.

A series of experimental (12 m^2) rectangular, shallow (25 cm) high rate ponds mixed with paddle wheels were operated at varying detention times, mixing speeds and biomass recycle rates to determine the pond operation conditions most favorable to maintaining algae cultures retained by 26 μ screens. At short detention times the microalgae cultures invariably became unharvestable; larger colonial algae were favored by relatively longer detention times. However, long detention times also reduced algae biomass production rates. Selective biomass recycling has been demonstrated to be an effective method of species control under specific conditions in the laboratory. In outdoor growth ponds biomass recycling is only marginally effective and also causes decreases in productivity. Increased mixing speeds (15 cm/sec) had a positive effect on harvestability of the cultures, however, they were not sufficient to allow efficient microstraining of cultures from short detention time-high productivity ponds. Higher mixing speeds tended to induce flocculation of microalgae.

The microalgae cultures suffered from instability due to zooplankton grazing, particularly in the summer. Coarse screens (150 μ) were used to prevent zooplankton blooms, but they were not successful. Zooplankton apparently helps algae culture harvestability due to preferential grazing of smaller algae. However, this effect is counteracted by loss of culture density and productivity. Short detention time ponds suffered less from zooplankton predation. Few, if any correlations could be made regarding species control; Micractinium replaced Scenedesmus from spring to summer in all ponds. Break-up or formation of the colonies of these algae determined relative dominance. Over a ten month period the highest productivity ponds averaged 13.4 gm/m^2/day. However, the most harvestable ponds averaged only 8.5 gm/m^2/day of which 7.2 gm/m^2/day was removed by the microstrainer. Overall, the experiments demonstrated that certain pond operations lead to predominance of microstrainable algae culture, however, optimization with biomass productivity was not achieved.

Effluents from these ponds were used to grow a second crop of algae in either batch or continuous cultures. The objective was to produce a low ammonia effluent suitable to grow nitrogen-fixing blue-green algae. This was achieved by either settling or microstaining the second algae crop. Ultimately a multi-pond system capable of advanced waste treatment is envisioned.

A large scale, 0.25 hectare pilot pond was operated and the effect of detention time on algae size control verified on a larger scale. In this large pond up to 19 gm/m^2/day were observed over an 18 day period during August-September at a detention time of 3 days. Also, zooplankton grazing was not noticeable to the extent observed in the small ponds; this must be verified by further experiments.

ACKNOWLEDGMENTS

The authors wish to acknowledge Dr. Roscoe Ward, Chief of Fuels From Biomass Program, Department of Energy, for his interest in this work. We thank Nick Grisanti, Thomas Tiburzi, Mary Riddle, Richard Spindel and Ben Hashel for help at various stages of the project, and Ms. Briggs Nisbet for typing the report. The staff of the Sanitary Engineering Research Laboratory and its director, Professor David Jenkins, are gratefully acknowledged for providing support and facilities.

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I. INTRODUCTION

Two major interelated problems confronting the United States and, indeed, much of the world, are a diminishing supply of fossil energy resources and the pollution of the environment with wastes. It is now a matter of urgent national concern that the discovery of new domestic natural gas reserves and production from existing sources is diminishing rapidly, and even now the actual supply of domestic natural gas is inadequate to meet our demand. Conventional waste treatment processes are energy intensive and often result in environmentally undesirable pollutants. A simultaneous solution to parts of these two problems is the subject of this project. The project deals with the development of an algal biomass production and conversion system which transforms solar energy and sewage into methane gas, reclaimed water and fertilizer. The potential economic advantage of this process over other proposed biomass production systems (e.g. terrestrial and ocean energy farms) consists of its integration with liquid waste treatment. This would allow a major portion of the biomass and methane costs to be covered by waste treatment credits. In addition, no higher uses for the algal biomass are apparent (at least in municipal sewage treatment). Thus there are no competitive non-energy demands for the biomass.

The production of methane has been associated with organic decomposition for about 100 years and applied as an art for over more than 75 years in sewage sludge digestion (1,2). The overall mechanisms of methane production by specific microorganisms have been elucidated for almost 40 years (3,4). Since 1920, methane from fermentation of sewage sludge has been widely utilized as a fuel for gas engines and for heating in sewage treatment plants (5). With this backlog of experience with methane production through fermentation, it is not surprising that anaerobic digestion has been the object of a surge in interest since the advent of current energy shortages.

The trouble is that, even complete methane fermentation of all of the

nation's day-by-day organic wastes, followed by a highly efficient use of the methane for energy, would meet less than 2% of the U.S. energy needs. Although limited by amounts of sewage (particularly its carbon content), methane production by algal biomass systems would be severalfold higher than achievable by digestion of raw sewage sludge. Through wastewater carbonation with power plant or other CO_2 emissions and through recycling of nutrients such as nitrogen and phosphorus, microalgae biomass systems could be theoretically expanded to any desirable size.

During earlier studies on the growth of algae on sewage(6-8) research was initiated on digestion of algae (9) and it was found that more than 50% of the light energy fixed in algae could be released in the form of methane. Although, during the 1960's, little evidence of national interest in the use of microalgae for energy production was apparent, the use of algae as a source of oxygen for waste oxidation and as a possible source of food or animal feed (10) and as part of life support systems in spacecraft (11) received considerable attention. As oxygen producers for waste oxidation, algae are the most efficient and economical agents presently available to man (12). Their efficiency in waste oxygenation is high because the oxygen produced in photosynthesis is directly available to oxidizing bacteria as dissolved oxygen without the problems of oxygen transfer from air to water. They are economical, as evidenced by their widespread use, because the energy for oxygen release to the water comes directly from solar energy. Photosynthetic oxygen produced by microalgae growing in waste to be oxidized costs less than one-fourth as much as oxygen introduced by the most economical mechanical means of waste oxygenation (13). Due to the rapid rise, since 1970, of fossil fuel prices, particularly natural gas, there has been renewed interest in processes by which microalgae and bacteria can be utilized to transform solar energy into the chemical energy of methane.

This led to a National Science Foundation-Research Applied to National

Needs-supported project which investigated different algae as substrates for methane fermentation. The results of that study (14) can be summarized as follows: The efficiency of the fermentation system for converting algal (Scenedesmus) biomass energy to methane ranged from 28 to 44.5%. The process suffers from ammonia feedback inhibition at higher loading rates. Nitrogen and other nutrients were retained in the digester. In experiments where 30% of other algae were mixed with Scenedesmus, the fermentability of Spirulina and Melosira appeared to be higher and Euglena and Micractinium similar to that of Scenedesmus. The fermentability of algae was a reliable process, no digester upsets were noted. The conclusions reached from that study were that algae, including the filamentous blue-greens, are suitable substrates for methane fermentation. Future research is required since these studies included only short term experiments and used cultures of dried or frozen algae.

Algae harvesting is the key technical-economic problem, not only for biomass production, but also in terms of Public Law 92-500, which dictates removal of solids in effluents (including algae) to 10 to 30 parts per million by 1977. Therefore, costs of algae harvesting will also be borne by waste treatment, and algal disposal credits could defray fermentation costs of the final methane gas product. Chemical flocculation processes, although expensive, have become the prevalent methods of large-scale algae harvesting because they are now the only reliable method aside from centrifugation. However, these methods were unsuitable for the purpose of this project because of large amounts of inorganic chemicals such as alum or lime required for flocculation. This makes the overall process prohibitively expensive and energy intensive.

Microstraining is an effective and economical method for algal removal from water supplies (15,16,17) and, as recently demonstrated at Clear Lake, California, for nuisance algal removal from lakes (18,19). However,

harvesting pond effluents with a microstrainer usually results in only small amounts of algae being removed (18,20,21,22). The reason for the effectiveness of microstraining water supplies lies in the difference in algal populations: filamentous and colonial algae often predominate in clean water reservoirs and natural bodies of water, whereas single-cell algae are the common form in sewage ponds. Because only filamentous or colonial algae are effectively harvested by microstraining (single-cell algae pass through or clog the screen fabric) use of this harvesting technique requires establishment of such algae populations in waste oxidation ponds. Filamentous blue-green or colonial green algae are often found in waste ponds, sometimes even predominating, however it is not yet known how such algae can be made to permanently become the predominant or exclusive type.

A review of the literature on algal populations in ponds does not reveal clear or obvious patterns. Although several qualitative surveys exist (23,24), the factors responsible for such algal population composition are not clear. There are no obvious geographical correlations and the algal population changes in waste oxidation ponds (as in natural bodies of water)are not reliably predictable. Although Euglena is often reported as predominating in winter and the filamentous Oscillatoria or colonial Microactinium frequently are bloom formers in spring and summer, these guidelines fail to apply in many (perhaps most) cases. One useful generalization is that when wastes are not yet stabilized there are fewer distinct species present, often effectively unialgal cultures are observed, while in ponds containing well-stabilized wastes, there is a relatively larger variety of algae genera. The list of the five most common algae genera found in ponds includes the single-cell type Chlorella, Scenedesmus, Euglena, Ankistrodesmus and the filamentous blue-green alga Oscillatoria.

Many reports from operating oxidation ponds contain mention of filamentous blue-green algal blooms, sometimes manifested by noxious scums of decomposing algae. In Windhoeck waste reclamation project study, a large colonial Micractinium

bloom was harvested for a few weeks by microstraining (25). Oscillatoria is often reported to form massive blooms at particular times. For example, Oscillatoria appeared in the Modesto (California) pond system during periods of large cannery waste flows. Occasionally almost pure Oscillatoria populations were observed at the Napa and Woodland oxidation ponds. Cultivation of Oscillatoria on organic wastes in the laboratory has been reported (26).

The occasional prevalence and frequent appearance of such microstrainable algae in presently operating ponds gave reason to believe that such algae could be encouraged and maintained by careful management and control of pond operations. However, even the more quantitative studies available on algal populations and pond operational parameters were insufficient to allow correlation between the algal population and the pond environment. The vast literature on algal ecology is, likewise, not directly applicable to waste pond systems. However, the more general findings, particularly with regard to nutrient preferences and competitive advantages of blue-green or green algae should hold as well in waste ponds as in other systems. Thus, pH, sodium, temperature, Fe, and other micronutrients seem to affect blue-green to green algae ratios and might be used for that purpose (27-30).

The possibility of developing microalgae species control techniques that would allow the selective cultivation in waste treatment ponds of algal species that could be cheaply harvested with microstrainers, and the potential of the harvested microalgal biomass in energy production, led to the ERDA contract in November 1975 "Species Control in Large-Scale Algal Biomass Production" (31). A basic premise of the project was that only high rate oxidation ponds permit sufficient control over the sewage pond environment to allow the establishment of the uniform and controllable conditions necessary to develop and apply microalgal species control techniques. High rate oxidation ponds differ from the more usual facultative oxidation ponds in being shallow (about 1 foot depth versus 3 to 6 feet

deep) and well mixed. Initially circular 3 m^2 ponds, mixed with paddle wheels were set up and operated throughout the spring and summer of 1976. Effluents from the ponds were fed through microstrainers and the harvestability of the algae determined. The initial inoculum of Oscillatoria did not grow well; the naturally appearing Micractinium harvested well and were maintained in the ponds with or without recycling of part of the harvested algae biomass. Such selective recycling was demonstrated, both theoretically and practically, to lead to dominance of harvestable algae under conditions where non-recycled ponds were not harvestable. However, Micractinium could not survive adverse conditions even with extensive recycling; other pond operations would be required to allow maintenance of such microstrainable algae.

The project reported below was proposed before the above experiments were initiated. The original work plan emphasized the cultivation of filamentous blue-green algae, which could not be demonstrated to grow well on secondary sewage under the conditions prevailing in Richmond, California. Therefore the aim of the project changed to the general goal of developing algae cultivation and harvesting technology which would allow combined treatment of wastewater and energy at a cost below conventional processes and resources. The ultimate aim was to develop an integrated system as shown in Figure I-1. A power plant is incorporated to allow use of CO_2 to maximize algae biomass production.

In most waste oxidation ponds, the yield of algae biomass is effectively limited by the carbon content of the wastes applied since nitrogen, phosphorus, and other nutrients are present in excess. If wastes are enriched in CO_2, the amount of algae that can be grown on the wastes will be determined by the next limiting nutrient, usually N, and the total amount of algal biomass produced will be, for municipal sewage, two to five times that produced without carbonation (32). However, the N limitation can also be overcome through cultivation of nitrogen-fixing blue-green algae which are going to have a selective advantage under N

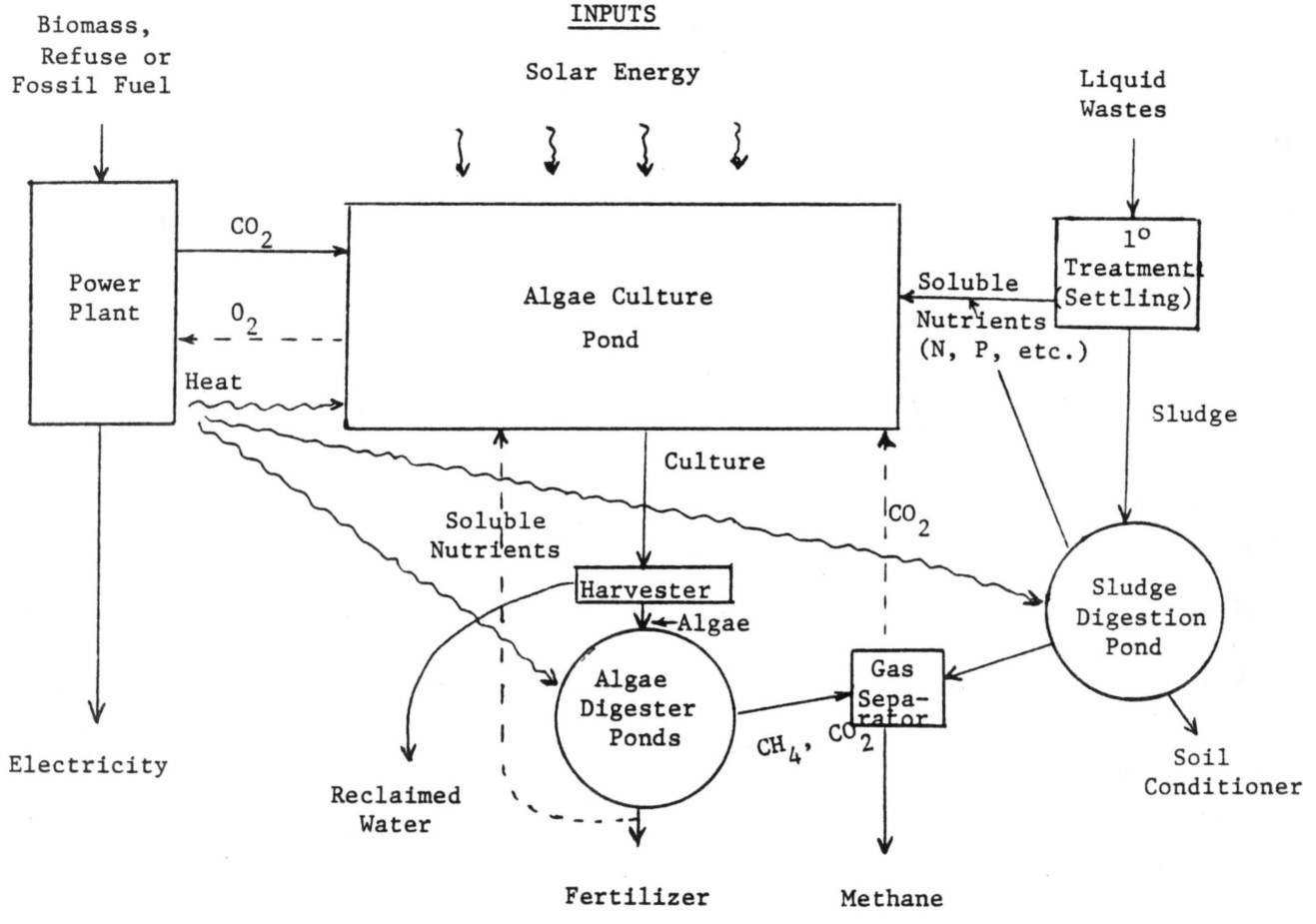


FIGURE I-1. SCHEMATICS OF ALGAE BIOMASS PRODUCTION AND METHANE DIGESTION INTEGRATED WITH POWER PLANT AND LIQUID WASTE TREATMENT

limitation conditions. The cultivation of N_2 fixing blue-green algae was studied under a separate contract with NSF-RANN and a Final Report is available (33). The overall system (Figure 1) can, therefore, be operated at the phosphorus algal growth potential of the wastes utilized with neither carbon nor nitrogen limiting.

The economics of algae biomass production as part of and separately from waste treatment systems have been subjected to repeated analysis. Most recently a detailed engineering design and cost analysis was undertaken for large-scale (100 mi^2) systems which concluded that a fully nutrient integrated system may be economically feasible (34). Other economic analyses were carried out under contract with ERDA, specifically during the project "The Photosynthetic Energy Factory" which analyzed the situation for waste treatment ponds integrated with silviculture energy farming (35). Therefore the economic analysis is not repeated herein.

This report details progress in the development of low cost algae biomass production systems using sewage as a source of nutrients and water. The research is still ongoing under a new contract "Large-Scale Freshwater Microalgae Biomass Production for Fuel and Fertilizer" with DOE. In this report the theory of species control through selective biomass recycle is refined and demonstrated in laboratory experiments (Appendix II). The outdoor pond system was further developed (Chapter II) and microstrainer effectiveness tested in multivariant experiments (Chapter III). The outdoor pond operations consisted of a series of experiments to test the effects of detention time, recycling and mixing on algae culture harvestability (by microstrainers) and productivity (Chapter IV). Batch and continuous secondary growth ponds were operated to result in low N pond effluents, after algae settling or microstraining (Chapter V). These effluents were used to grow nitrogen-fixing blue-green algae (33). The 0.25 hectare pond was operated to determine whether small-scale experimental results can be extrapolated (Chapter VI).

II. METHODS

POND SYSTEMS

The various ponds available for outdoor algal growth experiments included a 0.1 hectare facultative pond, a 0.25 ha high-rate pond, four 12 m^2 high-rate ponds, four 3.8 m^2 circular ponds, and additional smaller ponds. All but the facultative pond were utilized during 1977, with the 12 m^2 ponds serving as the major experimental units.

Sewage Supply

A continuous supply of fresh sewage was essential to the experiments. The sewage supply network is illustrated in Figure II-1. Sewage was pumped from a trunk sewer serving a section of Contra Costa County, California to a 12,000 liter primary clarifier located in the Sanitary Engineering Research Laboratory (SERL) pilot treatment plant. A portion of the clarified sewage flowed by gravity to a weir box which fed both the facultative pond and a coarse (2.8 mm slots) DSM screen. After passing through this screen, the sewage was fed to the 0.25 ha pond or pumped to a 3,000 liter clarifier which supplied the smaller ponds. A Jabsco Model 12040-0001 flexible impeller pump, operating at about 600 rpm for reduced wear, provided a constant rate of flow. The use of a second clarifier in series insured a more uniform strength sewage, since detention times in the SERL clarifier varied unavoidably. Flow through the 3000 ℓ clarifier was continuous at a detention time of about 45 minutes, thus freshly settled sewage was always available to the ponds. From the clarifier overflow pipe the sewage entered the ponds on demand with a system of float switches and solenoid valves providing dilution, at constant pond depth, during the harvest (see Figure II-4).

The sewage supply was part of a larger liquid transfer network (Figure II-2) constructed early in the year to facilitate movement of fluids throughout the pond system.

Sewage supply to the 0.25 ha pond differed fundamentally from the smaller

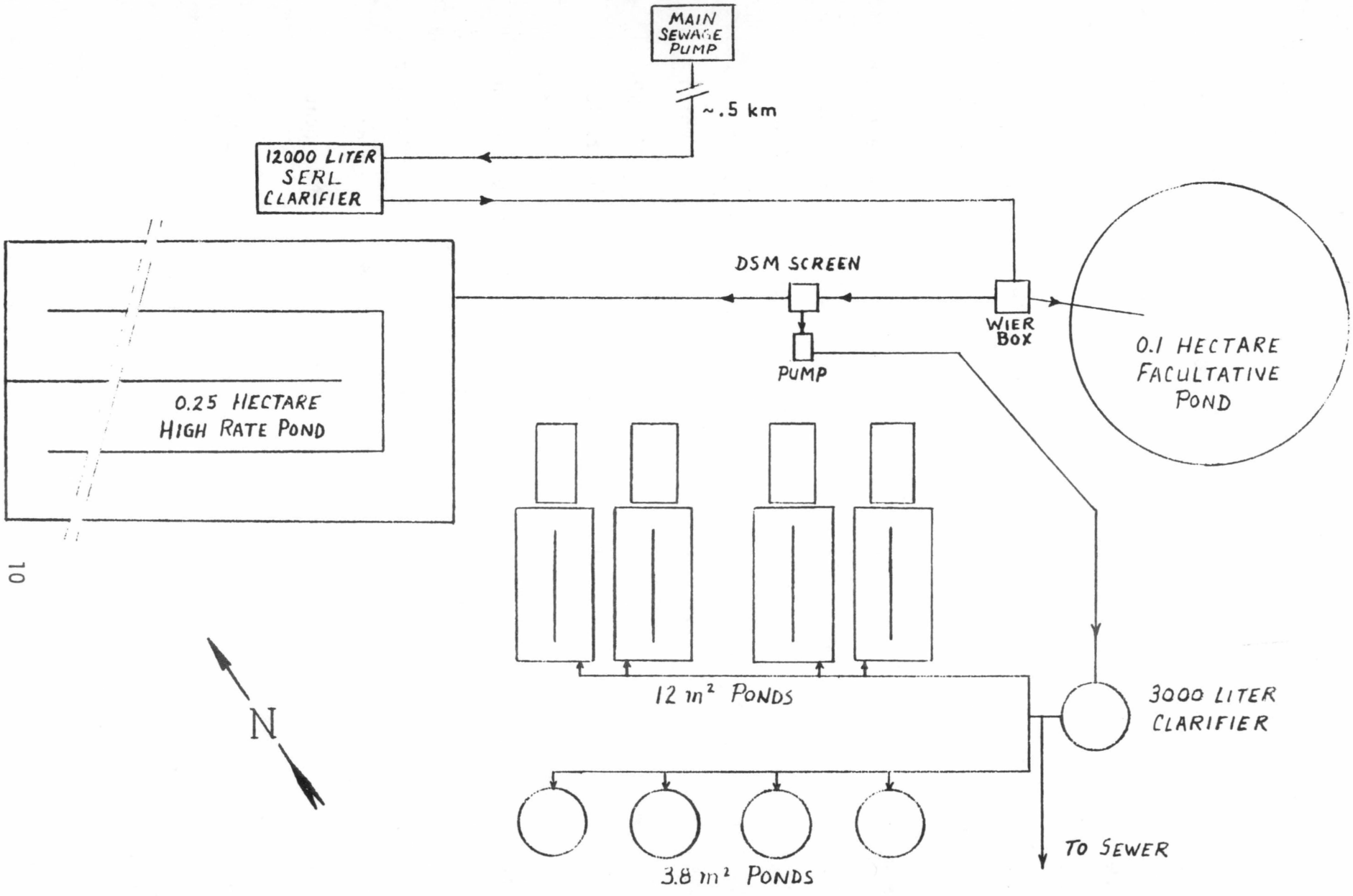


FIGURE II-1. SEWAGE SUPPLY NETWORK (PLAN VIEW)

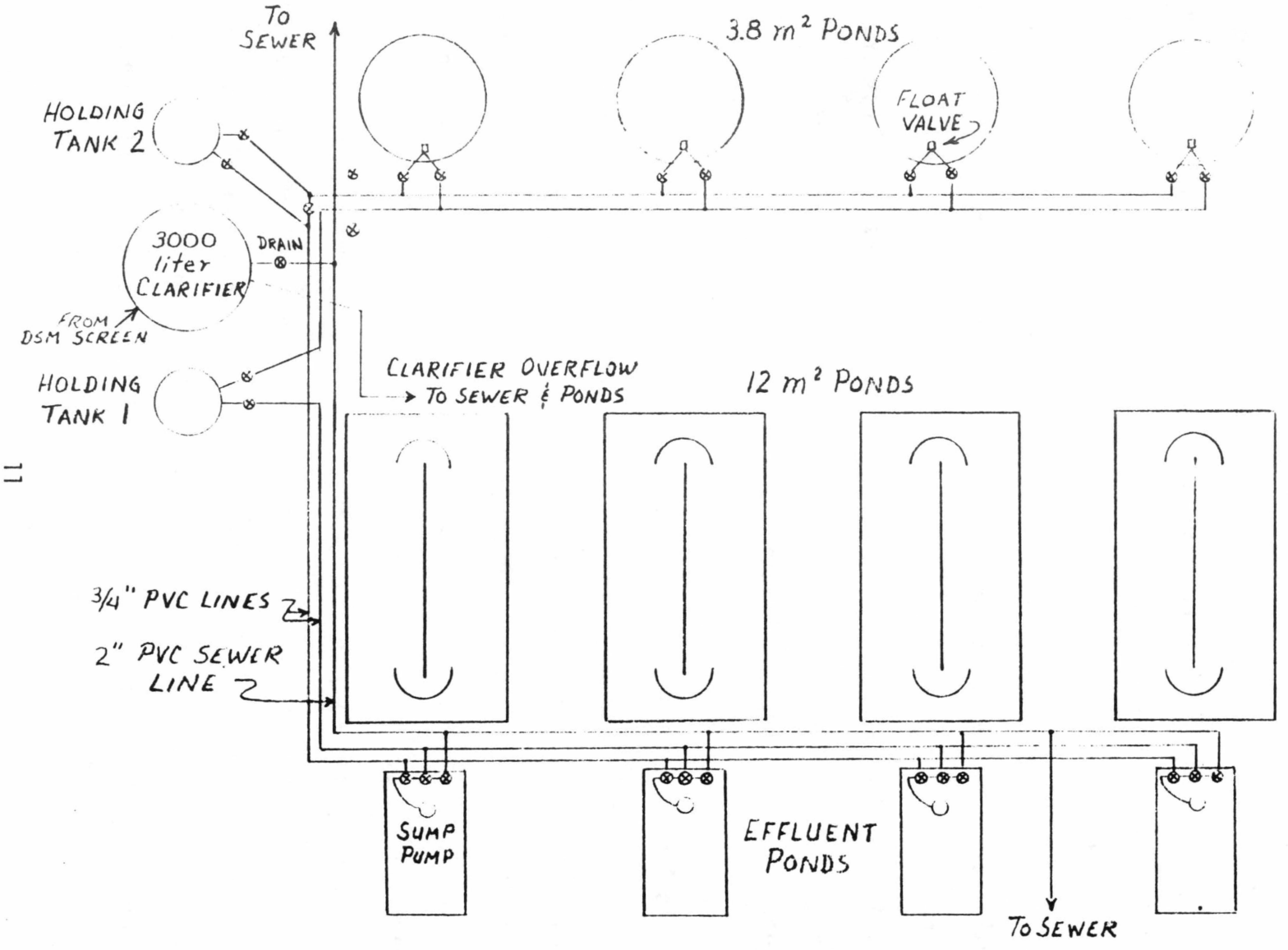


FIGURE II-2. LIQUID TRANSFER SYSTEM

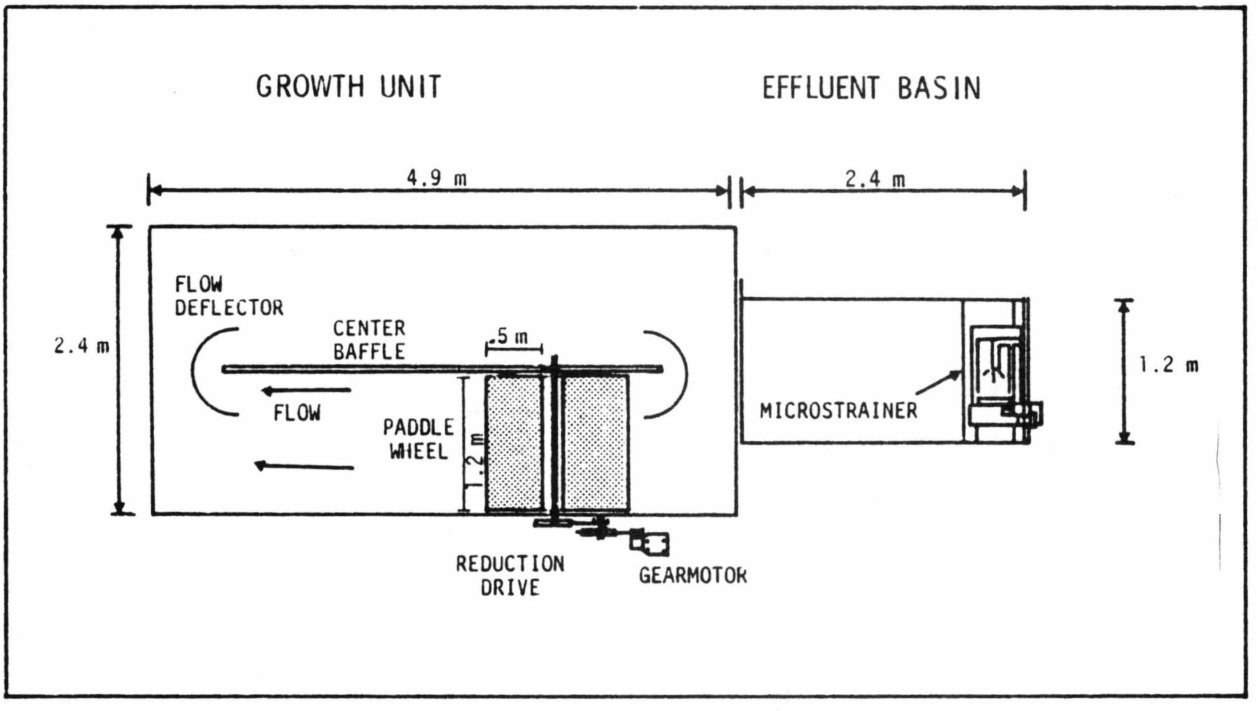
ponds in that the flow was by necessity continuous rather than on demand. Operation of the pond at reasonably short detention times required a fairly large flow of sewage continuously throughout the day. Since an overflow pipe held the pond at a constant depth, the daily volume of sewage entering the pond set the detention time. This inflow was controlled by a valve near the SERL clarifier, and was measured by the depth in the weir box (Figure II-1). A flow calibration curve was established and depths were recorded twice daily. The weir box was modified in March to permit higher flows to the large pond.

The method of flow metering described above was reasonably accurate as long as the flow to the SERL clarifier remained constant. However, since the latter condition could not be quaranteed, an alternate method for measuring detention times in the large pond was employed. The "on time" of the pond's effluent pump was recorded with an elapsed time meter. By calibrating the pump's discharge rate, pond detention times were calculated. The only drawback with this technique was that the discharge rate could change as a result of clogging, so frequent calibration was required.

12 m^2 High-Rate Ponds

In January work was completed on the 12 m^2 ponds, which thereafter served as the primary experimental units. Pond geometry is shown in Figure II-3. The adjacent smaller ponds were used to support the microstrainers, and to catch effluents, providing an accurate measure of the daily dilutions. The ponds were operated at a depth of 30.5 cm (3600 liter) during January and February, and at 24.5 cm (3000 liter) for the remainder of the year.

A single paddle wheel in each pond (Figure II-4) provided mixing velocities up to about 15 cm/sec. Two paddles in adjacent ponds were powered by a single DC gear motor (1/8 H.P. Bodine 40:1, driven by a Minarik W-53 speed controller). A multiple shaft arrangement provided further speed reduction and allowed the paddles to be operated at different speeds. Fluid velocities would be difficult, if not misleading, to report since pond geometry created



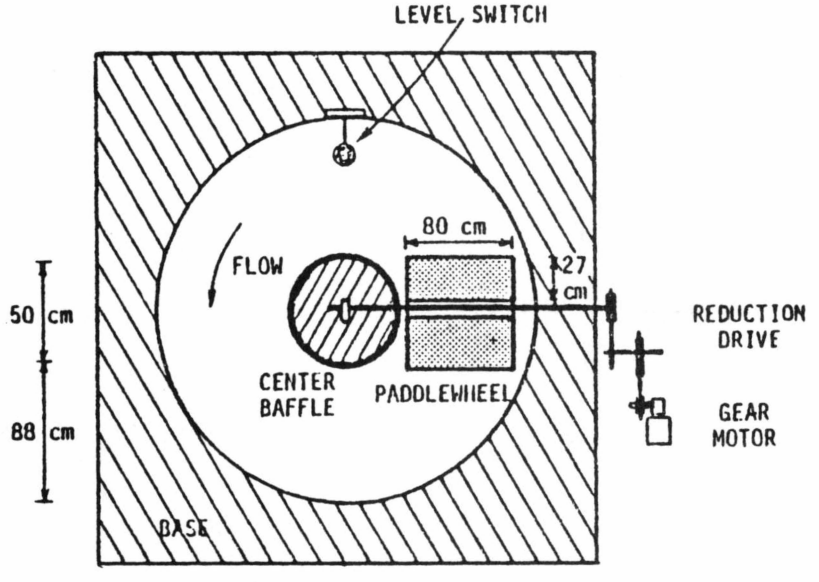


FIGURE II-3. PLAN VIEW OF ALGAL PRODUCTION PONDS 12 m^2 (above), 3.8 m^2 (below).

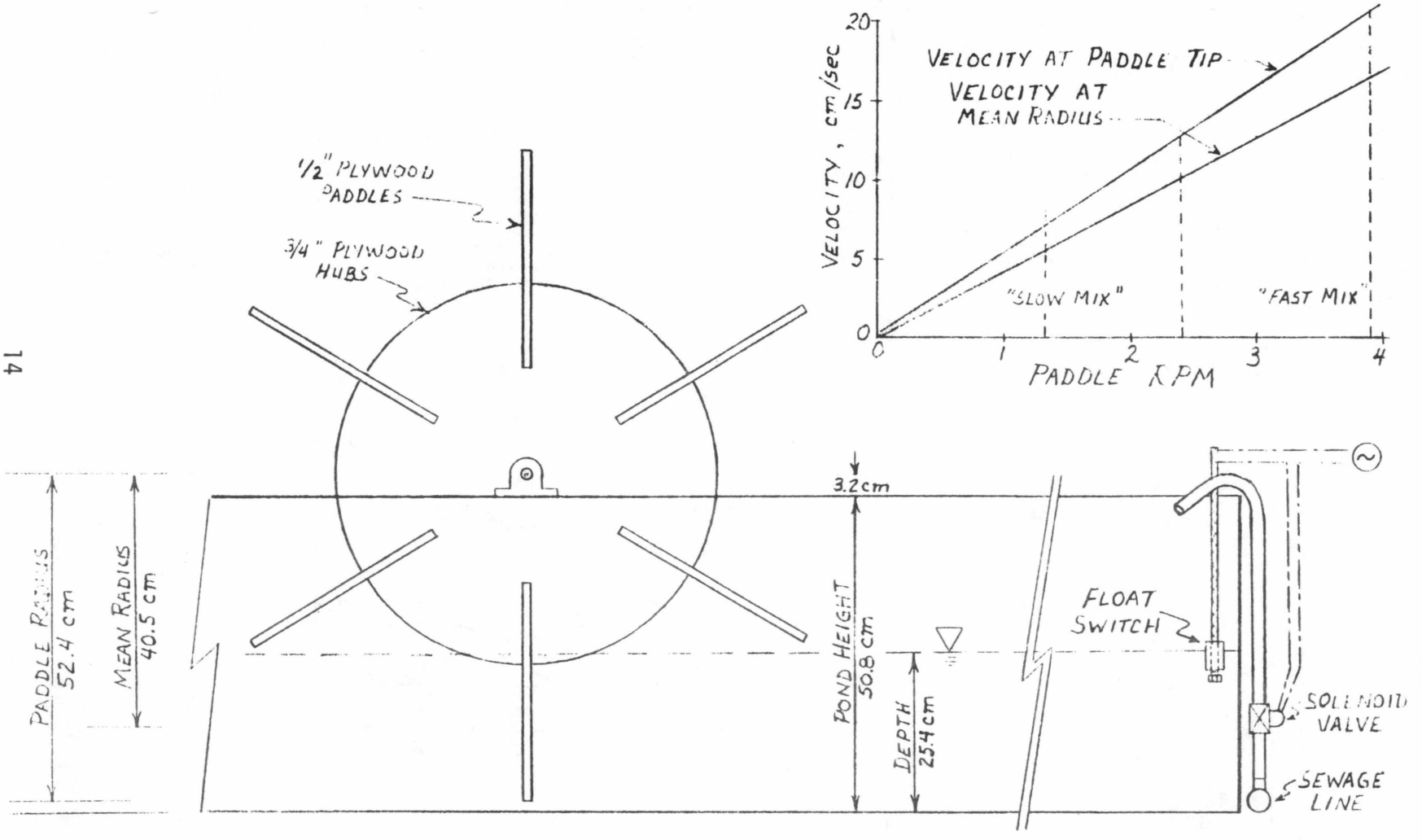


FIGURE II-4. PADDLE WHEELS AND SEWAGE SUPPLY

conditions of highly non-homogeneous flow. For this reason mixing speeds were measured in terms of paddle revolutions per minute. This method allowed quick and accurate monitoring. Throughout any given experiment, the paddles were operated at a constant rate.

The paddle wheels and pond freeboard produced appreciable shading, and thus reduced the available surface area of the ponds. However, production figures were reported on the basis of total pond surface area and are thus somewhat conservative.

3.8 m^2 Circular Ponds

Illustrated in Figure II-3, the 3.8 m^2 circular ponds were used extensively for the previous year's experiments ([31]). In 1977 these ponds were used for secondary and tertiary (nitrogen fixation) growth experiments. These ponds suffered two major disadvantages, one being their small volume, and the other their tendency, upon mixing, to concentrate algae in a ring around the center baffle. Shading reduced the effective pond area to 3.0 m^2.

0.25 Hectare High-Rate Pond

The 0.25 ha (0.6 acre) high-rate pond is illustrated in Figure II-5. This pond is asphalt-lined and can be operated between 15 and 60 cm depth. Pond volume at its normal operating depth of 27 cm is about 620,000 liters.

Three 5 h.p. low-head propeller pumps were available for mixing. Generally, only a single pump was used. In addition a submersible pump drew pond water from the mixing sump and directed a portion of its flow (about 1/10 of the pond volume per day) through a DSM screen (0.43 mm slots). This screen removed some large predator organisms and prevented debris from entering the microstrainers, which were fed from the screen's effluent line. The submersible pump, when used alone, provided a bare minimum of mixing for the pond.

Pond overflow, DSM screenings and microstrainer effluents were collected in a concrete sump and were discharged through a newly installed Kenco 129A automatic sewage pump.

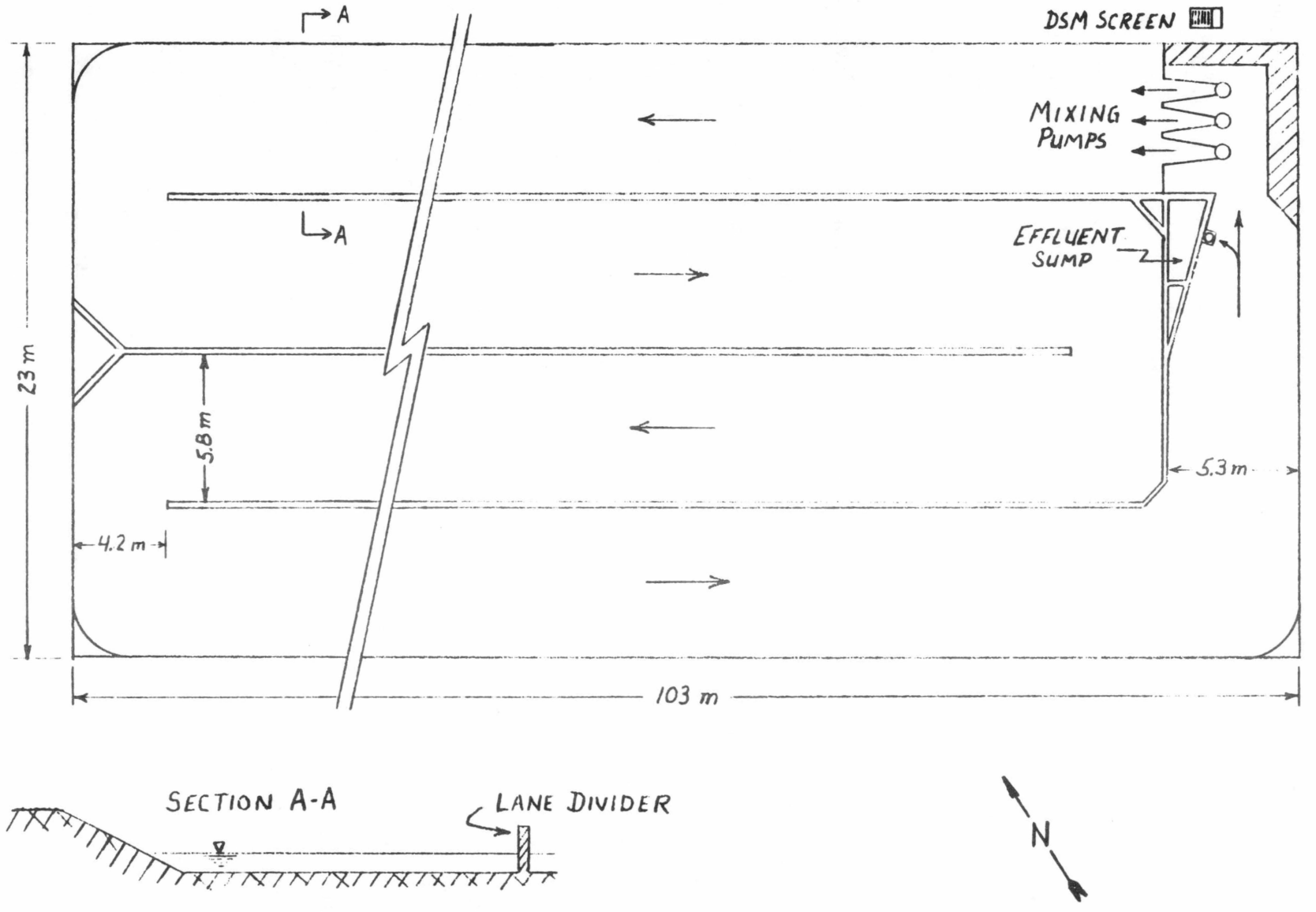


FIGURE II-5. .25 HECTARE HIGH-RATE POND (PLAN VIEW)

Mixing was one of the major operational dissimilarities between the large pond and the 12 m^2 ponds. Poor mixing characterized the large pond. As pond water rounded each of three baffles an extensive "shadow", or zone of very low flow velocity, was induced. The shadows extended as much as 30 meters downstream from the turns, and accumulated large amounts of settled algal and other solids. While this condition existed to some extent in the 12 m^2 ponds it was considerably more pronounced in the large pond.

The addition of paddle wheels and flow deflectors would vastly improve pond mixing and reduce solids accumulation. An additional improvement planned for this pond is the continuous monitoring of the effluent flows, in order to provide a more accurate measure of detention time.

Microstrainers

Four experimental microstrainers (0.2 m^2 straining area each), available from the previous year's work, were used to harvest algae from the 12 m^2 ponds. An additional unit was fabricated for use in other experiments and as back-up. Two larger microstrainers (0.9 m^2 screening area each) were also available for harvesting a portion of the large pond's effluent.

The 12 m^2 pond microstrainers were placed above the effluent ponds (see Figure II-3). The influent was pumped through flexible impeller pumps (Jabsco 17000-0037), driven by variable speed DC motors at 1-3 liters/min, depending on algal concentration. Drum rotation was also adjustable.

A more detailed description of microstrainers and their performance is included as Chapter III of this report.

Herbivore Screens

To test the concept of herbivore control by mechanical means an herbivore screen was fabricated. Various mesh sizes (100-315 microns) were investigated. In September four 150 micron screens were built (see Figure II-6). The 150 micron fabric represented a compromise between efficient rotifer removal (nearly

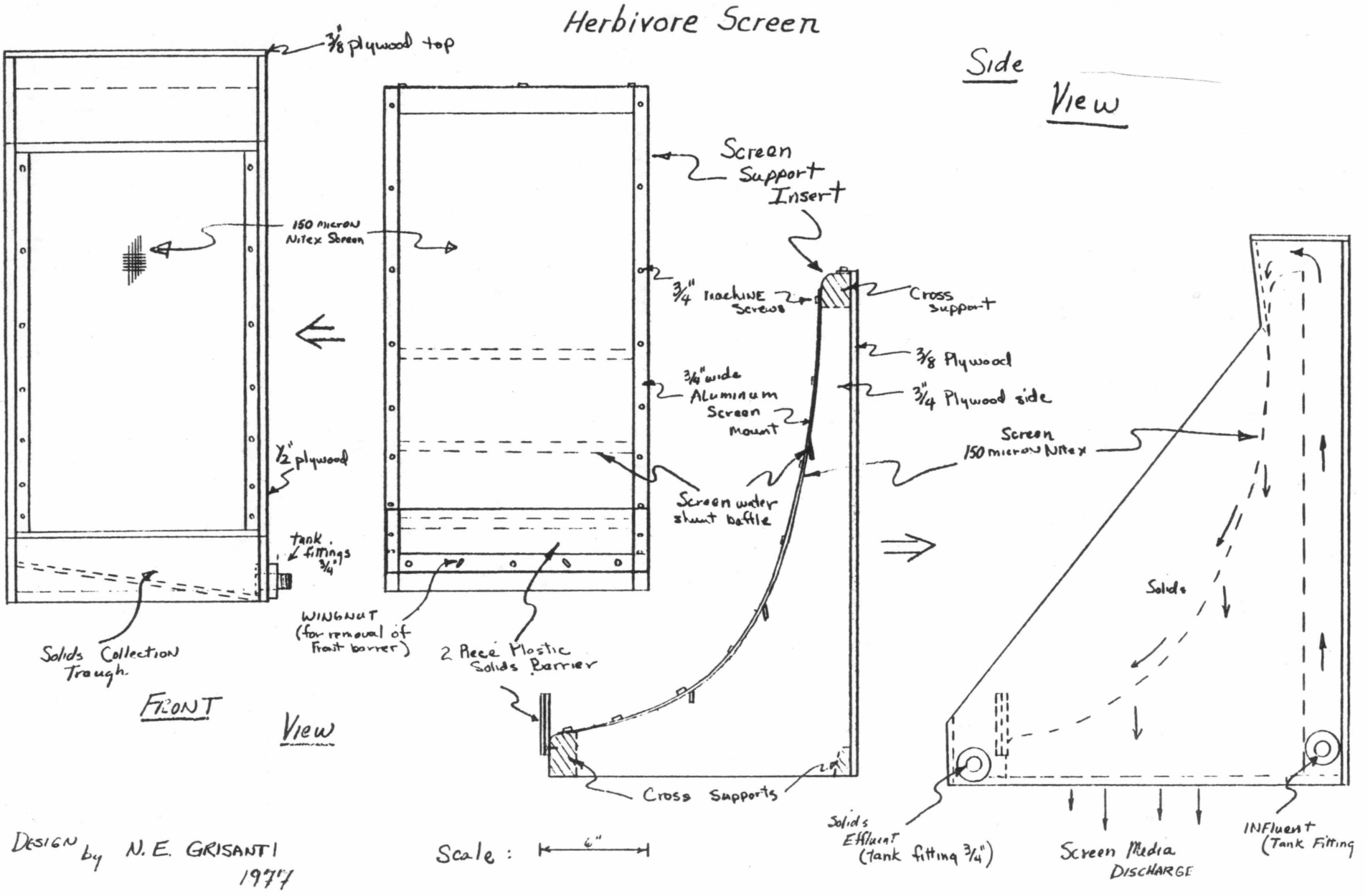


FIGURE II-6. 150 MICRON DSM SCREENS FOR HERBIVORE (ZOOPLANKTON) REMOVAL

100% effective at 100 microns) and the problem of solids accumulation (including large algae).

The herbivore screens were fed by a submersible sump pump (Geyser) suspended with the inlet about 10 cm above the pond bottom. Flow rates averaged 50 liters/min. Periodically, the front plastic barrier was loosened to allow washing the accumulated screenings into the collection trough. This cleaning was required every one to four hours, depending on rotifer concentration, sewage loading, etc.

Insolation

Insolation was measured with an Eppley 8-48A Pyranometer driving a strip type chart recorder. The daily curves were integrated with a hand planimeter. The latter was calibrated against the more tedious "counting squares" method, and found to be of sufficient accuracy.

Daily insolation graphs are presented with each experiment. Figure II-7 is a graph of monthly averages.

POND OPERATIONS

Daily Operations

In addition to the sample analyses described elsewhere, a record of pond temperature, depth and pH was made twice daily. The pH was measured with a Beckman Electromate pH Meter, standardized to the nearest buffer; fresh samples were always used for pH measurements. These observations were made before harvesting (0800 to 0900, depending on the experiment), and in the evening at about 1600.

A Klett-Summerson photoelectric colorimeter, equipped with a #66 (red) filter was available to provide an on-the-spot measure of pond density. During the summer experiments pond "Kletts" were measured each morning.

Harvesting and Dilutions

The 12 m^2 ponds were harvested daily by pumping the requisite volume

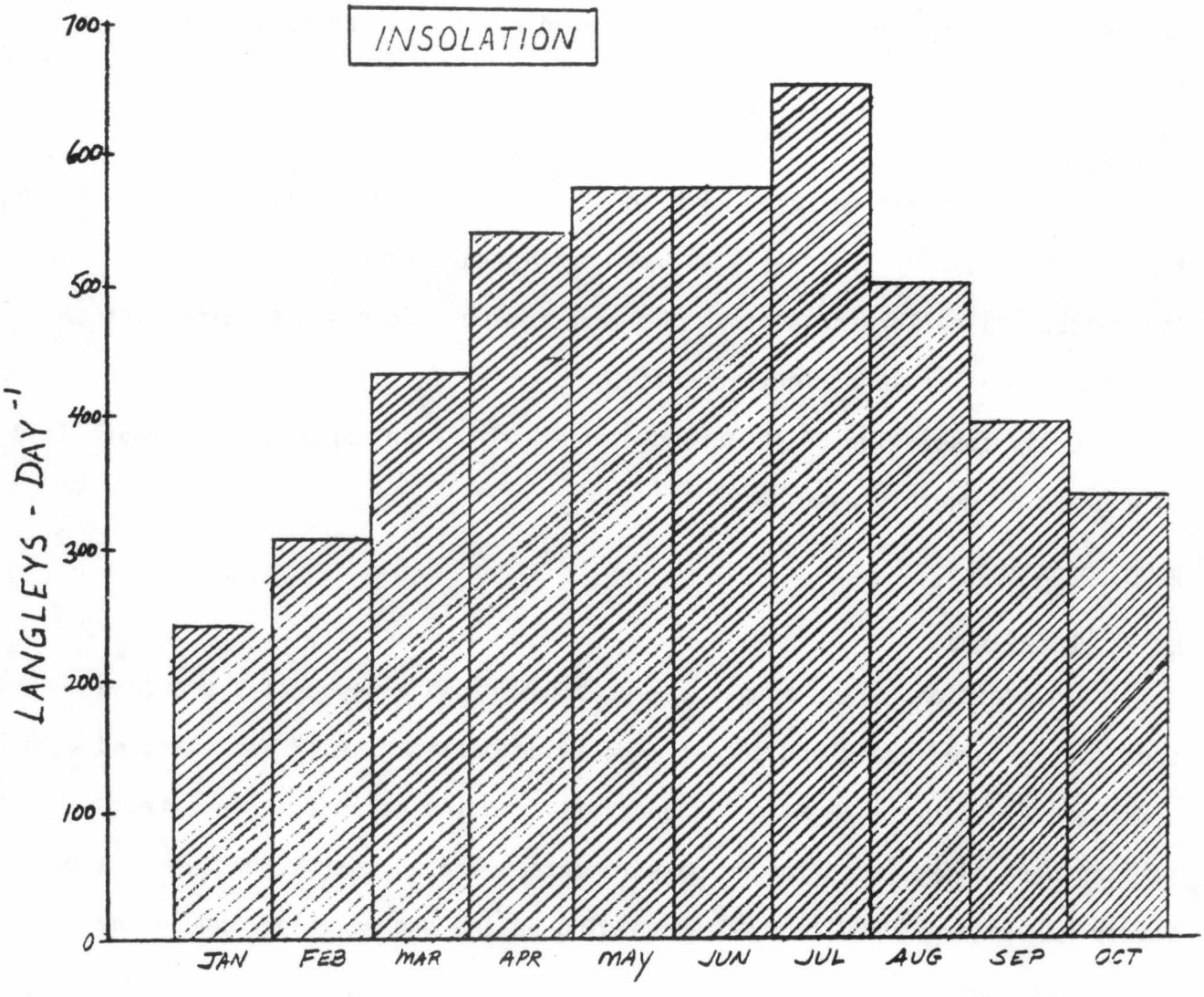


FIGURE II-7. MONTHLY AVERAGE INSOLATION FOR 1977, (Langleys/Day)

from a point 13 cm below the pond surface into the adjacent effluent pond. A level switch, responding to the decrease in pond depth, activated the solenoid valve to allow sewage into the pond. The level switch deadband was approximately 0.3 cm. On sample days the harvest volume was routed through the microstrainers. For the biomass recycle ponds the microstrainers were run daily.

Since harvest durations of at least six hours were considered desirable, and since the microstrainers required influent rates of at least one liter per minute, it was necessary to operate the strainers on an intermittent basis. An automatic timer, Cramer Model 540, was installed to actuate the dilution pumps and microstrainers for a preset fraction of each hour, adjustable from 0 to 100%. Each pond was programmed independently by adjusting the switching cams' position on the timer shaft. For example, an eight-day pond would be harvested at 25% time (15 min/hr), a four-day pond at 50% time, etc. The timers were set to operate the drum rotation and backwash for 5 minutes after the influent ceased to prevent fouling of the screen fabric. Level switches in the effluent ponds shut off the dilution pumps when the correct volumes were reached. The harvest volumes were adjusted for the addition of water from the microstrainer backwash.

Sampling

Samples of the microstrainer influent and effluent streams were collected three times weekly for laboratory analysis. Variations in pond densities over the harvest period, which were most pronounced in short detention time ponds where a large fraction of the pond is diluted with sewage, necessitated taking composite samples. Generally, three subsamples were collected at equal time intervals. Pond subsamples were taken directly from the microstrainer influent line; effluents were taken from the catch pan located below each microstrainer. Effluent densities were not corrected for backwash dilution since the error induced was always less than 10%. At each subsample period, microstrainer concentrate volumes were measured in 20 l graduated cylinders, and the appropriate

fraction was returned to those ponds operating with biomass recycle.

At least once each week, a composite sewage sample was taken from the same line that fed the ponds. Occasionally samples of unscreened and/or unsettled sewage were also collected.

All samples were kept under refrigeration until analyzed.

Sampling and harvesting procedures for the 0.25 ha pond were similar to those described above, the major difference being the sewage supply system as previously described. The large pond was also diluted over a longer period of time--eight to twelve hours depending on detention time.

ANALYTICAL METHODS

Definition of Terms

Harvestability and production were calculated from volatile solids and chlorophyll *a* concentrations using the following equations:

Harvestability (removal efficiency)

$$H = \frac{\text{Pond Density (chlorophyll a)} - \text{Effluent Density (chlorophyll a)}}{\text{Pond Density (chlorophyll a)}}$$

Harvestable Production

$$Ph = \frac{(\%\text{ of Concentrate Vol. Not Recycled})(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

$$= \frac{(H-Rc)(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

$$= \frac{H(1-rc)(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

Total Production

$$Pt = \frac{(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})} - H\,rc\,\frac{(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

$$= \frac{(1-Hrc)(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

$$= \frac{(1-Rc)(\text{Vol. Harvested})(\text{Pond Density})}{(\text{Pond Area})}$$

where Rc = fraction of total pond biomass recycled

rc = fraction of harvested biomass recycled (% of concentrate volume recycled)

H = Harvestability fraction (defined above)

Rc = H rc

Photosynthetic energy conversion efficiencies were calculated from the total production and daily insolation as follows:

$$\text{Sunlight Conversion Efficiency} = \frac{\text{Production}\left(\frac{\text{gm}}{\text{m}^2\text{-day}}\right) \times \text{Heat of Combustion}(5.5\ \text{Kcal/gm}) \times 10^{-4}\ \frac{\text{cm}^2}{\text{m}^2} \times 100}{\text{Total Insolation}\left(\frac{\text{gm-cal}}{\text{m}^2\text{-day}}\right) \times 10^{-3}\ \frac{\text{kcal}}{\text{gm-cal}}}$$

In computing productions no credit was taken for the solids which accumulated within both the 12 m^2 and 0.25 ha ponds. These bottom sludges consisted of sewage and bacterial solids, as well as a large amount of settled algae. All ponds were cleaned out between experiments.

Suspended Solids Analysis

Total Suspended Matter. Standard Methods (36) was used as a reference for this assay. Glass fiber filters (Whatman GFC) were washed in distilled water, dried at 105°C for one hour, ignited at 550°C for 15 minutes, and cooled in a dessicator to room temperature. Measured volumes of sample were vacuum-filtered through the preweighed filter disks, dried at 105°C for 30 minutes in a forced air drying oven, and weighed. Constant weights were found to be attained after 20 minutes in a series of tests to determine optimum drying times.

Volatile and Fixed Suspended Matter. The filter disks with total dried solids from the above determination were ignited at 550°C for 15 minutes in a muffle furnace. After cooling to room temperature in a dessicator, the ashed filter was reweighed; the difference between this weight and the total suspended solids weight was the volatile (organic) component. The difference between the ashed weight and the empty, preweighed filter disk was the fixed (inorganic) component.

Chlorophyll a

The methanol extraction method of Holden (37) was used. Measured volumes of 90% methanol using 15 ml centrifuge tubes were heated to boiling in a water bath. Measured volumes of sample were filtered under vacuum onto Whatman Glass fiber disks with 2 drops of magnesium carbonate solution added for pH control; these filters with residue were transferred into the boiling methanol for 45 seconds, shaken vigorously, and centrifuged for 15 minutes at 5000 g. Supernatants were pipetted off and their absorbances read at 665 nm using a Hitachi Model 100-60 double-beam spectrophotometer. A reading at 750 nm was used to subtract out the effect of turbidity from the other absorbance. As in all light absorption techniques, suitable volumes of sample to register absorbance readings in the 0.200-0.600 range were estimated from prior experience, as the Beer-Lambert Law is most well obeyed in this range.

Chlorophyll a was calculated in mg/l by $(D_{665}-D_{750})(13.9)(\frac{\text{ml MeOH}}{\text{ml sample}})$ where D = optical density and ml MeOH = final volume of methanol used minus the volume of the filter disk.

Biochemical Oxygen Demand (BOD) - Dilution Method

The technique consisted essentially of the determination of the dissolved oxygen (DO) content of the sample by the Winkler Method, azide modification from Standard Methods (36) before and after incubation for 5 days at 20°C. Dilution of samples was necessary to obtain desired DO depletions in the range of 40 to 70% after 5 days. Dilutions were generally 2 to 3% for sewage samples, 5 to 10% for pond samples, and 10 to 12% for pond effluents. Dilution water was prepared with aerated distilled water according to Standard Methods. BOD was calculated in mg/l by (initial DO-final DO) x 1/dilution. No seeding of the dilution water was necessary. See Appendix I for BOD data.

Chemical Oxygen Demand (COD)

The dichromate reflux method from Standard Methods (36) was used in

this study. Twenty ml quantities of sample were refluxed for 2 hours with 10 ml of 0.25 N potassium dichromate, 30 ml of concentrated sulfuric acid containing 22 g silver sulfate per 9 kg bottle, and 0.4 g of mercuric sulfate. The mixtures were diluted to approximately 150 ml with distilled water, cooled to room temperature, and the excess dichromate titrated with 0.10 N standard ferrous ammonium sulfate, using ferroin indicator. A distilled water blank was refluxed in the same manner. COD was calculated in mg/l by $\frac{(a-G)N \times 8000}{ml\ sample}$ where a = ml $Fe(NH_4)_2(SO_4)_2$ used for blank, G - ml $Fe(NH_4)_2(SO_4)_2$ used for sample, and N - normality of $Fe(NH_4)_2(SO_4)_2$. See Appendix I for COD data.

Total Kjeldahl Nitrogen and Ammonia Nitrogen

The determination of total Kjeldahl nitrogen basically followed that of Standard Methods (36), but was modified to digest and distill in 500 ml Kjeldahl flasks instead of 800. The final ammonia determination was done colorimetrically but changed to the indophenol method developed by Solarzano (38). Generally, an aliquot of the sample to be tested was filtered and reserved for the ammonia test, and the remainder of the unfiltered sample was used for the Kjeldahl digestion and distillation; then both sets of samples were tested for ammonia. These were reported as free ammonia and Kjelhahl nitrogen. The difference between the two was reported as organic nitrogen.

200 ml quantities of sample were boiled with 20 ml of concentrated H_2SO_4, one bag of Kelpak No. 2P, and 2 or 3 Hengar selenized granules in 500 ml Kjeldahl flasks. The digestion was carried out for approximately 40 minutes after the first fumes of SO_2 appeared. The solutions were cooled, diluted to about 300 ml with distilled, demineralized water, and 50 ml quantities of 50% NaOH were added. The mixtures were immediately distilled into receiving flasks containing 20 ml of 2% boric acid. After collecting about 150 ml of distillate, a range of dilutions of each sample was prepared with ammonia-free water in order to fall within the sensitivity range (10-500 µg/l) of the indophenol

test for ammonia.

A 5 ml aliquot of the sample to be tested for ammonia was measured into a test tube and the following reagents were added with mixing after each addition: 0.2 ml of a 10% phenol-ethanol solution (95% ethanol); 0.2 ml of a 0.5% sodium nitroferricyancide solution; 0.5 ml of alkaline citrate solution (200 g trisodium citrate dihydrate and 10 g sodium hydroxide in 1 liter of water) plus 5.25% sodium hypochlorite in a 4:1 ratio. Absorbances were read after one hour at 640 nm on a Hitachi model 100-60 double-beam spectrophotometer. Ammonium sulfate standards were prepared and run along with the samples. Ammonia values of the samples were then calculated through construction of a standard curve. See Appendix I for total-N and NH_4^+-N data.

Quality Control

Control procedures designed to maximize the quality of data were an integral part of this study. Laboratory services and apparatus were monitored routinely by laboratory personnel and maintained by factory representatives. Distilled water was de-ionized by mixed-bed ion exchange (conductivity < 2 micromhos). The Hitachi (model 100-60) spectrophotometer was calibrated for wavelength alignment regularly. Mettler semi-micro balances were serviced and calibrated periodically.

Sampling errors were minimized by compositing samples (excepting occa - sional grab samples) throughout the day for the laboratory. The effectiveness of this method was checked on three separate occasions by comparing two different sets of composites collected during the same pond operation (N = 3 sets of 2 composited samples). Percent differences between composites averaged less than 6% for TSS and VSS and less than 5% for chlorophyll a. The difference in means of composites were statistically not significant (95% confidence interval).

All assays were randomly checked for reproducibility by analysis of

samples in duplicate and triplicate. For duplicate runs of the same sample, errors were calculated in terms of percentage deviation of the two values from their mean ($\frac{\bar{X}-X}{\bar{X}} \cdot 100$). For suspended solids percentage errors ranged from 0.1 to 5.6% with a mean of 1.7% (N=74). Chlorophyll *a* errors ranged from 0 to 12.2% with a mean of 1.9% (N=57). In addition to reproducibility checks, the chemical oxygen demand assay was occasionally tested against standard solutions. A 500 mg/l COD standard prepared with potassium acid pthalate, analyzed on separate occasions by the same method, yielded COD values ranging from 470.5 to 510.5 mg/l with a mean of 495.5 mg/l (N=5). The ammonia assay was accompanied each time with a series of ammonium sulfate standards due to the slightly variable nature of the color development, thereby negating the effects of temperature and other environmental factors on the analysis. Reproducibility between ammonia duplicates ranged from 0 to 25% and averaged 5.3% (N=188).

MICROSCOPIC OBSERVATIONS

Algae

Algae from pond samples and harvester effluents were identified and enumerated. Due to the high algal densities, a hemocytometer technique could be directly utilized without cell concentration. Genus-specific algae biovolume was routinely estimated by measurement of randomly selected individuals of that type. Volume for each size class of each genus was established through the use of geometrical approximations for each class using the mean value of the above measurements. Although only algal volume data are presented, estimation of carbon biomass can be easily calculated from published equations (39). Initially, algal harvestability was estimated by measurement. Individuals with linear measurements larger than the average pore size of the harvester fabric were defined as harvestable. Algae which exhibited only one dimension larger than the average pore size (e.g. *Ankistrodesmus*) were defined

to have less than the total harvestability. Algae with no measurements larger than the average pore size were defined as non-harvestable.

One of the greatest difficulties in this study was the correlation of harvestability by microstraining and microscopic algae size. In general harvestability was overestimated by microscopic analysis, particularly at low harvestabilities. The error was apparent with the observation of effluent samples, most of which contained "harvestable" algae. Thus, in general, very low microstrainer harvestabilities still gave 30-60% microscopic harvestabilities. A larger enumeration effort would be required to make microscopic data more quantitative.

In order to assess the precision of the algae enumeration method, a variance estimate was completed. The data base for this estimate was 12 replicate counts of one pond sample. The enumeration technique assumes that the algae distribution on the hemacytometer will be random--a Poisson distribution. This type of distribution establishes that the mean value of any specific observation (e.g. species counts) must equal the variance of that data. This is mathematically stated as: $\bar{X} = \sigma^2$. From this distribution, the expected error of any sample (Xi) can be estimated by $2\sigma/X_i$ (e.g. if one was to count a community of 400 cells, one would expect a 10% error or 90% confidence limit). Means ($\bar{X}$) and standard deviations (σ) for each size class and total cell counts from each of the 12 replicate counts were calculated. As shown in Table 1 , the distribution on the hemacytometer may be described as slightly contagious (not precisely random). This data indicated that counts of more than 400 total cells would provide a reasonable estimate of the total algal volume. This standard was used for all enumerations, thereby providing data with an average variability of about $\pm$ 15% for the total algal biovolume. Individual genus expected errors are much larger than those stated for the total community due to the reduced number of cells counted. To obtain genus-specific expected errors approximating those derived for the total community

TABLE 1

MEAN vs. STANDARD DEVIATION OF REPRESENTATIVE REPLICATE MICROSCOPE COUNTS.

	Mean No. Cells	Standard Deviation
	($\bar{X}$)	(σ)
Scenedesmus		
Individuals	1.83	1.47
Small Colonies	5.25	3.31
Medium Colonies	41.83	23.03
Large Colonies	95.00	21.06
Micractinium		
Small Colonies	31.67	11.59
Medium Colonies	9.83	4.65
Ankistrodesmus	2.00	1.21
Chlamydomonas	1.25	1.14
TOTAL CELLS /COLONIES	189.0	23.34
Approximate Fit To: $\sigma = 0.52 + 4.46 \ln \bar{X}$		
If $\bar{X}$	σ	% Error
50	17.97	71.88
100	21.07	42.14
200	24.16	24.16
400	27.25	13.63
1000	31.34	6.27

would require the enumeration of about 400 individuals of that genus. High statistical variances found in some of the published data may be partially due to subsampling errors. The largest sampling errors probably occur during pond compositing.

The enumeration method was as follows: Algae samples were taken from composites of that day and placed in 60 ml Nalgene^{R} plastic bottles. When possible, enumeration occurred immediately to prevent bacterial decomposition and/or zooplankton predation and allow the observation of fresh, non-preserved material. After agitation, a small volume of the sample was withdrawn with a pipette. From this pipette, a single drop was randomly selected and immediately placed on an improved Neubauer-type hemacytometer. A cover glass was then placed on the hemacytometer, yielding a depth of 0.1 mm. Counts were done on a 9 mm^2 grid. Individual alga were tabulated on eight key recorders. Total size class per genus counts were accumulated on the recorders then transferred to the working data sheet for volume estimation.

In response to the overestimates of harvestabilities as compared to other methods (Klett, TSS,VSS, Chlorophyll a), the level of enumeration was increased to include pond effluent counts. Harvestability data after 20 March was calculated from the pond and effluent count data with the use of the formula: Harvestability = $\frac{\text{Pond - Effluent}}{\text{Pond}}$. This calculation resulted in better agreement with other harvestability estimates. In addition to the total harvestability, specific harvestability was calculated using data from the enumeration process. Specific harvestabilities allowed the added observation of which algae were being removed from the pond, not just how much of the algal community was harvested.

Zooplankton

Zooplankton populations were monitored at times throughout this study. When enumeration was done, samples were preserved in 4% buffered formalin, concentrated by centrifugation, and counted by major groups using a 1 ml

Sedwick-Rafter counting chamber. At least three fields per sample were normally counted in this manner.

Rotifers were by far the most numerous group, normally comprising more than 75% of the zooplankton biomass although ciliated protozoa occasionally became important. At least two and possibly several species of Brachionus, a ploimate rotifer, were dominant throughout the year. Other occasionally seen rotifera were Trichocerca, Synchaeta, Conochilus, Philodina, Rotaria and several unidentified genera. Protozoa were represented most commonly by Paramecium, Aspidisca, Euplotes, Tokophyra, Vorticella and several unidentified genera. Crustacean zooplankters such as Daphnia, Cyclops and ostracods were occasionally observed. Zooplankton biovolumes were estimated by geometrical approximation, e.g. the displacement volume of a ploinate rotifer was estimated by applying its measured dimensions to a hemisphere and a cylinder.

III. FACTORS AFFECTING PERFORMANCE OF MICROSTRAINERS IN REMOVING ALGAE

INTRODUCTION

Microstrainers, or microscreens as they are also called, are mechanical straining devices used to separate suspended particulate matter from water. They were originally developed in England about 1945 to provide for the removal of algae from raw water supplies (40). Their uses have since expanded to include treatment of industrial and domestic wastewaters. They have also proven particularly useful in removing suspended solids from biological treatment plant effluents . The construction and operation of microstrainers is comparatively simple. They consist of a rotary drum covered by straining fabric on its periphery. Water enters axially through one end of the drum and is passed out radially through the fabric. Particulate matter larger than the fabric openings is intercepted on the inner surface of the fabric forming a filtration matt or "schmutzdecke". Some particles smaller than the fabric openings are entrapped in this matt. All the materials deposited are removed by a high velocity backwash spray and are collected in a trough which passes them from the drum. Figure III-1 shows schematically the design and operation of a microstrainer. The low unit cost of microstraining ($20 to $40 per million gallons treated) (31) derives principally from the straightforward construction and simple operation of the equipment.

Despite their widespread acceptance, microstrainers have usually failed when applied to waste pond effluents. Golueke (20) was unsuccessful in removing Chlorella (3-5 μm) or Scenedesmus (5 x 30 μm) with a microstrainer having fabric openings of 35 x 40 μm. In their work at Firebaugh, the California Department of Water Resources (41) achieved removals of Scenedesmus of up to 30% with 25 μm fabric but attributed this result largely to algal settling in influent and effluent chambers of the microstrainer. van Vuuren et al (25) were able to effectively remove algae from a South African waste

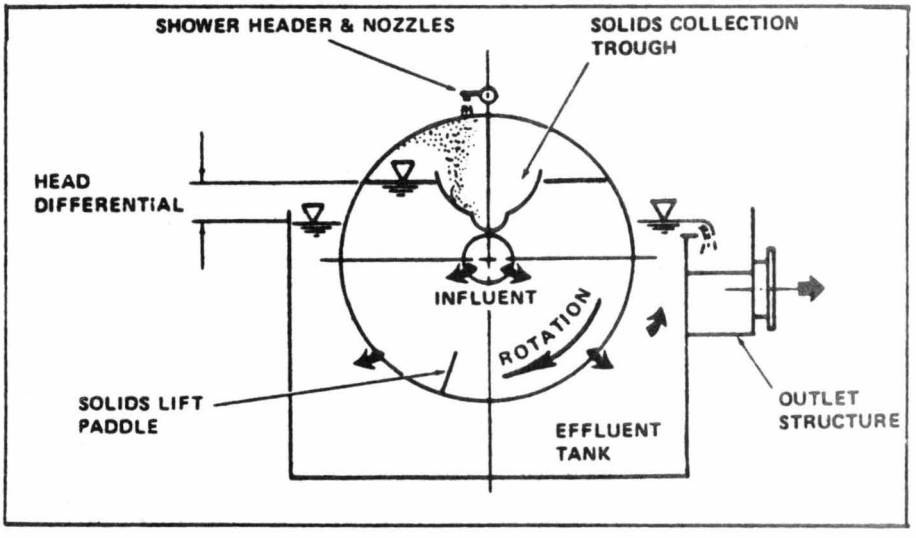


FIGURE III-1. SCHEMATIC DIAGRAM OF MICROSTRAINER OPERATION AFTER (43)

pond using 21 μm fabric during a period when Micractinium (effective size ca 50 μm) dominated. Later, when the Micractinium population was overtaken by Scenedesmus and Chlorella, the same fabric achieved very poor algal removals.

These observations confirm the obvious notion that microstraining will be ineffective when applied to algae smaller in size than the pore size of the straining fabric employed. If waste pond algae are to be removed by microstraining one of two strategies must be resorted to. The first is to utilize fabrics having openings smaller in size than the common waste pond algae. The second strategy is to control conditions within waste ponds so that larger algae will predominate. At the time the previously discussed studies were conducted the finest fabrics available were made of stainless steel and had openings of 23 μm. Recently, nylon and polyester fabrics have become available with openings as small as 1 μm. Honeycomb grid support systems such as those developed by Envirex Company (42) allow use of these fabrics without excessive flexing and resultant fatigue failures. However, because the proportion of open area within the fabric decreases with the smaller pore sizes, greater straining areas are required when finer fabrics are employed. For example, consider the difference in throughput rates observed between 25 μm and 10 μm mesh fabrics. Given that throughput rates are proportional to open area, as reported by Ewing (43), 10 μm mesh fabric would require approximately four times more straining area than 25 μm mesh fabric to treat equal flows. Currently, both Crane Company and Envirex Company are independently investigating the use of ultra fine (openings less than 10 μm) nylon and polyester fabrics in the removal of algae from waste pond effluents. The complementary strategy of developing mass culture technology for microalgae harvestable by microstraining is the topic of this report.

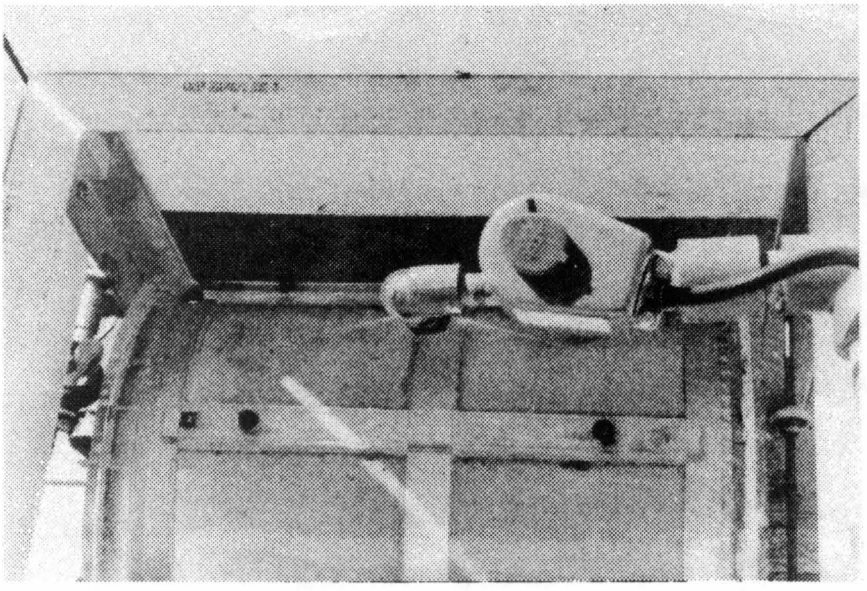
Recognition of this dichotomy in strategies led this project to neglect the development of microstrainer technology in favor of using microstraining as a tool in assessing the "harvestability" of different algal cultures. For

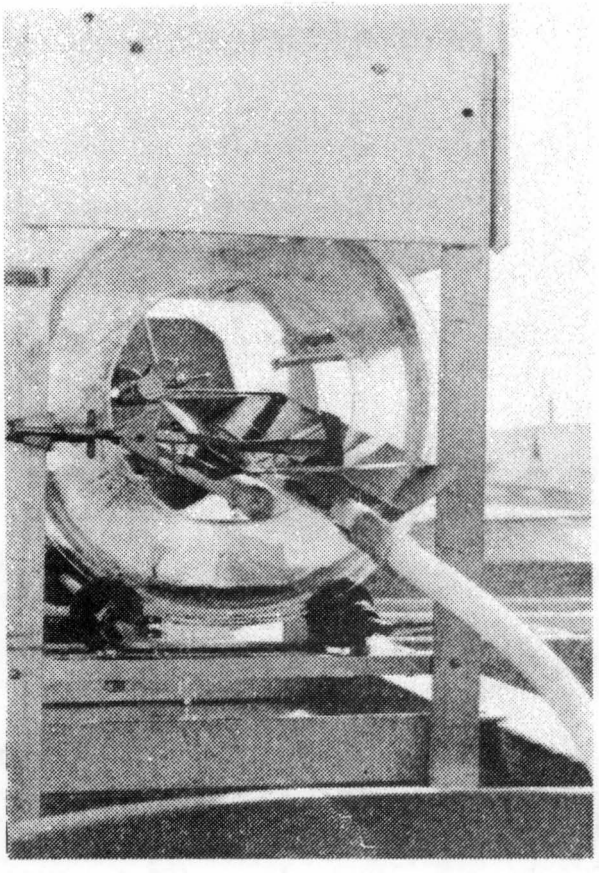
this purpose small pilot-scale microstrainers capable of treating several several liters of flow per minute were sufficient. Because microstrainers this small were not commercially available, these units were constructed at this laboratory. Nylon straining fabric having 26 μm openings was selected for use with the pilot-scale microstrainers for two reasons: (1) so that results obtained could be compared to previous studies where 23 or 25 μm mesh fabric was used and (2) because economic evaluation indicated that fabric area requirements became excessive when finer fabrics were employed. Because of their role in comparing harvestabilities of different algal cultures the pilot-scale microstrainers were constructed identically and were equipped with identical straining fabrics. However, the individual operational characteristics of the microstrainers could not be kept identical due to constraints imposed by culture densities and equipment limitations. In order to assess the influence of operational variables on microstrainer performance an experiment was conducted in which drum peripheral speed, drum pool depth and influent algal density were varied according to a preset schedule. The particle size distribution of the algal culture was maintained constant. The methods and results of this experiment are discussed below.

METHODS

Pilot-Scale Microstrainers

The experimental microstrainers were composed of three primary components: (1) the drum, fabricated from lucite plastic (1/4 inch thick), (2) the straining fabric, made of nylon with 26 μm openings (Tetko, Inc., 420 Saw Mill River Road, Elmsford, New York 10523) and (3) the frame, drive and backwash system. Photographs of a typical unit are shown in Figure III-2. The operation of the pilot-scale units differed from field-scale units in that the drum was not partially





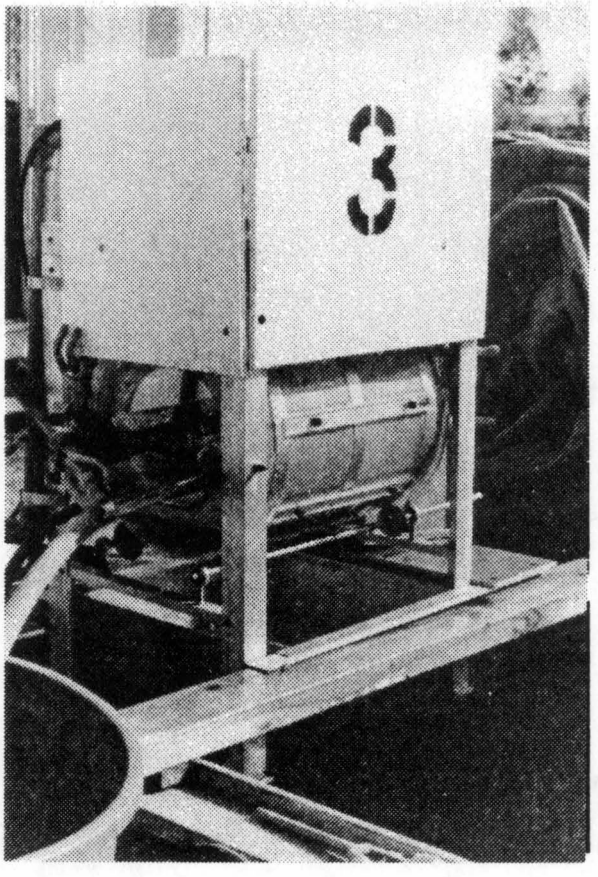


FIGURE III-2. EXPERIMENTAL PILOT-SCALE MICROSTRAINER

Views are (clockwise starting at top) of the solenoid valve-controlled backwash spray (looking down on the unit), perspective view of microstrainer (note algae on screen and drum pool) and face view showing the collection trough (note strained water dripping underneath the drum).

submerged. This had the effect of decreasing the proportion of available straining area utilized. Additionally, the maximum drum pool depth of the pilot units was 7.1 cm compared to a maximum of 60 cm for larger units, thus further decreasing unit throughput rates.

Design of the drum proved very important to separation efficiency, concentration performance and throughput rate. The final design incorporated eight narrow baffles, firmly attached to the straining fabric, running lengthwise inside the drum. Without the baffles the algae retained on the fabric tended to slide downward as the drum rotated. The baffles acted as barriers to catch the sliding algae and prevent them from falling back into the drum pool. These baffles were originally attached at an angle of 90° to the plane of the fabric, as shown in Figure III-3. However, it was later found that the baffles were more effective in trapping algae if they were inclined slightly (about 15°) in the direction of drum travel. All the microstrainer drums were then changed to incorporate this improvement.

Experimental Design

The question addressed by the experiment was what is the relative magnitude of performance variation attributable to variations in microstrainer operation? (e.g. how good is the "harvestability" data?). Because of the need to evaluate three variables over a multitude of levels, an orthogonal square experimental design was used (44). A 4 x 4 orthogonal matrix requiring 16 experimental trials was employed; such a matrix could have been used with as many as five variables but instead was restricted to three variables, each tested over four levels. For the experiment, a pilot-scale microstrainer of the design used to harvest the 12 m^2 algal growth ponds was fitted with a 30 cm diameter acrylic drum having 0.195 m^2 total straining area. Nylon straining fabric with 26 μm openings (the same fabric used in all the experiments in this report) was used.

Various influent densities were obtained by successively diluting the

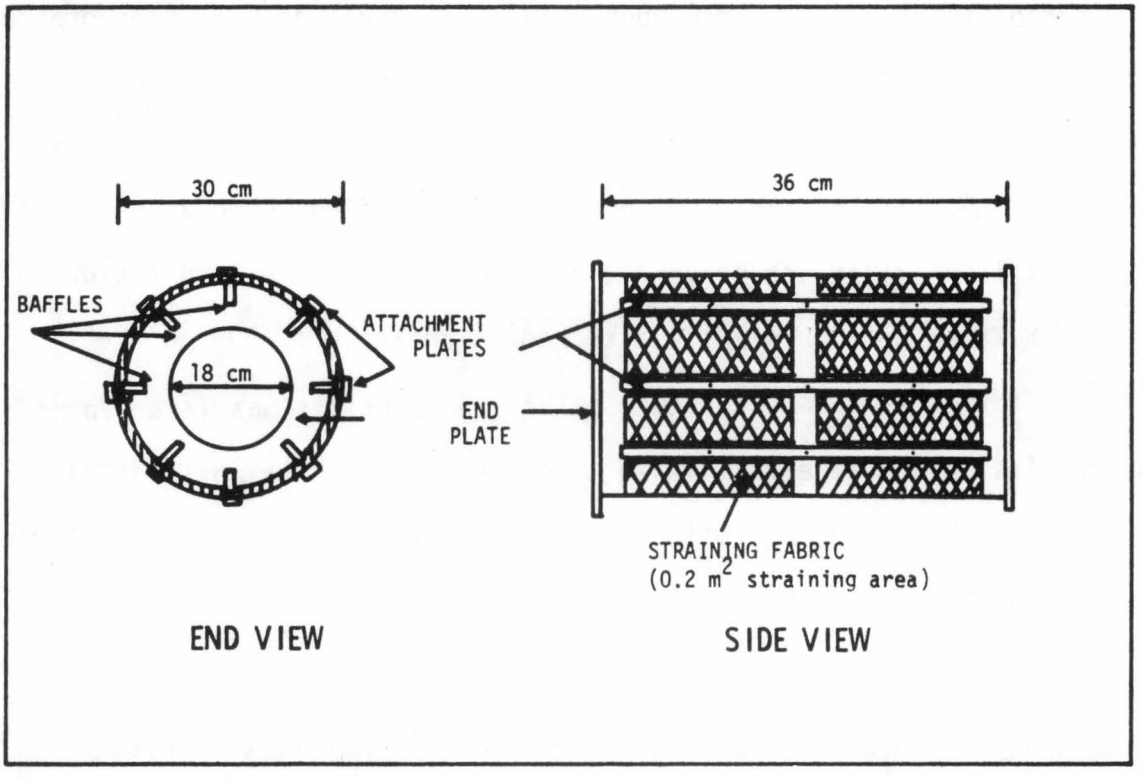


FIGURE III-3. DESIGN OF LUCITE DRUM USED IN PILOT-SCALE MICROSTRAINERS

original algal culture with potable water of the same temperature. Biologically, the culture was composed mostly of Scenedesmus and Micractinium, with smaller concentrations of Ankistrodesmus and pigmented flagellates present (see Table 2). Colony volumes for the Scenedesmus and Micractinium were moderately large, 1400 μm^3 and 1200 μm^3 respectively. Based solely on colony dimensions, it would be expected that about 99% and 80% of these respective algal colonies could be removed by straining fabric with 26 μm openings.

Trials were carried out for 5 or more minutes each. During each trial, composite samples of the influent, effluent, and concentrate streams were taken. The densities of each stream were measured using a Klett-Summerson photoelectric colorimeter equipped with a #66 (red) filter. Stream densities in terms of volatile suspended solids were calculated according to the regression determined previously (31),

$$VSS = 1.8 \times Klett \qquad (1)$$

RESULTS

The results obtained from each combination of experimental levels tested are given in Table 3 . After sorting and averaging these results according to the format defined by the 4x4 orthogal matrix the data were plotted to show the individual effects of each variable.

Separation Efficiency (Figure III-4)

The extent of schmutzdecke formation was observed to depend on the duration of fabric immersion in the drum pool. Lesser drum velocities, which provided longer immersion periods, resulted in higher separation efficiencies. At the higher drum velocities the backwash spray was not completely effective in removing algae from the inner surface of the fabric thus allowing some algae to be carried over back into the drum pool. This carryover tended to increase the probability of algae passing through the fabric. The range of separation efficiencies caused by varying drum peripheral velocity was

TABLE 2

BIOLOGICAL CHARACTERISTICS AND PARTICLE SIZE DISTRIBUTION OF THE ALGAL CULTURE USED IN MICROSTRAINER EVALUATION

Algal Species	Proportion by Volume %	Average Colony Volume μm^3
Scenedesmus	52	1400
Micractinium	44	1200
Ankistrodesmus	2	100
Pigmented Flagellates	2	200

TABLE 3

PERFORMANCE OF PILOT-SCALE MICROSTRAINERS UNDER VARIOUS OPERATIONAL CONDITIONS

Trial	Operational Variables			Performance			
	Drum Peripheral Velocity cm/min	Drum Pool Depth cm	Influent VSS mg/ℓ	Product VSS mg/ℓ	Separation Efficiency %	Inflow Rate ℓ/min	Harvest Rate gm/min
1	190	7.1	157	1,770	44	6.0	0.42
2	155	5.4	157	1,560	52	3.3	0.27
3	115	3.6	157	1,120	59	2.2	0.20
4	75	1.8	157	460	62	1.0	0.10
5	155	3.6	118	1,050	56	2.9	0.19
6	190	1.8	118	720	50	2.3	0.14
7	75	7.1	118	1,330	59	3.3	0.23
8	115	5.4	118	1,240	57	5.0	0.34
9	115	1.8	74	630	51	2.9	0.11
10	75	3.6	74	640	55	2.9	0.12
11	190	5.4	74	1,060	47	6.7	0.23
12	155	7.1	74	1,330	51	6.7	0.25
13	75	5.4	53	710	55	5.0	0.14
14	115	7.1	53	1,180	48	6.7	0.17
15	155	1.8	53	560	48	4.0	0.10
16	190	3.6	53	730	43	5.7	0.13
				Mean 1,005	52	4.2	0.20

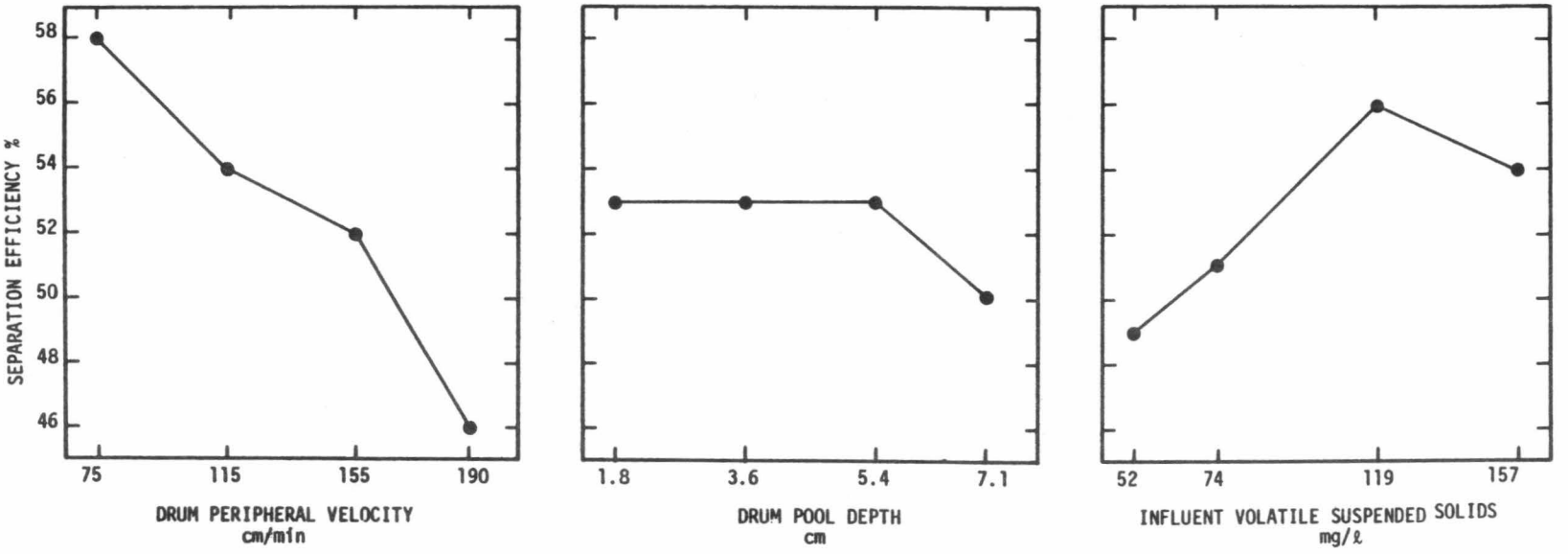


FIGURE III-4. EFFECTS OF DRUM PERIPHERAL VELOCITY, DRUM POOL DEPTH, AND INFLUENT VOLATILE SUSPENDED SOLIDS ON SEPARATION EFFICIENCY

larger (46-58%) than that caused by either drum pool depth or influent density. Schmutzdecke formation was also more extensive at higher drum pool depths because of greater wetted fabric areas. However, separation efficiencies did not reflect this behavior, in fact, they declined at the highest depth. Possibly the increase in pressure associated with the higher depths offset algal entrapment in the schmutzdecke by tending to force more algae through the fabric openings. The range in efficiencies caused by variable pool depths was relatively small (50-53%). Two factors, matt formation and backwash effectiveness, apparently interacted to produce an optimum separation efficiency at the influent density of 119 mg/l. Up to 119 mg/l the efficiency increased due to the improved schmutzdecke formation but above 119 mg/l there was a decrease in separation efficiency apparently because the backwash could not adequately clean the fabric when the filtration matt became too thick. The range of separation efficiencies effected by influent density (49-56%) was intermediate to that caused by drum velocity and pool depth.

Product Algal Density (Figure III-5)

Drum pool depth exerted the strongest influence on product density. The relationship was approximately linear, ranging from a density of 594 mg/l at the 1.8 cm depth to 1400 mg/l at the 7.1 cm depth. Apparently a greater compaction of the filtration matt resulted from the greater pressures associated with higher pool depths.

The drum peripheral velocity was least important in determining product density. A velocity of 155 cm/min gave the best product density (1120 mg/l) whereas the poorest product density (775 mg/l) was obtained at 75 cm/min. The falloff of product densities at both extremes of drum velocities appeared to be due to a dilution effect at the higher velocities caused by carryover of underwatered algal suspension into the product trough and to a different dilution effect at the lower velocities caused by an increased efficiency of fabric penetration by the back-wash spray.

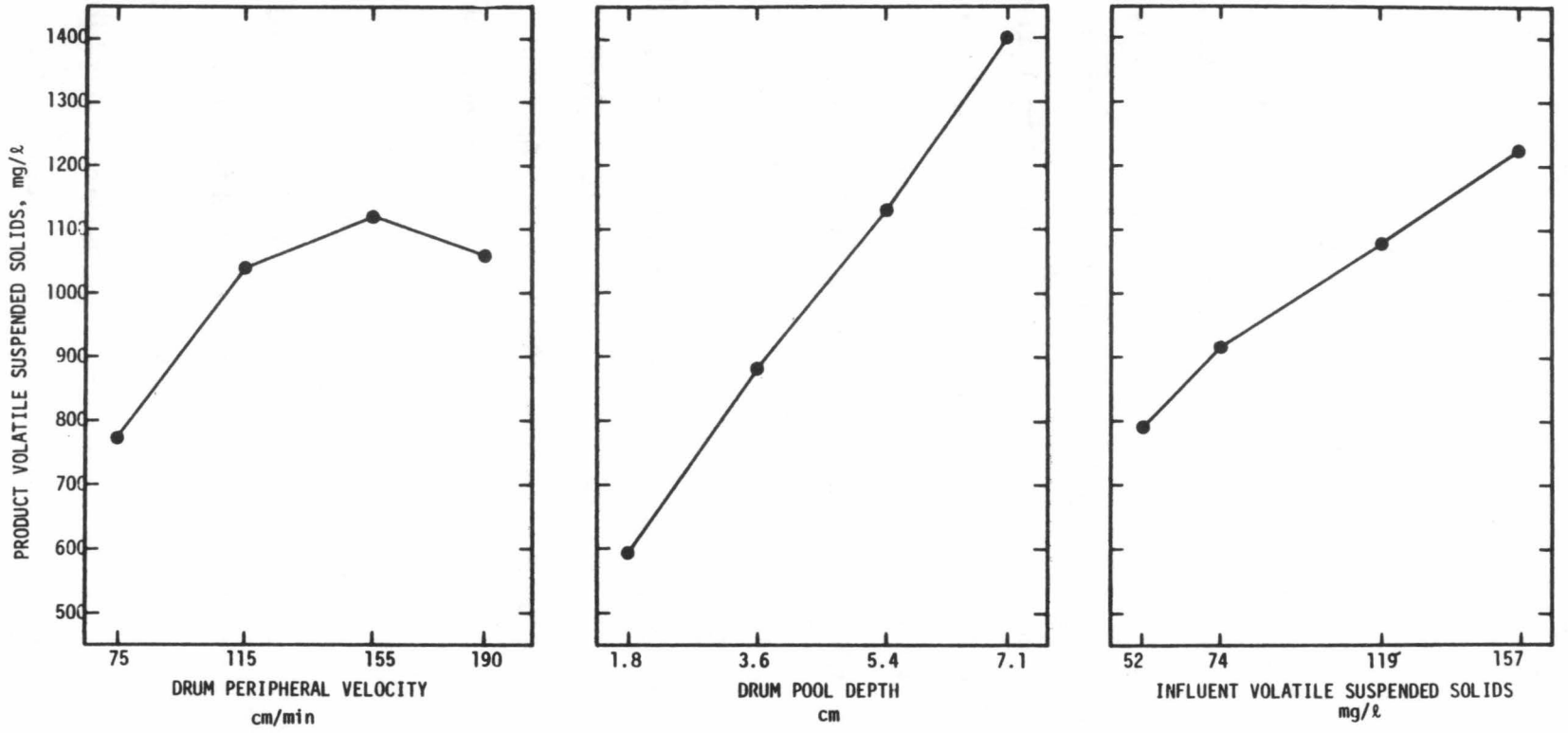


FIGURE III-5 EFFECTS OF DRUM PERIPHERAL VELOCITY, DRUM POOL DEPTH, AND INFLUENT VOLATILE SUSPENDED SOLIDS ON PRODUCT VOLATILE SUSPENDED SOLIDS

An approximately linear change in product densities was observed with increasing influent densities. The range of variance in product density (792 mg/l to 1220 mg/l) induced by the influent VSS was intermediate to the range effected by the two other variables tested.

Inflow Capacity (Figure III-6)

The rate of fabric renewal was important to the inflow capacity as evidenced by the approximate linear relationship between drum velocity and capacity. The step in the plot perhaps indicates that backwash efficiency partially limited capacity at the higher velocities. The range of capacity for this variable was 3.1 to 5.2 l/min. Inflow capacity varied directly with drum pool depth, ranging between 2.6 and 5.7 l/min. Conversely, an inverse effect on capacity was seen with inflow VSS. An inflow of 5.3 l/min was possible with an influent VSS of 52 mg/l whereas only 3.1 l/min of 157 mg/l inflow could be processed.

Harvest Rate (Figure III-7)

The harvest rate is the product of separation efficiency, influent density and inflow capacity. It is the parameter to be optimized with respect to algae-removal for the purpose of biomass production. As the plots indicate, the maximum harvest rates were obtained at the highest levels of drum peripheral velocity, drum pool depth and influent VSS. Pool depth most strongly influenced the harvest rate. The shape of all three curves indicates that the effects of the operating variables were beginning to level out at the highest levels. Backwash efficiency is most likely the ultimate limiting factor to harvest rates; an increase in backwash intensity would result in upward displacement of each harvest rate curve.

DISCUSSION

In order to assess the relative influences of the operational variables the respective ranges in microstrainer performance were compared to the mean

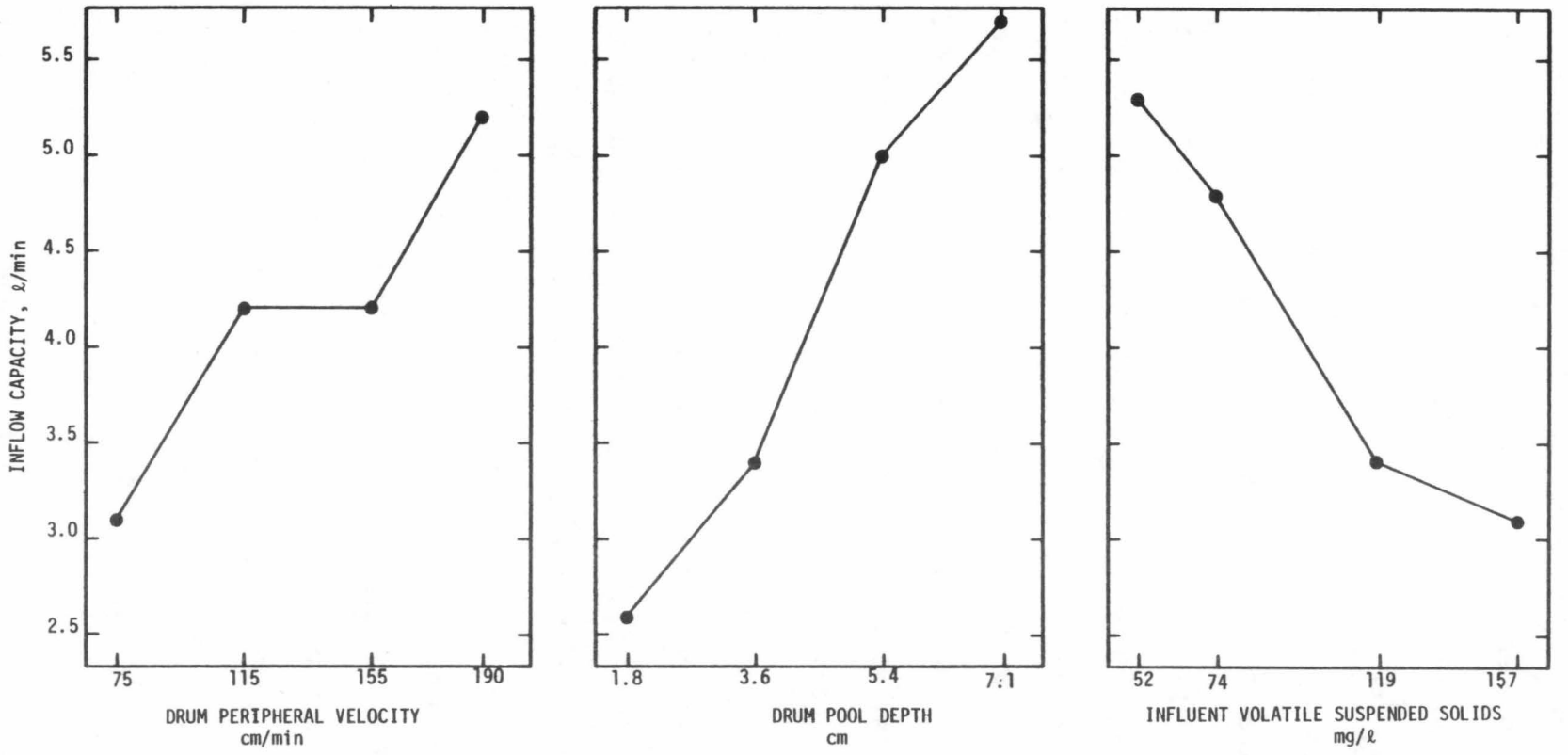


FIGURE III-6. EFFECTS OF DRUM PERIPHERAL VELOCITY, DRUM POOL DEPTH, AND INFLUENT VOLATILE SUSPENDED SOLIDS ON INFLOW CAPACITY

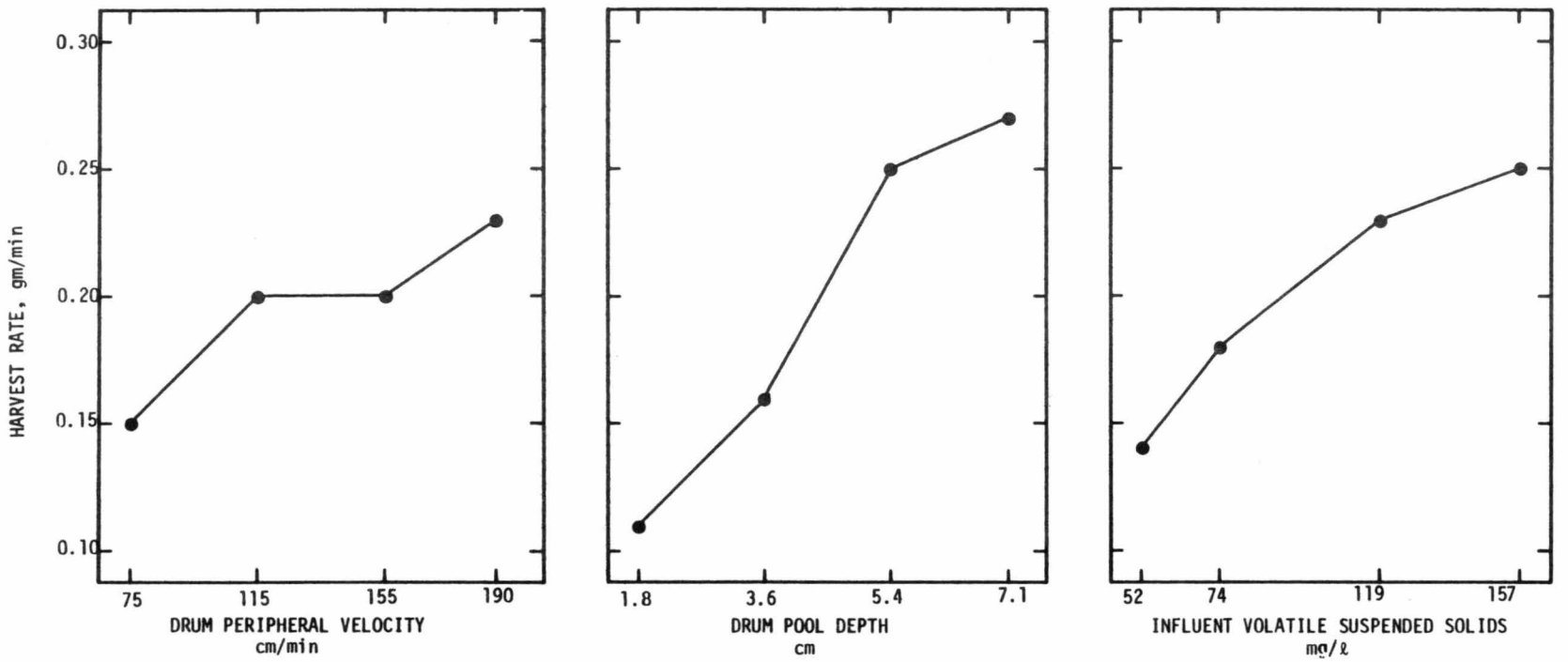


FIGURE III-7. EFFECTS OF DRUM PERIPHERAL VELOCITY, DRUM POOL DEPTH, AND INFLUENT VOLATILE SUSPENDED SOLIDS ON HARVEST RATE

performance value. For example, the range in separation efficiencies caused by drum peripheral velocity (58-46% or 12%) was equal to 23% of the mean separation efficiency (52%) obtained by averaging all 16 trials. The relative performance ranges (expressed as % of mean performance) are given in Table 4.

Separation efficiency of the four performance parameters evaluated was least affected by changes in operational variables. Inasmuch as separation efficiency was the parameter most critical to evaluation of culture "harvestability" this result was most advantageous. The results also indicate that during operation it is most important to maintain common drum peripheral velocities between microstrainers, letting drum pool depth vary. In addition, it appears that culture densities need not be identical to produce intercomparable results.

The remaining performance parameters were all strongly affected by the operational variables. Drum pool depth was the single most influential variable. Drum peripheral velocity and influent density exerted lesser influences. Because of the disparity in influence of the operational variables on separation efficiency and the remaining three performance parameters, it appears that microstrainer operation to produce intercomparable "harvestabilities" will necessarily produce non-intercomparable values for algal product density, inflow capacity and harvest rate. Therefore, use of these parameters to characterize algal cultures is not justified.

TABLE 4

EFFECTS OF OPERATIONAL VARIABLES UPON MICROSTRAINER PERFORMANCE

Operational Variable	Induced Ranges in Performance*, % of Mean Performance			
	Separation Efficiency	Algal Product Density	Inflow Capacity	Harvest Rate
Drum Peripheral Velocity	23	34	50	41
Drum Pool Depth	6	80	74	82
Influent Density*	13	43	53	56

*The size distributions of algae were identical between different densities of cultures.

IV. EXPERIMENTAL POND OPERATIONS

INTRODUCTION

Work conducted during the summer of 1976 (31) demonstrated that large, colonial forms of algae of the genera Scenedesmus and Micractinium could be efficiently removed with microstrainers. The experiments described in the following pages are all concerned with determining how much control selected pond operations can exert in maintaining these types of algae as predominant forms in the ponds, and what specific factors are most effective.

Operational variables of interest, identified in the previous study (31) included biomass and effluent recycling, hydraulic detention time and mixing. Each was tested for its capability of transforming harvestable pond cultures into unharvestable cultures and/or vice versa. Since herbivore blooms occurred zooplankton grazing and its relationship to the above variables was investigated as a determinant of harvestability as well as a factor contributing to the instability of intensive algal cultivation.

Many of the experiments described were designed to allow simultaneous correlation of the variables with productivity and sewage treatment, as well as harvestability. The experiments described in this chapter used the set of four 12 m^2 ponds. The experiments were intended to be carried out for periods of several, at least three, detention times and were terminated after definite differences or trends occurred. New experiments were started by intermixing selected ponds and starting on a new operational schedule. A set of seven different experiments was carried out during the period January to October 1977.

EXPERIMENT 1 RESULTS *

Using four ponds, two variables can be tested, each at two different values. In this experiment, ponds M1 and M3 were operated at a hydraulic detention time of 6.7 days and ponds M1 and M4 at 15 days. M3 and M4 were biomass recycled 50% (actual recycle), while M1 and M2 were not recycled. Since recycle makes hydraulic and cell detention times distinguishable for recyclable forms, this experiment could allow partial separation of their effects.

To provide the starting cultures, stationary-state cultures containing 75% harvestable Scenedesmus (as determined by microstraining) were intermixed and slowly diluted with sewage to fill the four ponds. The stationary-state cultures originated from two ponds which were left undiluted from mid-November, at which time they had been predominantly composed of non-harvestable Micractinium (left over from the previous year's experiments--see reference 31). The experiments during the previous summer and changeover from non-harvestable to harvestable populations in stationary ponds prompted this investigation of the effects of hydraulic and cell detention times on algal colony size distribution.

All four ponds were slow-mixed at a paddle wheel rotation of 1.3 rpm and operated at a depth of 12 inches. Figures IV-1-8 show the data collected and Table 5 provides a convenient (but, as discussed below, sometimes misleading) summary. As expected, both long detention time and recycling led to increased pond density. Pond M2 was expected to attain an average density comparable to M3, but, as shown in Figure IV-1, several weeks into the experiment, the pond density decreased precipitously. It became necessary to "spike" this pond with algae. Harvestable algae from M3 and M4 concentrates were used. The crash was characterized by the development of

* In this chapter all figures and tables follow the discussion of individual experiments.

large clumps of algae and a relatively transparent medium. The recovery of this pond remained doubtful. The density eventually started to decline again. In addition, the colonies were significantly larger in this pond than the colonies used for spiking. Subsequent experience has correlated this type of decline in pond density with proliferation of grazers (see below), but grazers were not counted at this time. It seems apparent that grazing was significant in this pond. Hence the effect of grazing on this experiment is uncertain.

Effluent densities (after microstraining) were low in both of the long detention-time ponds (17 mg/ℓ in M2 effluent, 21 mg/ℓ in M4 effluent). The effluent from the short detention, recycled pond (M3) tended to be lower than that from the short detention time, non-recycled pond (M1), but was consistently higher than the effluents of long detention-time ponds. The harvestability was greater than 80% for these latter two ponds (M2, M4) and 75% for M3. This short detention-time, recycled pond was thus much more harvestable than the non-recycled, short detention-time pond (M1) which was only 48% harvestable.

The total production was less than 5 gm/m^2/day in all ponds and influenced more by detention time than by recycle. A recycle of 50% reduced total production by 20% at 6.7 days and by 10% at 15 days. Harvestable production was similar in all ponds except M4 in which it was lower. Photosynthetic efficiency is proportional to total production and, hence was also low in these dense cultures. Since insolation during January and February was low, and the cultures were dense, high productivities were not expected.

Table IV-1 shows that the chlorophyll a content per unit of ponds solids was greater in denser ponds, a self-shading response that is usually observed in algal cultures. The ratio of percent chlorophyll in the effluent solids to percent chlorophyll in pond solids correlated with growth rate; the slower-growing populations exhibited a lower ratio.

There was little discernable difference in the species dynamics in this experiment. In all ponds, Scenedesmus was the predominant algal type at the beginning and end of the experiment. However, its proportion declined in all ponds during the course of the experiment. Micractinium was the sub-dominant type at the end in all ponds except M2 which had experienced the decline in density (Table 5). Counts of the ponds and effluents at the end of the experiment showed that M2 and M4 had almost exclusively large colonies and that the effluents also contained large colonies. Large colonies of Scenedesmus encompassed any colony of four or more cells of the large-body Scenedesmus and eight or more of the small-body Scenedesmus. M2 had many more very large colonies. In M1 and M3 about 80% of the Scenedesmus were in large colonies, but in M1 only two-thirds of the Micractinium were large, whereas over 90% were large in M3.

The sewage treatment data indicate that long detention time was the major determinant of the extent of treatment with recycle having a small additional effect. Both long detention time pond effluents were below 30 mg/ℓ total suspended solids and also low in NH_3-N and COD. BOD was not measured in this experiment.

pH also exhibited the expected trend of increasing with detention time and recycle. The average pH of the 6.7 day non-recycled pond (M1) was about 8.0 in the morning (9 AM) and 8.7 in the afternoon (4 PM). The 15-day, non-recycled pond (M2) averaged 8.9 in the morning and 9.3 in the afternoon. Recycled ponds were over one-half a pH unit higher than non-recycled ponds. Differences between morning and afternoon pH's were less on cloudy days. The temperatures of all ponds were the same with morning temperatures 3°-7° lower than afternoon temperatures.

At the conclusion of Experiment 1, the ponds were intermixed and another detention time-recycle experiment started. The experiment was carried out for only a short time (10 days) so that only transients were observed

(Figure IV-6). The short detention time ponds, whether recycled or not, produced high density effluents and, thus, harvested poorly. The two longer detention time ponds--7 days, no recycle and 13.6 days, 33% recycle--produced effluents of approximately equal quality. Productions were reduced 25% (from 8 to 6 $g/m^2/day$) by 33% recycling at 4.5 day detention time, although averages from this transient experiment are of dubious significance.

EXPERIMENT 1 CONCLUSIONS

The main observation during this experiment was that the different values for recycling and detention time did not have much effect on species composition. The dominant algal type remained dominant with no significant differences between ponds. Apparently the physicochemical environment and the range of operational parameters covered maintained the initial algal types. However, algal colony size distribution did differ significantly between ponds. This is easily seen by taking harvestability as an operational definition of the ratio of large colonies to small colonies and effluent density as a measure of the density of small colonies in the pond. Both of these are very rough measures. Effluent counts showed that almost all of the small colonies were unharvestable, but that the smaller number of unharvested large colonies still could dominate the effluents on a volume basis when the pond had few small colonies. The trend was that a pond with higher harvestability had a higher ratio of larger-to-smaller colonies, and that the effluent was an overestimate of the number of small colonies. The short detention time, non-recycle pond (M1) had the lowest ratio of large to small and the greatest number of small colonies. M3, the other short detention time pond, but 50% recycled, had the next highest number of small colonies. The selective recycling of large colonies would cause a continuous decrease, and eventual washout, of small colonies from this pond unless the pool of small colonies was continuously replenished from

the pool of large colonies through colony breakdown. (Although there were always several types of Scenedesmus, distinguished by larger and smaller cells for example, each of these types occurred in both large and small colonies.) Rates of colony breakdown and formation could be affected by sewage detention times and cell growth rate. Thus, the rate of colony breakdown can be written as as $A^{L\rightarrow S}(\Theta,\mu^{L})X^{L}$ and the rate of colony formation as $B^{S\rightarrow L}(\Theta,\mu^{S})X^{S}$, where X denotes cell density; Θ is hydraulic (sewage) detention time; μ is specific growth rate; S refers to small colonies; L refers to large colonies; and $A^{L\rightarrow S}$ and $B^{S\rightarrow L}$ are specific rates. Any influence of hydraulic detention time on $A^{L\rightarrow S}$ and $B^{S\rightarrow L}$ was equalized between M1 and M3 because Θ was 6.7 days for both. If these specific rates were dependent on growth rate such that a slower growth rate increased the net formation of colonies, then this might account for the fewer number of small colonies observed in M3 compared to M1. If a lower growth rate did not change A and B, or if it changed them similarly, then one might have expected more small colonies in M3 because the recycling increased the ratio of large to small. But recycling increased this ratio by lowering the steady state levels of smaller colonies. The recycling of large colonies increased the pond density thus lowering growth rates of all algae without reducing the washout rate of small colonies. Thus, recycling by itself could lead to fewer small colonies in M3 versus M1 just by decreasing their rate of production through growth alone. So it is not possible to determine, from this experiment, the dependence of colony formation on growth rate. If all of the algae in M3 were recycled (non-selective recycle), then the only effect of the recycle would be to increase density. Any change in the ratio of large to small, i.e., harvestability, would yield information about the dependence of colony formation on growth rate.

The experiment was designed to elucidate the effect of hydraulic detention on the net rate of colony formation through a comparison of two ponds (M2 & M3) in which the specific growth rates were expected to be equal.

However, μ was not the same in both ponds since the densities were so disparate. Also, the apparently anomolous density decline in M2 makes any comparison tenuous. The rate of washout of small colonies was faster in M3 and their rate of growth was slower; the ratio of large colonies to small colonies was lower in M3, and yet the pool of small colonies was larger. One may tentatively conclude that either the longer hydraulic detention time in M2 decreased the net rate of colony breakdown (leaving unexplained the decline in density) or that grazers depleted the pool of small colonies at a selectively faster rate than the pool of large colonies, thereby increasing harvestability. Both are possible.

M4 demonstrated that greatly increasing both hydraulic and cell detention times has limited additional benefit. It may be that senescence of the culture (increased cell death) or additional nutrient limitation (see below) also affect colony size distribution.

Some effect of hydraulic (sewage) detention time on colony size can also be seen by comparing M1 (θ = 4.6 days, 33% recycled) with M2 (θ = 4.4 days, no recycle) from the short-term experiment shown in Figure IV-6. Both ponds started out about 75% harvestable and both showed similar time courses in becoming less than 50% harvestable. Thus, slower or equal growth rates (as judged by densities) and selective recycling of M1 did not result in increased net rates of colony formation in M1. The shorter (as compared with the previous experiment) hydraulic detention times may have overriding effects on these processes.

Algal cultures require energy input just to maintain the culture at zero growth rate. The insolation during the period of this experiment averaged only 263 langleys, leaving little solar energy available for net productivity. The low photosynthetic efficiencies of all ponds reflect this. The pH's indicate that carbon dioxide availability may also have limited the growth

of these cultures and thus may have resulted in a further reduction in efficiency, especially at the long detention times. The lower efficiency of the long detention recycle pond may be a consequence of N limitation as well (because ammonia levels dropped to about 1 mg/ℓ), although the decrease in efficiency at such high density could be predicted on the basis of light and carbon dioxide limitation alone. The difference in efficiency (total production) between M2 and M3 is predominantly a consequence of the decline in density of M2.

As shown by the data and explained in Appendix II, biomass recycling usually decreases productivity at least somewhat by increasing pond density. Since harvestable algae are selectively recycled harvestable production is lowered more than total production (compare M2 and M4 in Table 5). Thus, recycling should only be practical in situations where its positive effects on harvestability are significant. This experiment indicates that these effects may be significant at moderate detention times but that a short detention time pond cannot be made harvestable through recycling and that a long detention time pond is harvestable without recycling. Of course, the classification into short, moderate, and long depends on climate, season and species.

TABLE 5

EXPERIMENT 1 SUMMARY TABLE

Dates 1/17-2-21
Insolation (Langleys/day) 263
Temperature (°C) AM 8.8 PM 13.5
Depth (cm) 30

	M-1	M-2	M-3	M-4
Detention Time (days)	6.7	15	6.7	15
Mixing Speed (paddle wheel, rpm)	1.3	1.3	1.3	1.3
Recycle Fraction (%)	0	0	50	50
Pond Density (VSS mg/ℓ)	85	101	134	167
Effluent Density (VSS mg/ℓ)	44	17	33	21
Harvestability (% chlor. removal)	48	83	75	87
Total Production (gm VSS/m^2 day)	3.9	1.9	3.1	1.7
Harvestable Production (gm VSS/m^2 day)	1.7	1.7	1.6	1.2
% Chlorophyll (by weight)				
Pond	1.8	1.8	2.1	2.6
Effluent	1.8	1.1	1.4	1.3
Conversion Efficiency (%)				
of Total Sunlight	.8	.4	.6	.4
of PAR	1.9	.9	1.5	.8
Dominant Algae (beginning)	Scen. 73%	Scen. 89%	Scen. 82%	Scen. 81%
Sub-Dominant Algae (beginning)	Chlamydomonas 21%	Mic. 8%	Mic. 12%	Mic. 18%
Dominant Algae (end)	Scen. 69%	Scen. 54%	Scen. 64%	Scen. 54%
Sub-Dominant Algae (end)	Mic. 14%	Ankist. 40%	Mic. 34%	Mic. 29%
Effluent NH_3 mg/ℓ/% Removal*	15.3/43	5.6/79	11.9/56	3.4/87
Effluent COD mg/ℓ/% Removal*	170/56	72.68	166/57	96/75

*% of pond influent

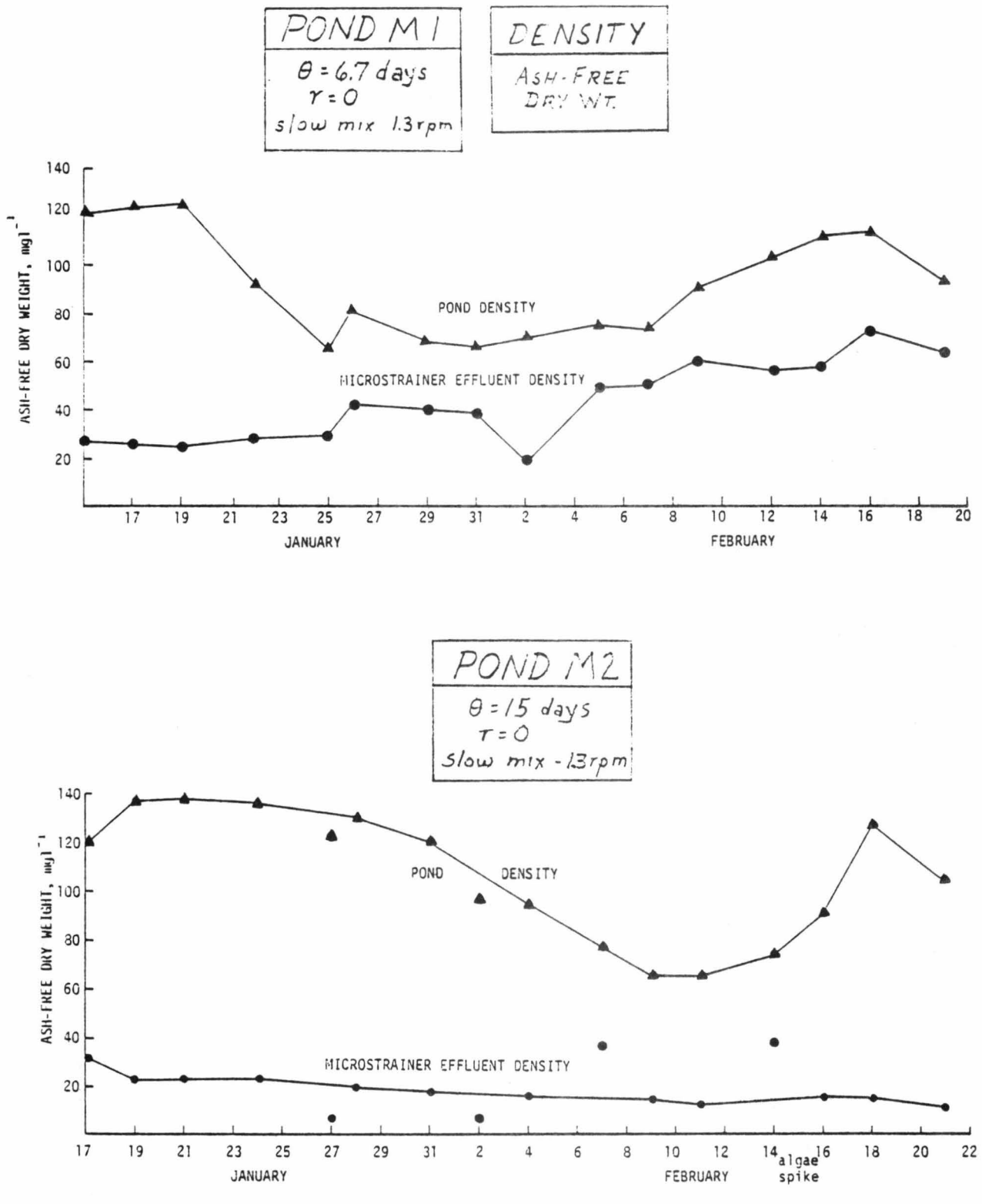


FIGURE IV-1. M-1 and M-2 POND AND MICROSTRAINER EFFLUENT DENSITY: EXPERIMENT 1

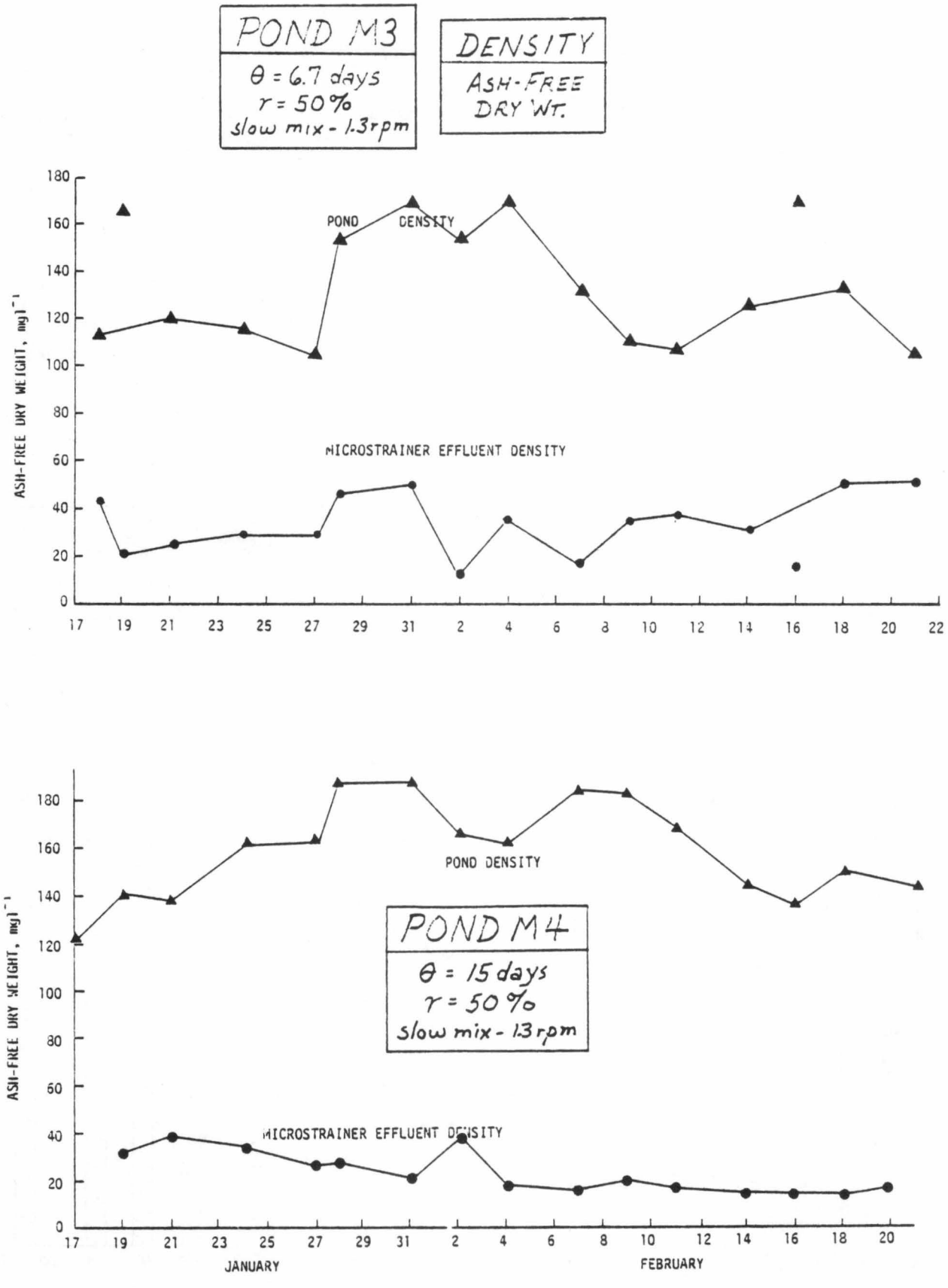
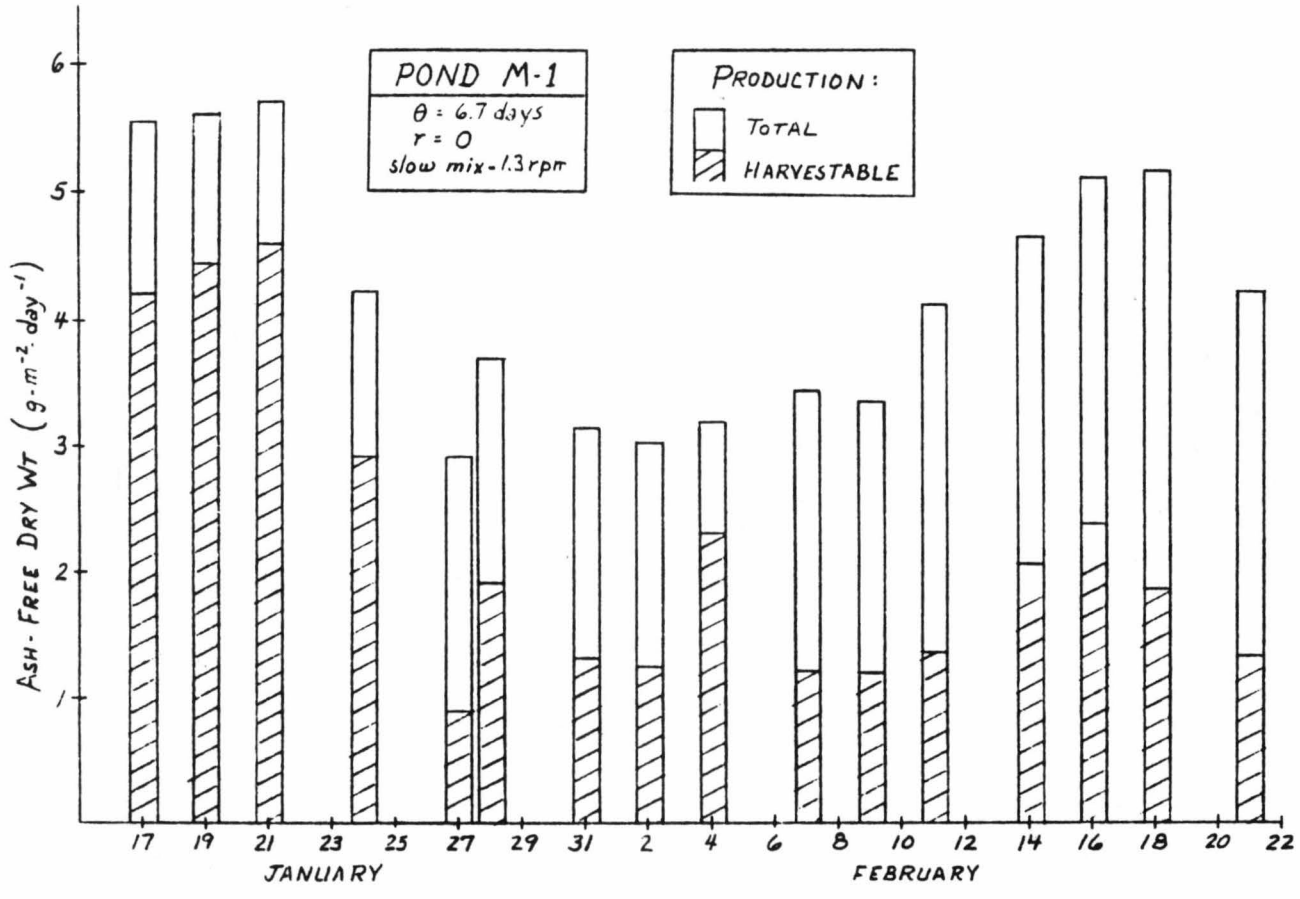


FIGURE IV-2. M-3 and M-4 POND AND MICROSTRAINER EFFLUENT DENSITY: EXPERIMENT 1



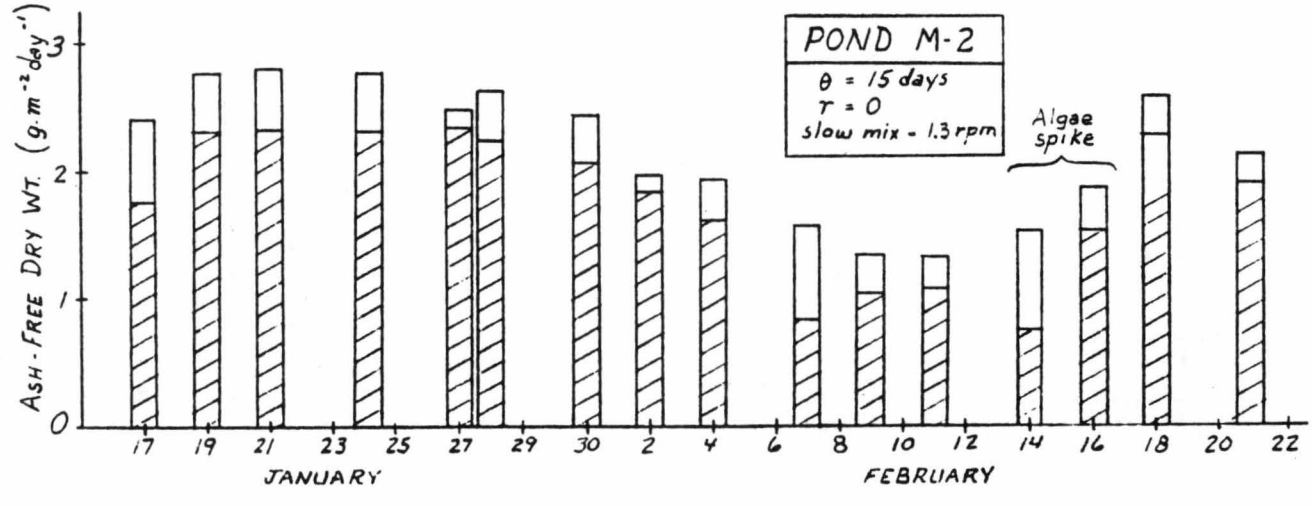


FIGURE IV-3. M-1 AND M-2 PRODUCTIVITY: EXPERIMENT 1

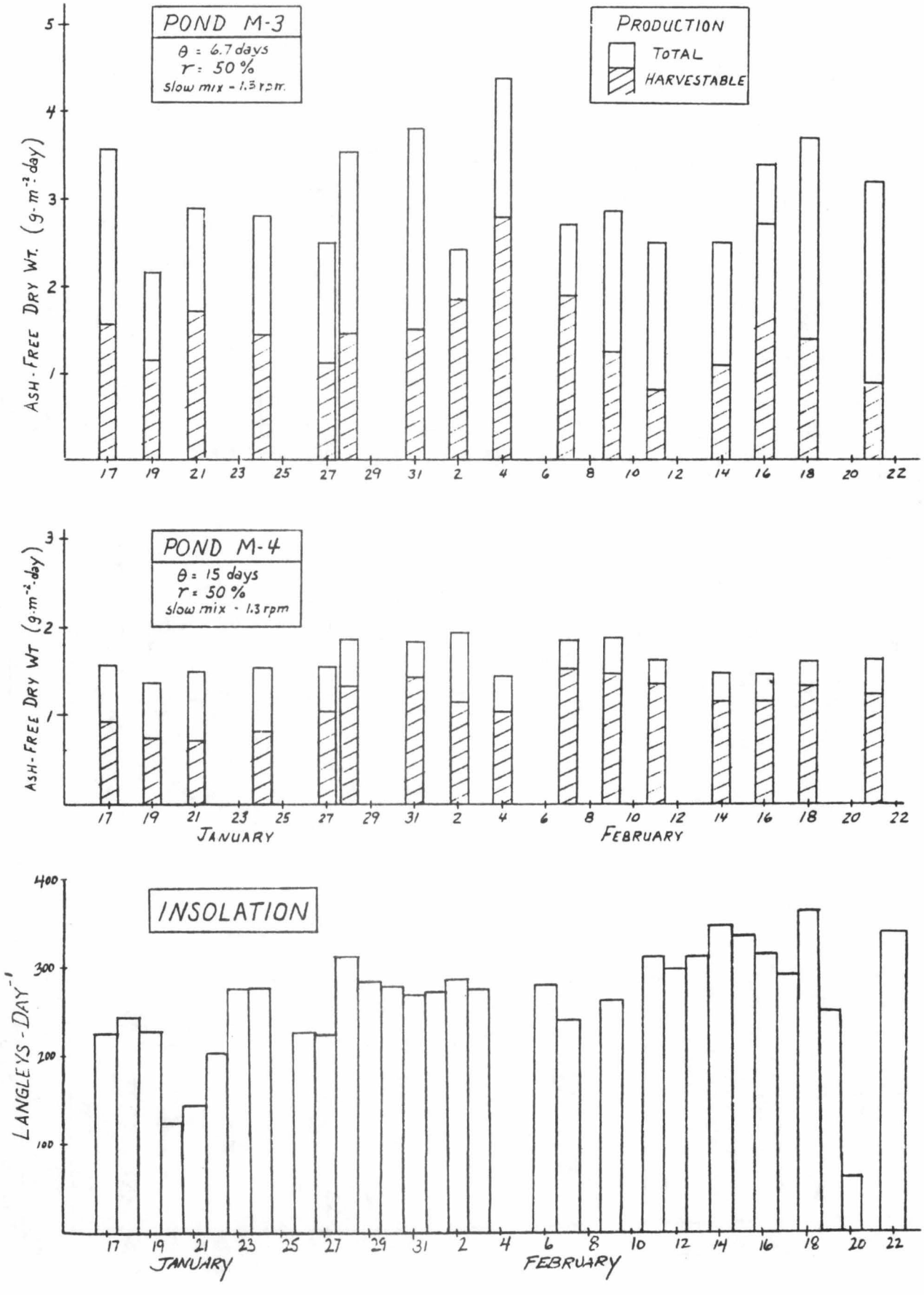


FIGURE IV-4. M-3 AND M-4 PRODUCTIVITY, INSOLATION: EXPERIMENT 1

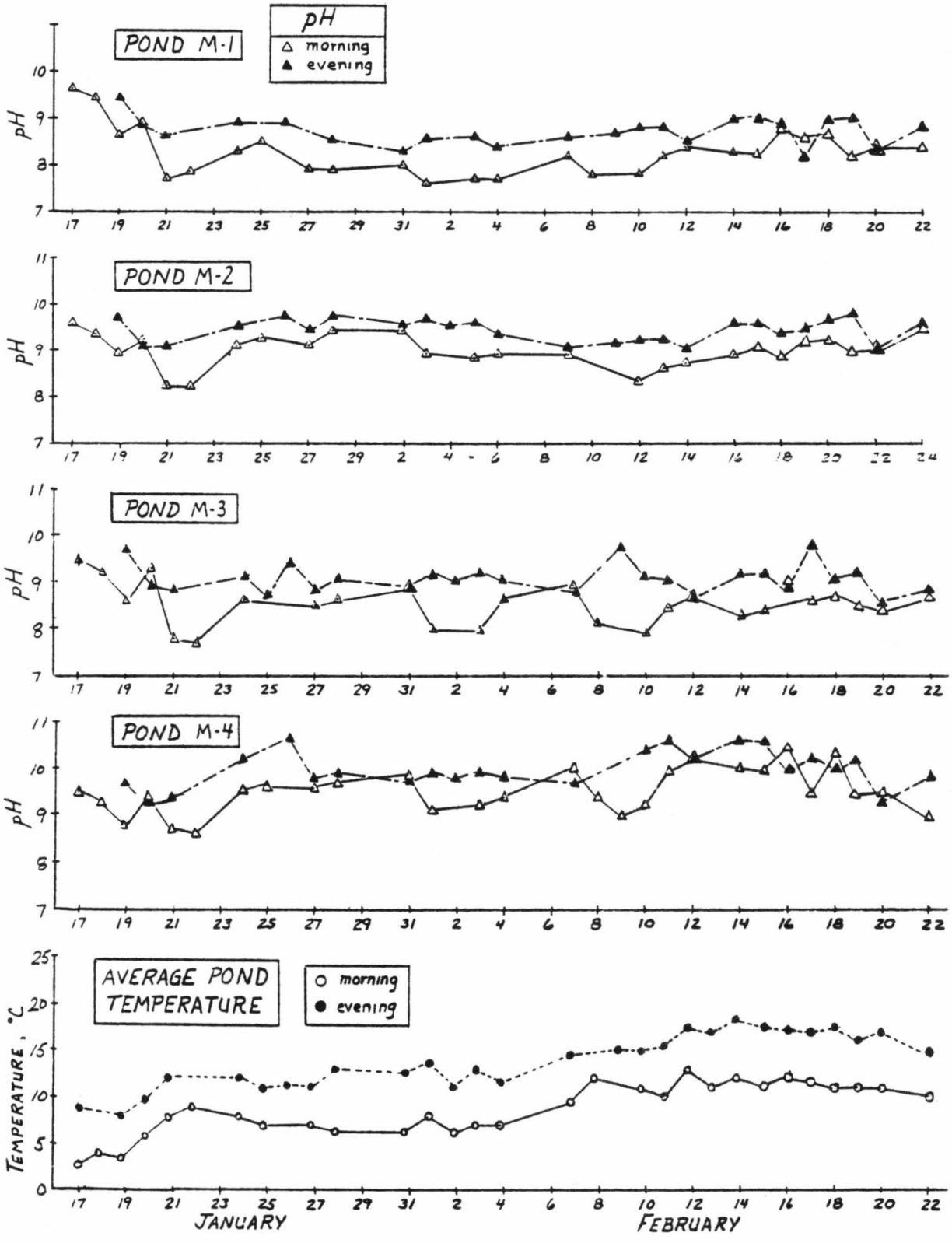


FIGURE IV-5. POND pH, AVERAGE POND TEMPERATURE: EXPERIMENT 1

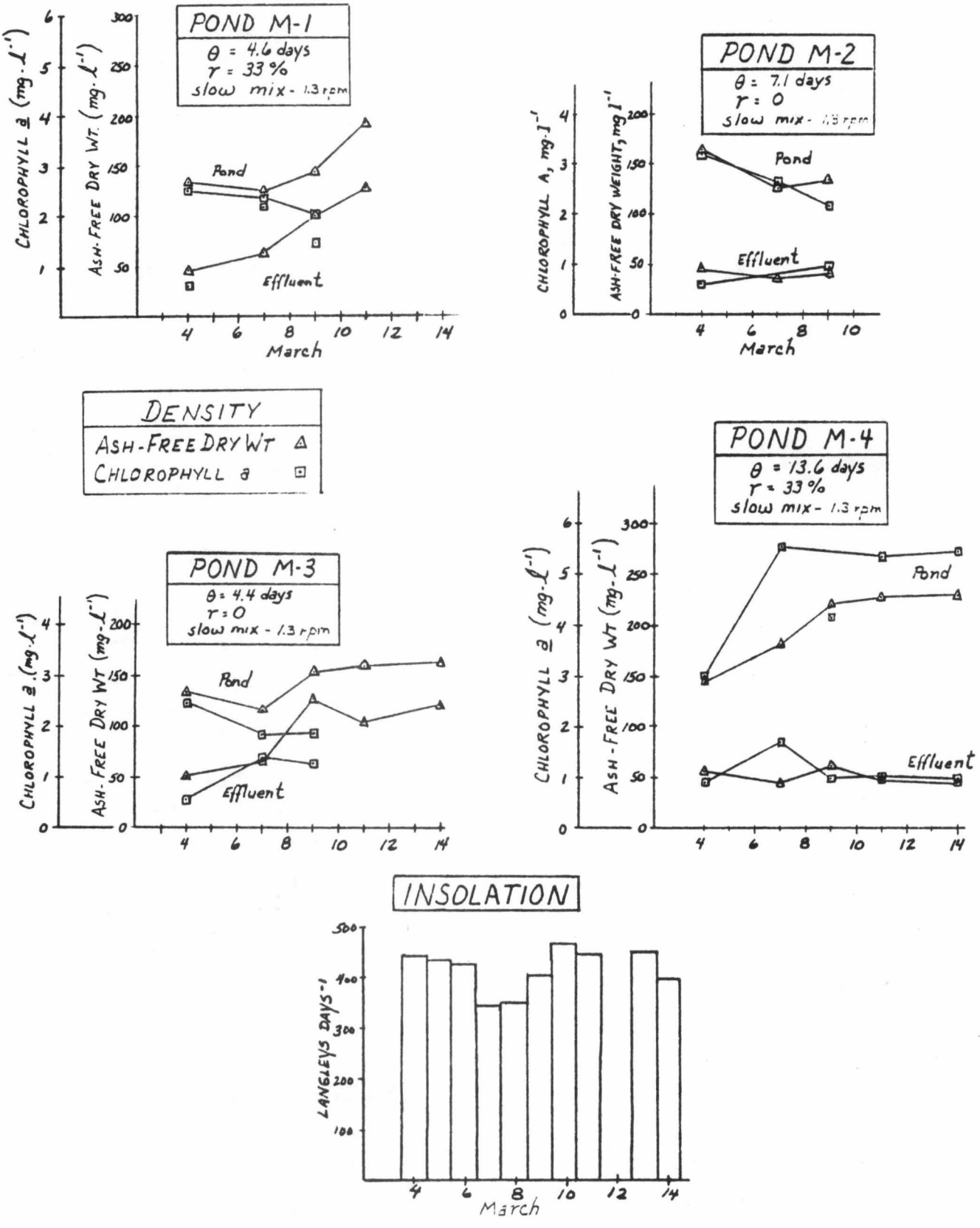


FIGURE IV-6. POND AND MICROSTRAINER EFFLUENT DENSITY, INSOLATION: EXPERIMENT 1

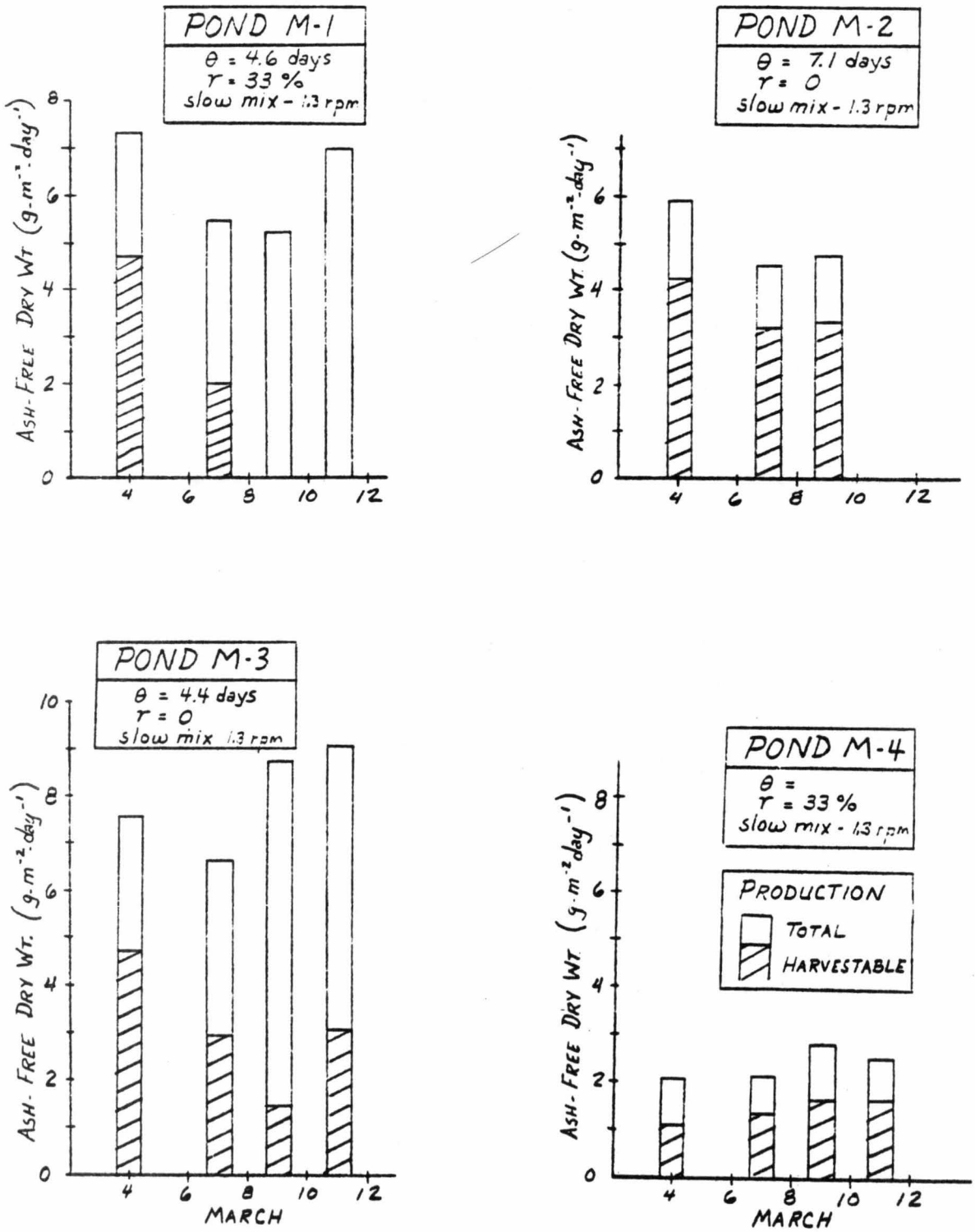


FIGURE IV-7. PRODUCTIVITY: EXPERIMENT 1

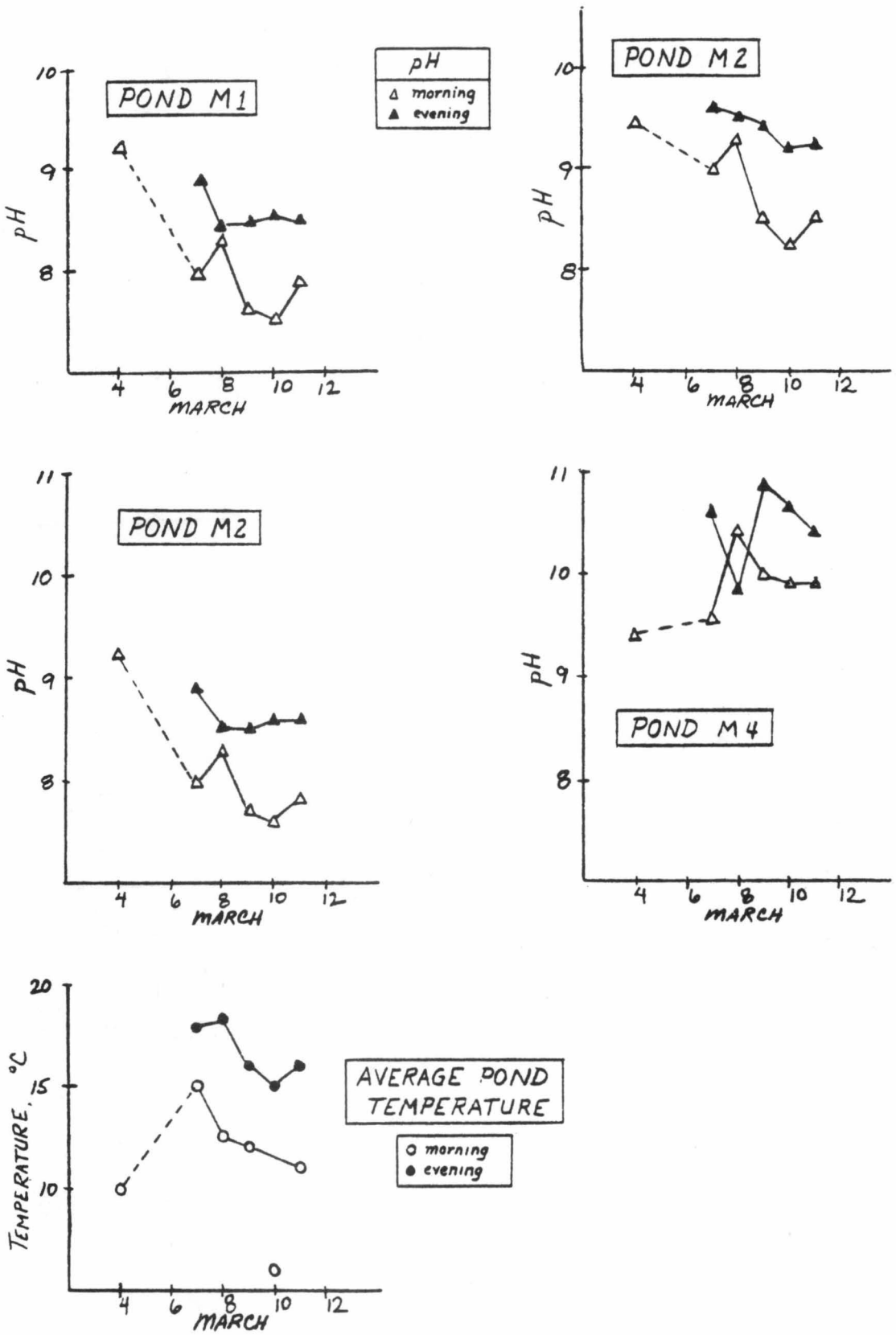


FIGURE IV-8. POND pH, AVERAGE POND TEMPERATURE: EXPERIMENT 1

EXPERIMENT 2 RESULTS

In this experiment a third operational parameter, mixing speed, was tested for its effects on particle size distribution, selection of algal types, and productivity. Ponds M1 and M3 were cleaned and intermixed one week prior to the beginning of the experiment and thus had similar starting points. Both ponds were operated at a short detention time, 4.4 days, with M1 fast-mixed (paddle wheel rotation equal to 4.0 rpm) and M3 slow mixed (1.3 rpm). These correspond to linear velocities of 15 and 5 cm/sec at the average immersion radius of the paddle wheel. Neither pond was biomass recycled. M4 was continued from the previous experiment (i.e. not intermixed with any pond), but the detention time was reduced to 9.4 days. This change was in response to anticipated higher insolation and ambient temperature. The pond was recycled 33% (actual). All ponds were operated at a depth of 10".

M4 served as a harvestability control. The previous experiment had indicated that a long detention time, recycled pond would harvest well. The steep initial decline in density of this pond can be partially attributed to the sudden reduction in detention time and partially to grazers (rotifers were observed microscopically). The pond did not continue to decrease in density and showed no other signs of heavy grazing. Still, as with the previous experiment, the importance of grazing in maintaining harvestability could not be determined without quantitative zooplankton determinations.

As shown in Figure IV-9, M1 and M3 were initially similar but diverged quickly. The high concentration of VSS (ash-free dry weight) in M1 on 21 March was due to the initiation of the fast mix that day which suspended the sludge that accumulated from 15 March to 21 March (settled algae, bacteria, and detritus). The amount of non-algal sludge that was resuspended by the fast mix can be approximately calculated from the VSS and chlorophyll data on 21 March. Assume that M1 and M3 were identical just prior to 21 March.

operated similarly until 21 March. Also assume that the chlorophyll a content of the algae resuspended by fast mixing in M1 was about the same as the chlorophyll content of the algae already suspended. (This need be only approximately true since the chlorophyll data from M1 and M3 indicate that fast mix caused only a 25% increase in chlorophyll.) Then the concentration (mg/ℓ) of non-algal VSS that was resuspended by fast mix in M1 is equal to the concentration (mg/ℓ) of VSS in M1 on 21 March minus the product of the chlorophyll a concentration in M1 on 21 March and the ratio of VSS to chlorophyll a in M3 on 21 March. It may be greater than this because the VSS to chlorophyll ratio used assumes all of M3 suspended solids were algae, which is obviously not so. Thus, about 100 of the 230 mg/ℓ VSS in M1 was resuspended non-algal sludge. The fast mix pond continued to have more non-algal VSS than the slow-mix pond. This was reflected in the lower percentage, by weight, of chlorophyll a in M1 when compared to M3 (1.4% vs. 1.8%). The initial sludge resuspension washed out after a couple of detention times and, thus, had little effect on the average ratio of chlorophyll to suspended solids. This trend is shown graphically in Figure IV-9 where the gap between the ash-free dry weight and chlorophyll curve is larger throughout the experiment in Pond M1 than in Pond M3.

It is difficult to calculate the average amount of non-algal solids present in M1 from the data. As mentioned above, chlorophyll content, expressed as percent chlorophyll a in Table 6 ,is the amount of chlorophyll in all of the pond solids, algal plus non-algal. Therefore, one can only attempt to calculate the "extra" amount of non-algal solids present in M1 due to fast mixing but absent from the slow mixed M3. Even so, the non-linear response of the chlorophyll content in algal cells to increasing density (algal plus non-algal) poses a problem. If it is assumed that the

chlorophyll a content per unit weight of algae was the same in M1 as in M3 despite the increased shading in M1, then there were only 239 - $\frac{(1.4)(239)}{1.8}$ = 53 mg/ℓ of "extra" non-algal solids in M1 due to fast mixing. A more realistic assumption might be that the chlorophyll content of algae in M1 was 2.5% of the VSS, giving 239 - $\frac{(1.4)(239)}{2.5}$ = 105 mg/ℓ "extra" non-algal solids in M1 if none are assumed in M3. In any case, although the total amount of chlorophyll was less in M3 than in M1 (2.9 mg/ℓ versus 3.3 mg/ℓ), it is most likely that the algal suspended solids concentrations were similar.

The same calculations can be made using the production data. The total chlorophyll a production was .19 g/m²/day from M1 compared to .16 gm/m²/day from M3. Yet, this may represent as little as 7.6 g/m²/day of algae produced by M1 if the algae were 2.5% chlorophyll. By contrast, M3 may have produced close to 9 g/m²/day of algae. On a suspended solids basis alone, fast mixing increased productivity from 9.0 g/m²/day in M3 to 13.6 g/m²/day in M1.

The effluent density from M1 was much lower than that from M3. The harvestabilities (M1 was 85% harvestable, M3 only 22%) reflected this difference as well as the differences in pond densities. The ratio of percent chlorophyll in the effluent to percent chlorophyll in the pond was somewhat lower for M1 than for M3. M1 effluent remained low in suspended solids throughout the experiment. M4, the harvestability control also remained harvestable throughout the experiment, producing a lower density effluent than M1.

The species dynamics were different in all of the ponds. M1 and M3 were initially 50% Micractinium (about 1/3 of the colonies were large according to counts of the ponds and effluents) with Scenedesmus (greater than 70% large) and Ankistrodesmus sub-dominant. In the fast-mix pond, after one week (30 March), Scenedesmus replaced Micractinium as the dominant algal type with

Ankistrodesmus dropping below 10%. In the short detention time, slow-mix pond, Micractinium increased its proportion at the expense of Scenedesmus while the percent of Ankistrodesmus remained unaltered. In both ponds, most of the Scenedesmus were in large colonies and all of the other algae were in small colonies. In M4, the slow-mix harvestability control, Ankistrodesmus and Scenedesmus were co-dominant initially. The former was 85% harvestable and aggregated into groups of at least 2-3 cells. Almost all of the Scenedesmus were in large colonies. The proportion of Ankistrodesmus doubled, becoming greater than 90% of the biovolume. This alga remained harvestable and aggregated.

M1 became highly flocculated with algae caught up within a non-algal matrix. This was visible to the naked eye (the flocs were up to several mm in size) and confirmed through microscopic examination. These flocs were not present in the two slow-mixed ponds. This flocculation phenomenon was a very striking, visual difference between the ponds (M1 and M3) observed soon after initiation of fast mixing.

The sewage treatment data presented in Table IV-2 , are based on one sample (taken on 6 April near the end of the experiment). There was little difference in NH_3-N removal between M1 and M3, but considerable difference in COD removal. The latter is measured on unfiltered samples and thus the poor harvestability of M3 led to high COD in the effluent. The harvestable control exhibited 95% NH_3-N removal, as was expected in a long detention time pond. The COD removal was comparable to M1.

EXPERIMENT 2 CONCLUSION

In this experiment, the different modes of operating ponds appeared to select for different algal types. Scenedesmus was at a relative disadvantage in the slow-mixed ponds. Its proportion fell significantly in these ponds while it rose significantly in the fast-mixed pond. Presumably, this alga was less competitive when its suspension in the water column was not aided by mixing. The comparison between M1 and M3 is especially noteworthy because of the similar initial species composition and history (e.g. intermixing) of these ponds. Micractinium was more successful than Ankistrodesmus in both M1 and M3. That Micractinium was not present in M4 is not considered significant. The different history of this pond and the expected resistence to invasion in a short period of time only allow cautious comparison between M4 and the intermixed ponds. Ankistrodesmus appears in greatest numbers in the spring and fall, indicating that selectivity by a chemical medium or operational parameter is greatly influenced by seasonal factors (as would be expected).

The productivity data show that fast mixing increased suspended solids production by 50% at a 4.4 day detention time. However, the 13.6 g/m^2/day total production obtained from M1 should not all be considered as biomass directly produced photosynthetically. Of this, 6.0 g/m^2/day (13.6-7.6 g/m^2/day, see Results Section) may have been non-algal solids derived from influent suspended solids or from tranformation of solubilized nutrients (in the inflow) into non-algal suspended solids. Hence, the photosynthetic efficiencies listed for M1 are overestimated. Total productivity of 9.0 g/m^2/day was obtained from M3 (slow mix) despite the low insolation (371 langleys/day) and temperature. The photosynthetic efficiencies, 1.1% of total, 2.4% of PAR, are good approximations although chemical energy inputs from any photoheterotrophic growth were ignored. The long detention time,recycle pond produced much less biomass (3.4 g/m^2/day) as expected.

The picture is different when harvestable production is considered because the fast mixing increased harvestability. Although algae may have comprised only about 6.0 of the 10.9 g/m^2/day harvestable production from M1, this is still much greater than the 1.9 g/m^2/day harvestable production from M3. That the short detention time, slow-mixed pond did not harvest well is consistent with the results of the previous experiment.

Flocculation is facilitated by increased inter-particle collision frequencies (and at the same time is limited by shear forces at higher mixing speeds). In the fast-mixed pond, the positive effects of mixing on flocculating algal and non-algal material seems to have been somewhat offset by the additional non-algal material appearing in the effluent (as seen from the low chlorophyll a content). How efficiently flocs form depends on the properties of the matrix material and the backbone material, as well as their relative abundances. If the non-algal material is thought to provide adhesiveness (a viewpoint supported by microscopic observations of the flocs) and the algae serve as the backbone, then the efficiency of flocculation may depend on the ratio of algae to matrix material. It is possible that in this experiment there was somewhat too much matrix for the algae and that the mixing speed was not optimal, causing non-algal suspended solids in microstrainer effluents. A fast mix, longer detention time pond might have a higher algal-to-matrix ratio and produce an effluent freer of non-algal suspended solids. (This was observed in the subsequent experiment No. 3 , see below.)

The data from this experiment indicate that significant settling of algae occurs at the slow mixing speed. A higher settling rate for Scenedesmus appears to explain its concomittent decline in the slow-mix ponds and increase in the fast-mix pond. The 25% higher chlorophyll level in M1 over M3 just after the onset of fast mixing roughly corresponds to 1-2 g more algal settling out per m^2 per day in a slow mix than a fast-mix pond. This estimate is probably conservative since much of the algae and chlorophyll may

have been degraded while sitting on the pond bottom for six days before it was resuspended by initiation of fast mixing. In addition to its effect on species composition, fast mixing facilitates floc formation, and thus harvestability.

TABLE 6

EXPERIMENT 2 SUMMARY TABLE

Dates March 16-April 8
Insolation (Langleys/day) 470
Temperature (°C) AM 10 PM 17
Depth (cm) 25

	M-1	M-3	M-4
Detention Time (days)	4.4	4.4	9.4
Mixing Speed (paddle wheel, rpm)	3.9	1.3	1.3
Recycle Fraction (%)	0	0	33
Pond Density (VSS mg/ℓ)	239	159	160
Effluent Density (VSS mg/ℓ)	46	134	34
Harvestability (% chlor. removal)	85	22	79
Total Production (gm VSS/m^2 day)	13.6	9.0	3.4
Harvestable Production (gm VSS/m^2 day)	10.9	1.9	2.5
% Chlorophyll (by weight)			
Pond	1.4	1.8	2.6
Effluent	1.1	1.5	1.7
Conversion Efficiency (%)			
of Total Sunlight	1.6	1.1	0.4
of PAR	3.7	2.4	0.9
Dominant Algae (beginning)	Mic. 51%	Mic. 51%	Scen. 45%-Ankis. 46%
Sub-Dominant Algae (beginning)	Ankis. 30%-Scen. 17%	Scen. 24%-Ankis. 23%	
Dominant Algae (end)	Scen. 50%	Mic. 62%	Ankis. 98%
Sub-Dominant Algae (end)	Mic. 38%	Ankis. 23% Scen. 15%	Scen. 6%
Effluent NH_3 mg/ℓ/% Removal*	11.9/55	15.0/44	1.3/95
Effluent COD mg/ℓ/% Removal*	119.2/75	405.9/16	129.7/73

*% of pond influent

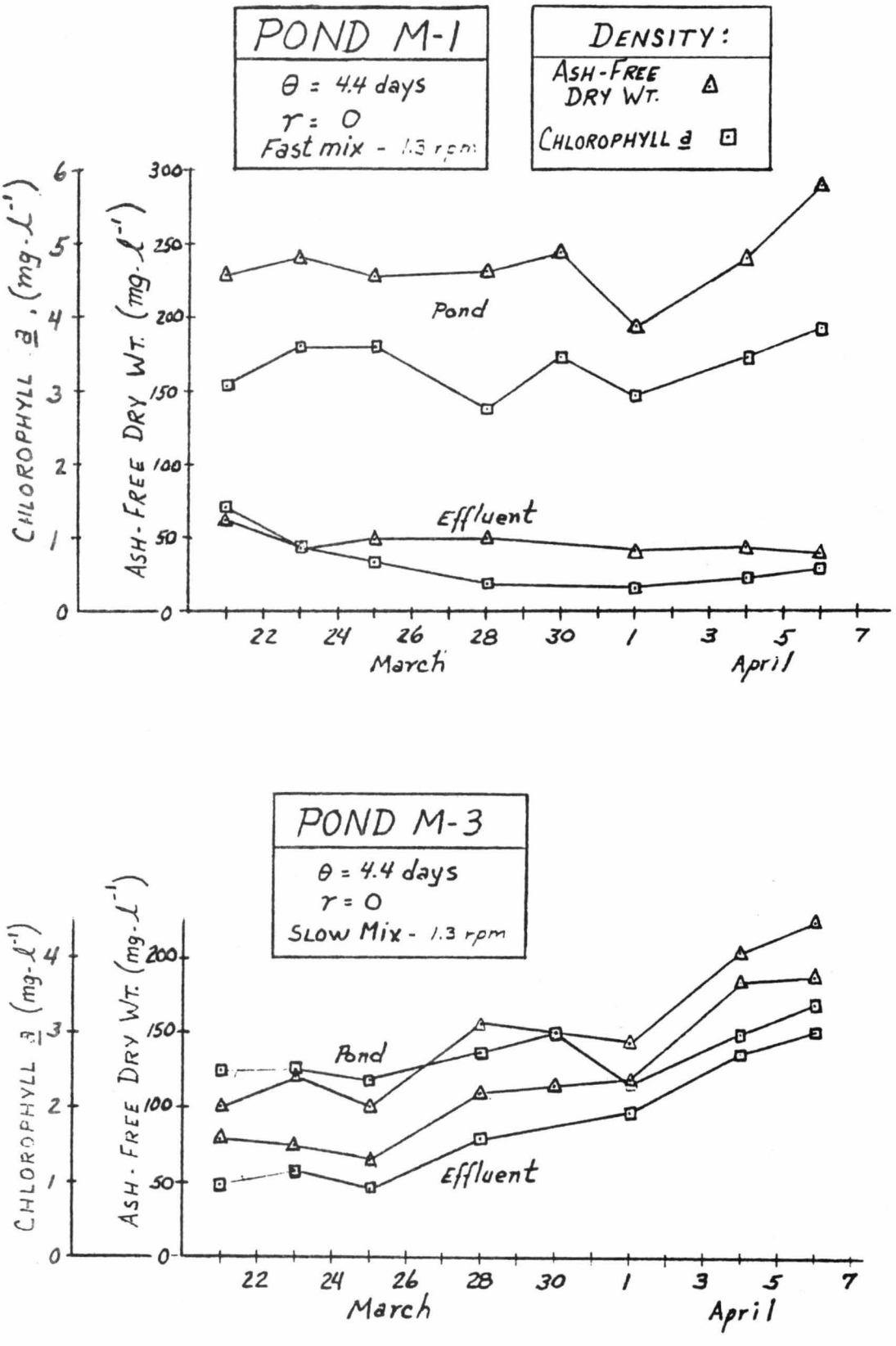


FIGURE IV-9. M-1 AND M-3 POND AND MICROSTRAINER EFFLUENT DENSITY EXPERIMENT 2

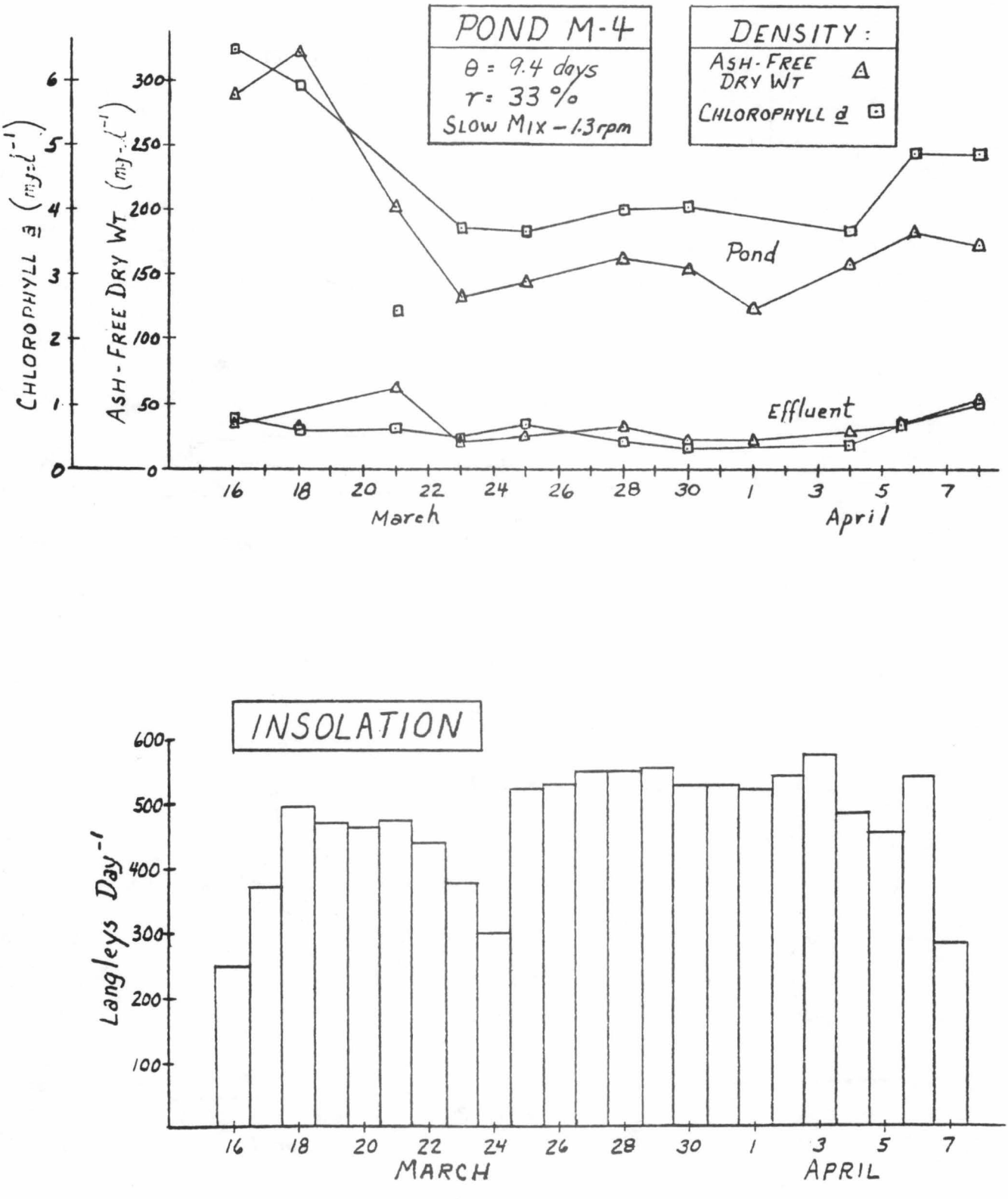


FIGURE IV-10. M-4 POND AND MICROSTRAINER EFFLUENT DENSITY, INSOLATION: EXPERIMENT 2

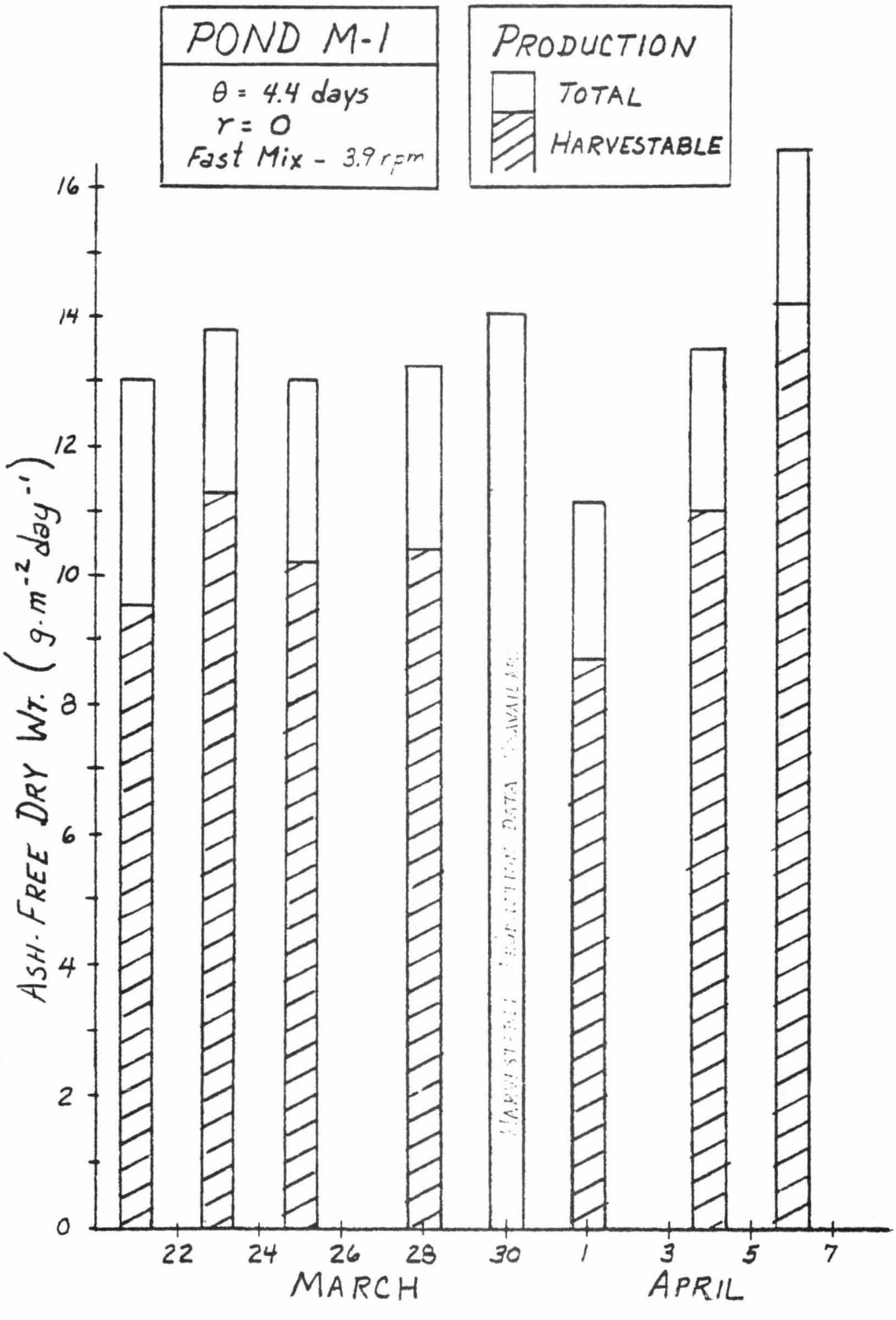


FIGURE IV-11. M-1 PRODUCTIVITY: EXPERIMENT 2

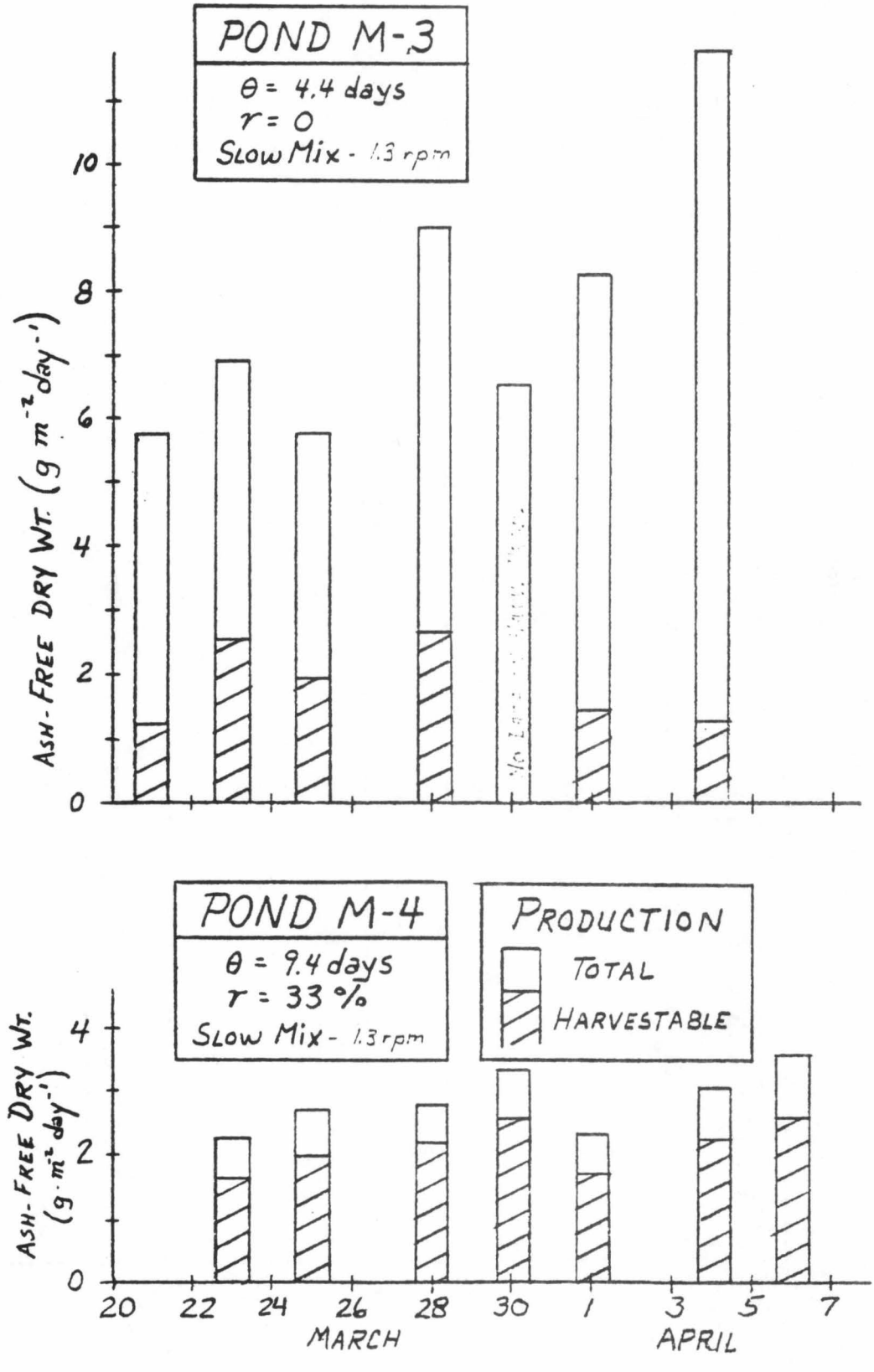


FIGURE IV-12. M-3 AND M-4 PRODUCTIVITY: EXPERIMENT 2

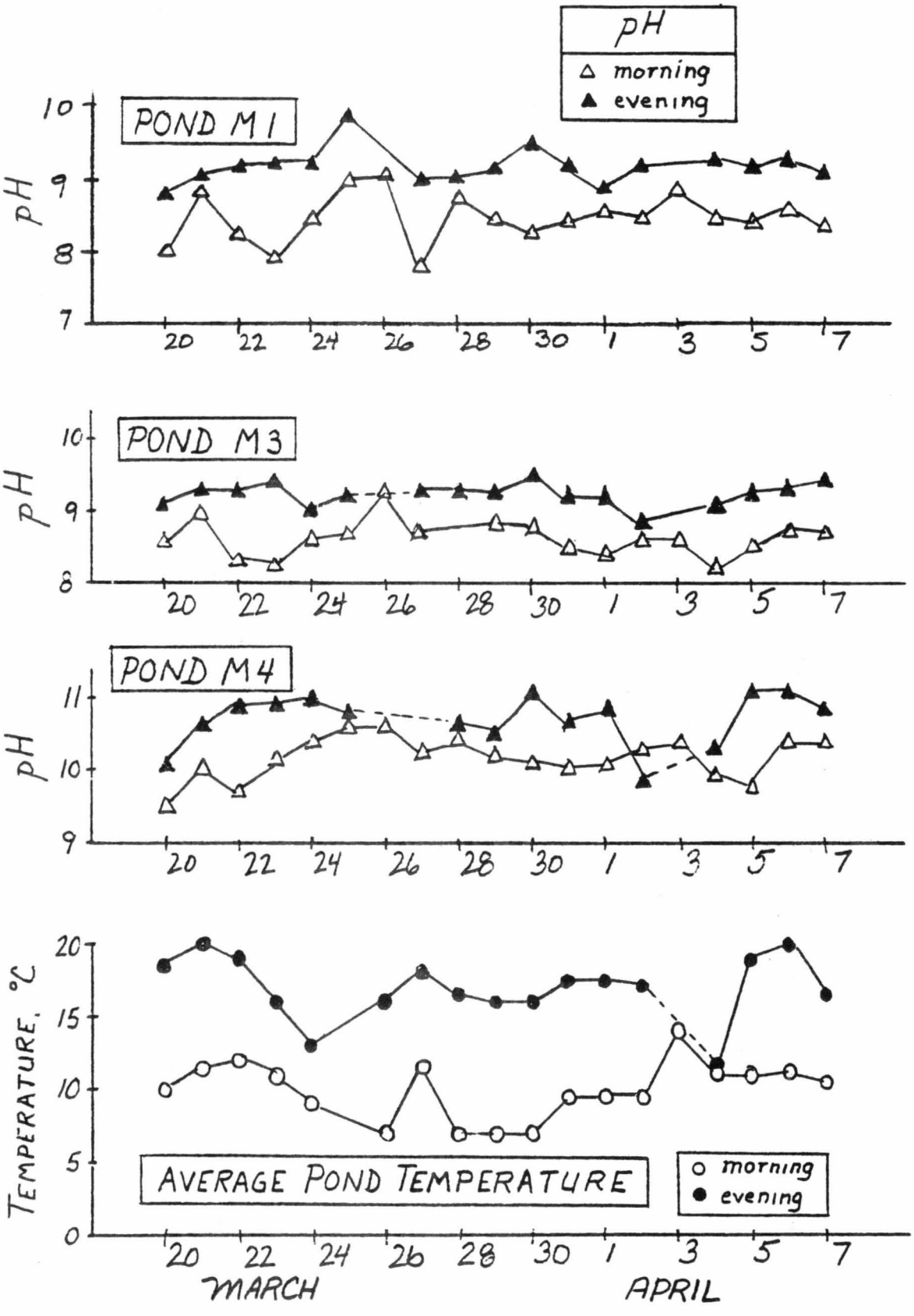


FIGURE IV-13. POND pH, AVERAGE POND TEMPERATURE: EXPERIMENT 2

EXPERIMENT 3 RESULTS

More experiments with fast mixing were required to evaluate its effects. M1 was operated at a short detention time (4.4 days) and fast-mixed to see whether mixing would reliably produce low-density effluents. For comparison M2 was run at a longer detention time (8.2 days) and fast mixed. Slow mix and fast mix were compared at the longer detention time by operating M3 at 8.2 days with slow-mixing. A short detention time, slow-mixed pond was left out of the experimental regime since this mode of operation had always resulted in poor harvestability in previous experiments. M1, M2, and M3 were intermixed on 12 April. M4 was operated the same way as M3 but was not intermixed, to obtain data on the effects of historical differences on pond performance and species selection.

Figure IV -14 shows that steady densities were attained in M1, M2, and M3. M4, which had its detention time shortened just prior to the beginning of the experiment, decreased in density during the first detention period. Subsequently, its density climbed, approaching the density of M3. The average densities in M1 and M2, which were both fast-mixed, were similar despite the difference in detention time. M3, which was slow mixed at the long detention time, was two-thirds as dense as M1 and M2. The chlorophyll percentage of pond solids increased from M1 to M2 to M3 with the greatest difference occurring between the latter two ponds.

Total production, on a suspended solids basis, was highest at the short detention time with fast mix (16.3 $g/m^2/day$). Lengthening the detention time (M2) reduced total production 40% to 9.7 $g/m^2/day$. Slow mixing at this longer detention time (M3) reduced it another 30% to 6.6 $g/m^2/day$. Harvestable production was 10.9 $g/m^2/day$ in M1, 8.7 $g/m^2/day$ in M2, and 4.6 $g/m^2/day$ in M3. Fast mixing at the longer detention time improved harvestability from 79% to 92%. The quality of the effluent was vastly improved. M2

microstrained effluent contained only 32 mg/ℓ VSS versus 66 mg/ℓ in the effluent from M3. The fast mix did not lead to reliably efficient removal of suspended solids (by microstraining) at the short detention time. Even the 60% average efficiency of solids removal is misleading, since the average effluent density was a high 94 mg/ℓ. Table 8 shows the "extra" non-algal contributions to pond density, total production, and harvestable production in the fast-mix ponds. The values were calculated as outlined in the Results section of Experiment 2. Two values for the percent chlorophyll in algae were assumed. One was taken the same as the percent chlorophyll in M3. The other was taken higher to account for the increased self-shading in M1 and M2 which were more dense than M3. The non-algal contribution to VSS was assumed to be zero in the slow-mixed pond (M3), as before.

The average chlorophyll to VSS ratio for the pond effluents are shown in Table 7. In the fast-mixed pond, this ratio was higher in the effluent than in the pond at the short detention time. This is opposite to the fast-mixed, short detention time pond from Experiment 2 as well as the longer detention time ponds in this experiment. This was particularly true at the time when M1 harvested most poorly.

The insolation was considerably higher during this experiment, averaging 565 langleys/day. Pond temperatures were also higher, averaging 13°C in the morning and 20°C in the afternoon. The morning and afternoon pH were lower in the short detention time pond (averaging about 8.5 and 9.5 respectively) than in the long detention time ponds. pH's were very similar in these ponds, averaging about 9.2-9.5 in the morning and 10.0 to 10.2 in the afternoon.

Algal types were found in different proportions in the fast-mixed ponds as compared to the slow-mixed ponds. M1, M2, and M3 all started out with similar proportions of Scenedesmus (about 70% large colonies) and

Micractinium (mostly small) with less Ankistrodesmus (present in both a short cell form and an aggregated large cell form). The proportions of Scenedesmus and Micractinium did not change significantly in M1 and M2. Although it appears from Table 7 that Scenedesmus was somewhat more competitive in M2 (long detention time, fast mix), the counting errors were sufficiently large to warrant caution in interpreting these data. Ankistrodesmus remained sub-dominant in both fast-mixed ponds. The major difference between the short and long detention time fast mixed ponds was in colony size distribution. Eventually greater than 50% of the colonies were small (for all types) in M1, whereas almost all were large in M2. In the slow-mixed M3, Ankistrodesmus greatly increased its proportion while Micractinium and especially Scenedesmus declined. Only Micractinium occurred to any significant extent in small colonies. M4, which was not intermixed with other ponds, started out two-thirds Ankistrodesmus with most of the rest Scenedesmus. The former decreased to about 50% of the biovolume while the latter nearly disappeared. Micractinium increased in frequency, becoming co-dominant with Ankistrodesmus. All types except Micractinium were about 80% harvestable at the beginning of the experiment. The harvestability of the Micractinium improved greatly in the course of the experiment.

EXPERIMENT 3 CONCLUSION

The results of this experiment confirm that fast mixing has a positive effect on harvestability but that fast mixing may not make a short detention time pond reliably harvestable. Fast mixing did significantly improve effluent quality at an 8.2 day detention time. Colonies were larger in the long detention time pond than in the short detention time pond. Some of the effects of mixing on floc formation were discussed in the conclusions to Experiment 2. In that experiment, the chlorophyll to VSS ratio was lower in the effluent than in the ponds (and very low in both), suggesting that there was more

non-algal bridging material than was necessary to flocculate the algae. In this experiment, the short detention time fast-mix pond had a higher ratio of chlorophyll to VSS in the effluent than in the pond. This did not occur in any other pond. The effluent chlorophyll density was also higher than in any other effluent. Thus, the M1 effluent contained a high proportion of unharvestable algal to non-algal solids and this may have been due to a sub-optimal ratio of matix material to algae in the pond. Indeed, the percent chlorophyll was much lower in the 4.4 day, fast-mix pond of Experiment 2 than in the 4.4 day, fast-mix pond in this experiment. The higher temperature and insolation during the period of this experiment could explain the greater extent of transformation of non-algal suspended solids into algal suspended solids.

The specific growth rates for algae in M1 and M2 can be assumed similar because the pond densities were similar. The larger number of small colonies (measured by counts and by the much greater effluent density) and greater ratio of small to large (from counts and harvestabilities) in M1 as compared to M2 is evidence that just decreasing detention time decreases the net rate of colony formation. The problem is confounded by the possibility that small colonies may contain less chlorophyll and may be faster growing than large colonies, but this interpretation is consistent with all of the data from previous experiments. M2 was more dense than M3 implying that specific growth rates were lower in M2. Since the dilution rate was the same, and the ponds attained steady densities, the average settling rate in M2 must also have been lower than in M3. Assuming similar growth rate-density relationships in the different algal types, fast-mix can decrease the settling rate as significantly as 50% higher density can decrease the growth rate ($D = \mu^{M2} - S^{M2} = \mu^{M3} - S^{M3}$). Again this assumes that, at the same pond density, Scenedesmus and Ankistrodesmus have similar specific

growth rates. Thus, mixing may tend to increase harvestability and improve effluent quality in several ways: (1) the ratio of large colonies to small colonies is increased since mixing decreases the settling rate. Since larger colonies settle faster than small colonies settling is more important for larger colonies. (2) Flocculation of algal and non-algal material is facilitated by mixing. (3) The ratio of large colonies to small colonies may be increased because specific growth rates are lowered as a consequence of the lowering of light levels through the suspension of "extra" non-algal solids. It was suggested in Experiment 1 that lowered growth rate may increase the net rate of colony formation. Which of these factors is dominant may depend on the detention time and environmental conditions. For example, floc formation may be most important at a short detention time when colonies are small, whereas decreased settling may be more important at long detention times when colonies are large.

The observation that the long detention time, fast-mix pond was not significantly more dense than the short detention fast-mix pond indicates that the amount of non-algal solids is significant enough to flatten the density-detention time response of an algal culture. The same or more algal suspended solids were present in M2, but a substantial amount of non-algal solids remained. This flattening of the density-detention time curve would be expected whenever the growth medium contributes turbidity. Mixing aggravates this situation.

TABLE 7

EXPERIMENT 3 SUMMARY TABLE

Dates 4/12-5/6
Insolation (Langleys/day) 565
Temperature (°C) AM 12.2 PM 20.7
Depth (cm) 25

	M-1	M-2	M-3	M-4
Detention Time (days)	4.4	8.2	8.2	8.2
Mixing Speed (paddle wheel, rpm)	3.9	3.9	1.3	1.3
Recycle Fraction (%)	0	0	0	0
Pond Density (VSS mg/ℓ)	287	318	216	187
Effluent Density (VSS mg/ℓ)	94	32	66	57
Harvestability (% chlor. removal)	60	92.5	79	78.5
Total Production (gm VSS/m^2 day)	16.3	9.7	6.6	5.7
Harvestable Production (gm VSS/m^2 day)	10.9	8.7	4.6	3.9
% Chlorophyll (by weight)				
Pond	1.9	2.1	2.6	2.4
Effluent	2.2	1.4	1.8	1.5*
Conversion Efficiency (%)				1.2-1.7
of Total Sunlight	1.6	.9	.6	.6
of PAR	3.6	2.0	1.4	1.4
Dominant Algae (beginning)	Scen. 44 Mic. 42	Scen. 44 Mic. 42	Scen. 40 Mic. 39	Ankist. 65
Sub-Dominant Algae (beginning)	Ankist. 14	Ankist. 14	Ankist. 16	Scen. 26 Mic. 7
Dominant Algae (end)	Mic. 51 Scen. 36	Scen. 55 Mic. 30	Ankist. 63	Ankist. 48 Mic. 46
Sub-Dominant Algae (end)	Ankist. 12	Ankist. 16	Mic. 26 Scen. 11	Scen. 5.5
Effluent NH_3 mg/ℓ/% Removal*	11.5/65	5.6/87	11.4/66	7.1/79
Effluent COD mg/ℓ/% Removal*	188/65	112/73	171/59	168/60

*% of pond influent

TABLE 8

ALGAL CONTRIBUTION TO POND SUSPENDED SOLIDS PRODUCTION

Pond	Chlorophyll *a* mg/ℓ	Chlorophyll/ VSS, %	VSS, mg/ℓ	Algal VSS @ 2.6% Chloro-phyll *a*, mg/ℓ	Algal VSS @ 3% Chloro-phyll *a*, mg/ℓ	Total Pro-duction $gm/m^2/day$	Harvestable Production $gm/m^2/day$
M1	5.4	1.9	287	210	182	16.3	10.9
M2	6.7	2.1	318	257	223	9.7	8.7
M3	5.6	2.6	216	216	—	6.6	4.6

Pond	Total Algal Production @ 2.6% Chlo-rophyll *a* $gm/m^2/day$	Harvestable Algal Pro-duction @ 2.6% Chlor. *a* $gm/m^2/day$	Total Algal Production @ 3% Chlor. *a* $gm/m^2/day$	Harvestable Algal Pro-duction @ 3% Chlor. *a* $gm/m^2/day$
M1	11.9	8.0	10.3	6.9
M2	7.8	7.0	6.8	6.1
M3	6.6	4.6	—	—

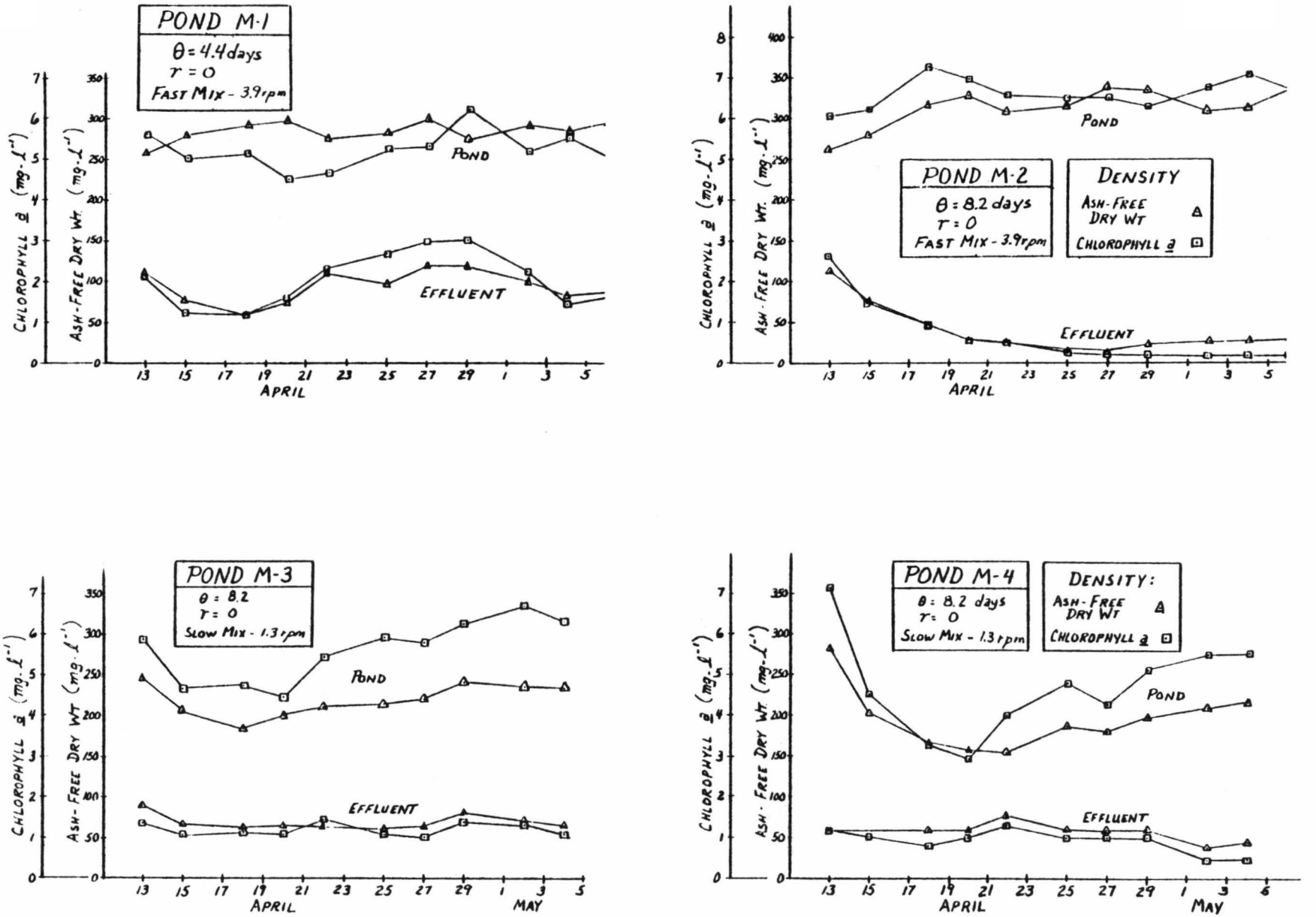


FIGURE IV-14. POND AND MICROSTRAINER EFFLUENT DENSITY: EXPERIMENT 3

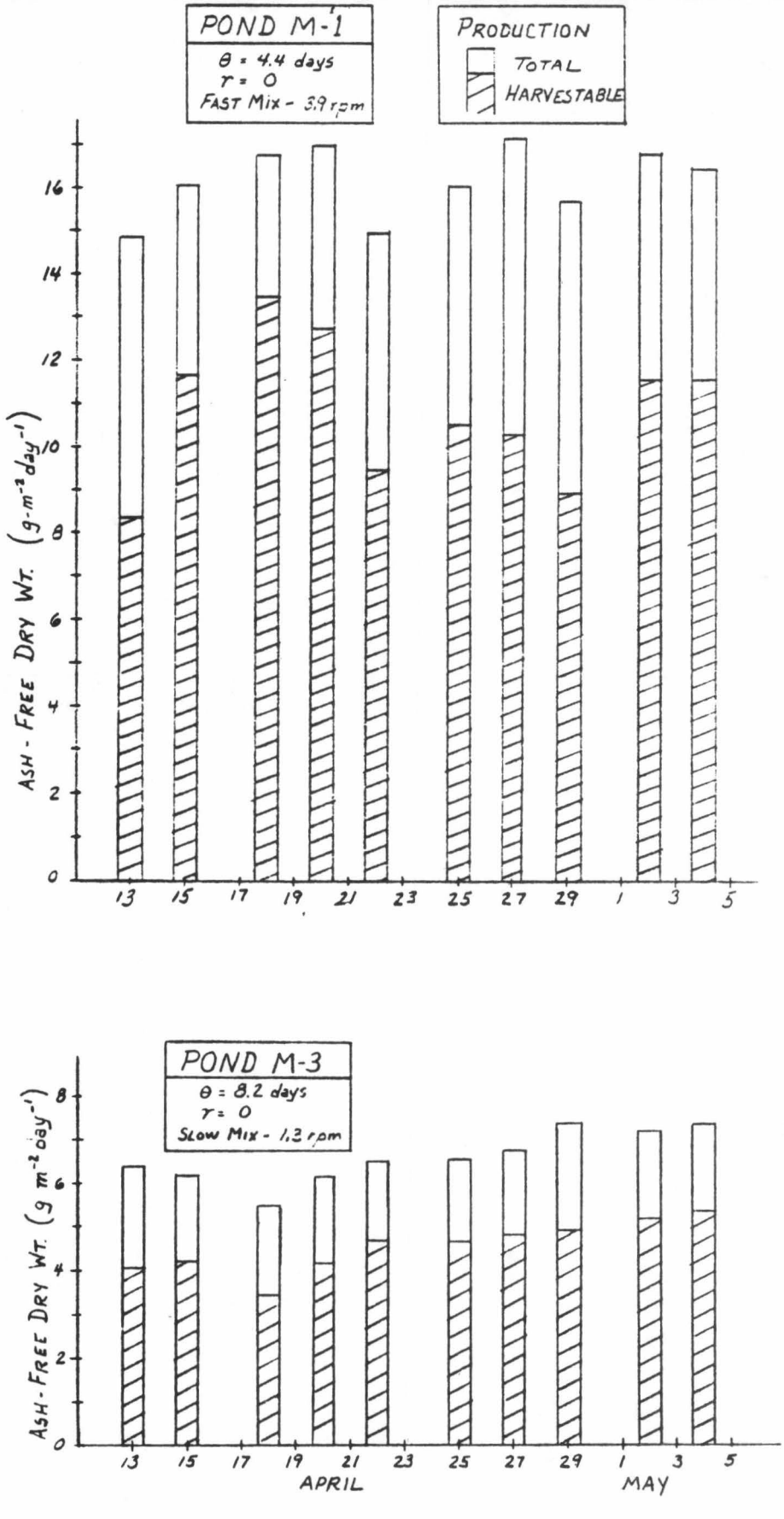


FIGURE IV-15. M-1 AND M-3 PRODUCTIVITY: EXPERIMENT 3

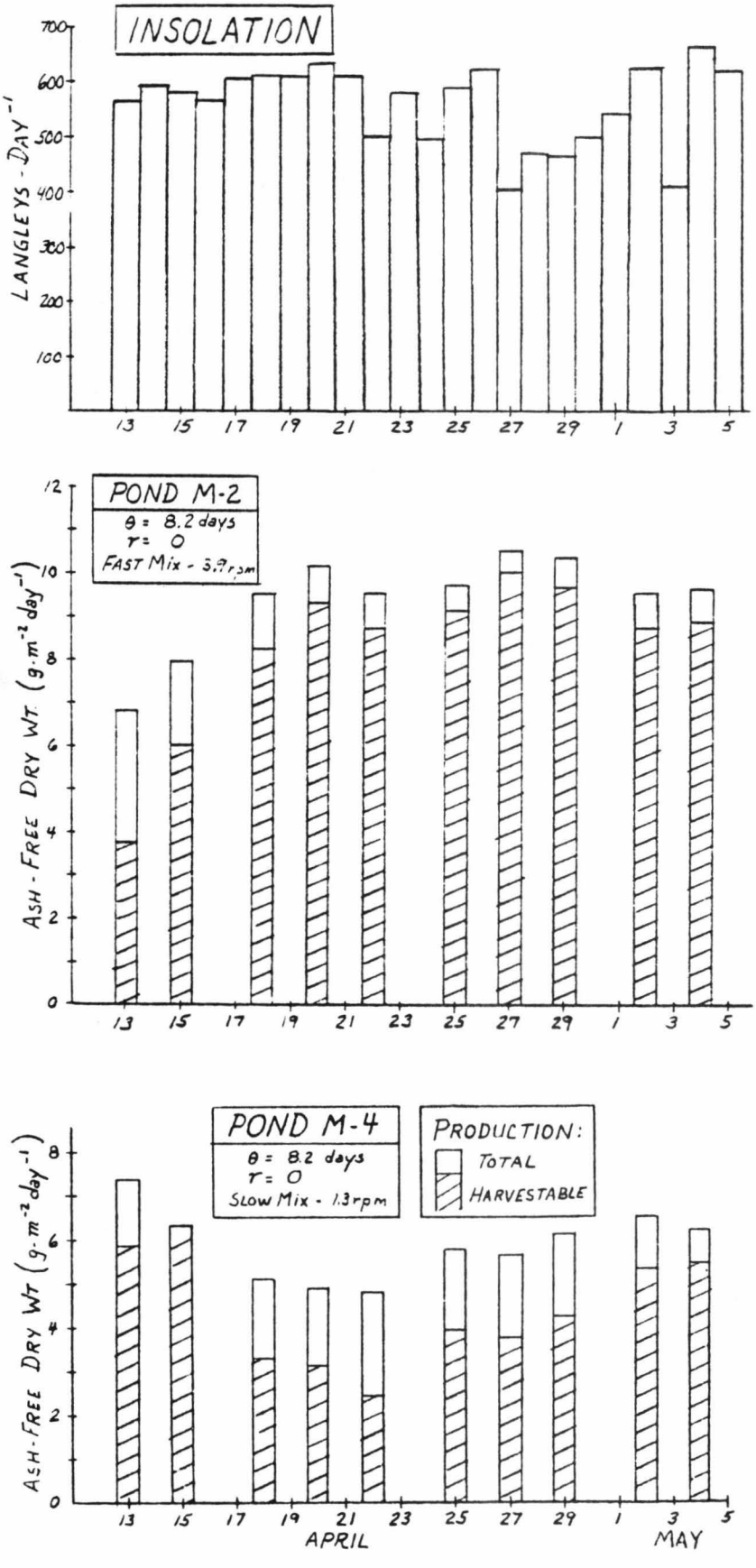


FIGURE IV-16. INSOLATION, M-2 AND M-4 PRODUCTIVITY: EXPERIMENT 3

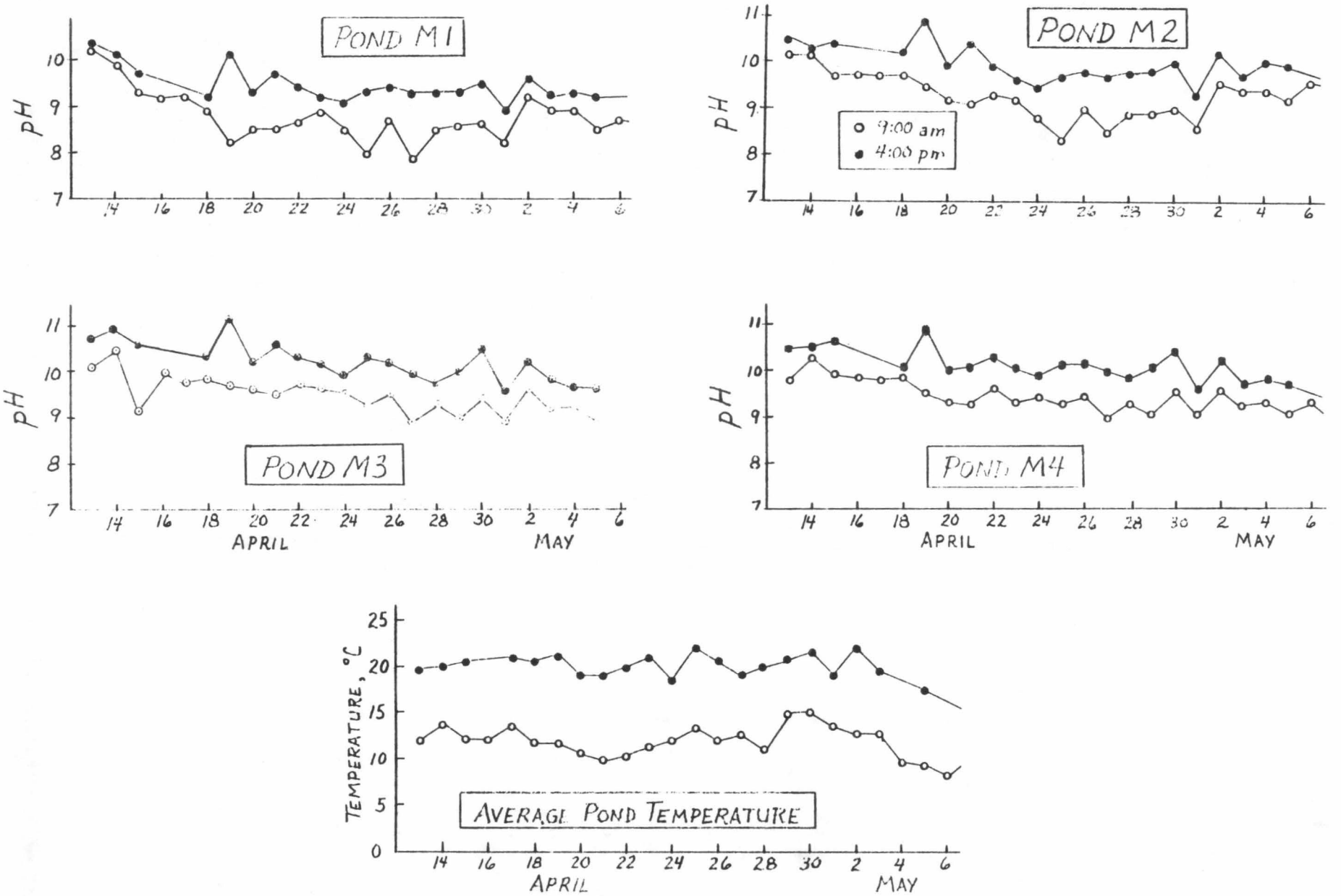


FIGURE IV-17. POND pH, AVERAGE POND TEMPERATURE: EXPERIMENT 3

EXPERIMENT 4 RESULTS AND CONCLUSIONS

Two ponds, M1 and M2 were continued from the previous experiment. The operation of M1 was modified on 6 May to include recycle of the microstrained effluent. The pond was harvested nominally at a detention time of 4.6 days. It was diluted with sewage during the first half of the harvest period. The microstrainer effluent collected was used for dilution during the second half of the harvest. The sewage detention time was approximately 8.4 days as was the detention time of non-harvestable algae. The detention time of harvestable algae was about 4.6 days. M2 was run as a harvestable control. The detention time was reduced from 8.2 days to 6.8 days on 16 May. Both ponds were fast mixed and were 10 inches deep.

As shown in Figure IV-18, M2 was about 85% harvestable and M1 remained inconsistently harvestable. The chlorophyll density of M1 remained fairly constant while that of M2 increased during the period of high insolation (13 May on). The period after the shortening of the detention time of M2 was too short to note any trends. The chlorophyll to VSS ratio of M1 increased after effluent recycle was started. This ratio in the effluent did just the opposite. However, both of these ratios were substantially biased in favor of VSS since the composite sampling was nearly complete by the time the inflow was changed from sewage to recycled effluent.

Total production declined in M1 when effluent recycling was practiced. Harvestable production also declined but not as much. As expected, production increased in M2 when detention time was shortened (insolation also increased).

Both ponds contained the same three familiar types of algae: Scenedesmus, Micractinium, and Ankistrodesmus. On 6 May, the Scenedesmus and Ankistrodesmus were mostly in large colonies in both ponds. The Micractinium were mostly small in M1 and mostly large in M2. The size distribution did not change significantly in M2 throughout the experiment. The proportion of Scenedesmus increased from about 5% to 80%, with the other two algal types

decreasing. Scenedesmus and Ankistrodesmus underwent very little change in colony size in M1 also. Micractinium changed from mostly small on 6 May to about two-thirds large on 20 May. It accounted for about 60% of the biovolume for most of the experiment.

Effluent recycle increases the rate of washout of algae which are removed by the microstraining. Small, non-colonial unicellular algae would be washed out most slowly because these have no tendency to aggregate and become microstrainable. Any loss through microstraining increases the average rate of washout of an algal type. An increase in the proportion of algae with a slow rate of washout tends to increase the density of a pond. This causes a decline in the number of algae with faster washout rates since these must grow faster, at the same total pond density, to keep up. However, unicellular algae were not observed in M1 and the pond density did not increase after effluent recycling was started. It is possible that the experiment was not continued long enough for new algae types (small, unicellular algae) to invade the pond. Alternatively, it is possible that even with the advantage of twice the cell detention time, unicellular algae could not compete with the colonial algae. That is, the combination of other factors (sewage, insolation, temperature, pH, etc.) may have strongly selected against invasion by small algae.

A predominance of small colonies did not occur either. This, too, may be due simply to the short duration of the experiment. However, when colonial algae are considered, factors affecting colony formation must also be considered. If the number of small colonies increased in an effluent recycle pond, then the pond density would tend to increase because the average washout rate for colonial (small and large) algae would decrease, allowing slower average growth rates. A reduction in growth rate might increase the net formation of large colonies which are microstrainable. This would increase

the average washout rate, lower density, increase growth rates, and favor the small colonies. Obviously, a relatively unharvestable pond might result. This was observed during the two weeks of the effluent recycle experiment. There may be a range of values of hydraulic detention time and fraction of the effluent recycled which would allow populations of large colonies to be maintained in a stable condition. That is, a sufficiently long detention time and slow enough growth rate could select for large colonies. If long detention time is more selective for these colonies than slow growth rate, then an effluent recycle pond could be harvestable, moderately productive, and, at the same time, effective in treating sewage. The fast mixing in M1 was another factor confounding analysis of this experiment since it decreases the loss of large colonies through settling and increases the loss of small colonies through flocculation. Obviously, more experimentation is necessary. At a given detention time, productivity can be increased by effluent recycling if harvestable forms are maintained. As mentioned above, this increase in productivity can be gained without decreasing the effectiveness of sewage treatment since the sewage detention time is unaltered. In this experiment, productivity decreased when effluent recycling was practiced because the pond density did not increase even though the average cell detention time did. Unharvested algae in the recycled effluent increased the average cell detention time. The productivity decrease was more pronounced for total production than for harvestable production since unharvestable algae were selectively recycled. It is not clear why the pond density did not increase when the density of the recycled effluents increased. Additional nutrients becoming limiting could be a factor. Grazing was another factor which became especially important near the end of the experiment. Rotifers were first observed in this pond on 26 May but could have easily escaped notice earlier when their numbers were few. The increase in rotifer density would have been slowed by their selective removal by microstraining.

The pond chlorophyll density did decline significantly on 27 May but rose again on 29 May. Rotifers were visible in large numbers. The effluent densities, especially on a chlorophyll basis, decreased on 27 May and 29 May. During this time, algal colonies became larger and the proportion of Scenedesmus increased.

Pond 2 also decreased in density and improved in harvestability by the time rotifers were observed. The proportion of Scenedesmus rose to nearly 100% by 3 June. All of the colonies were large.

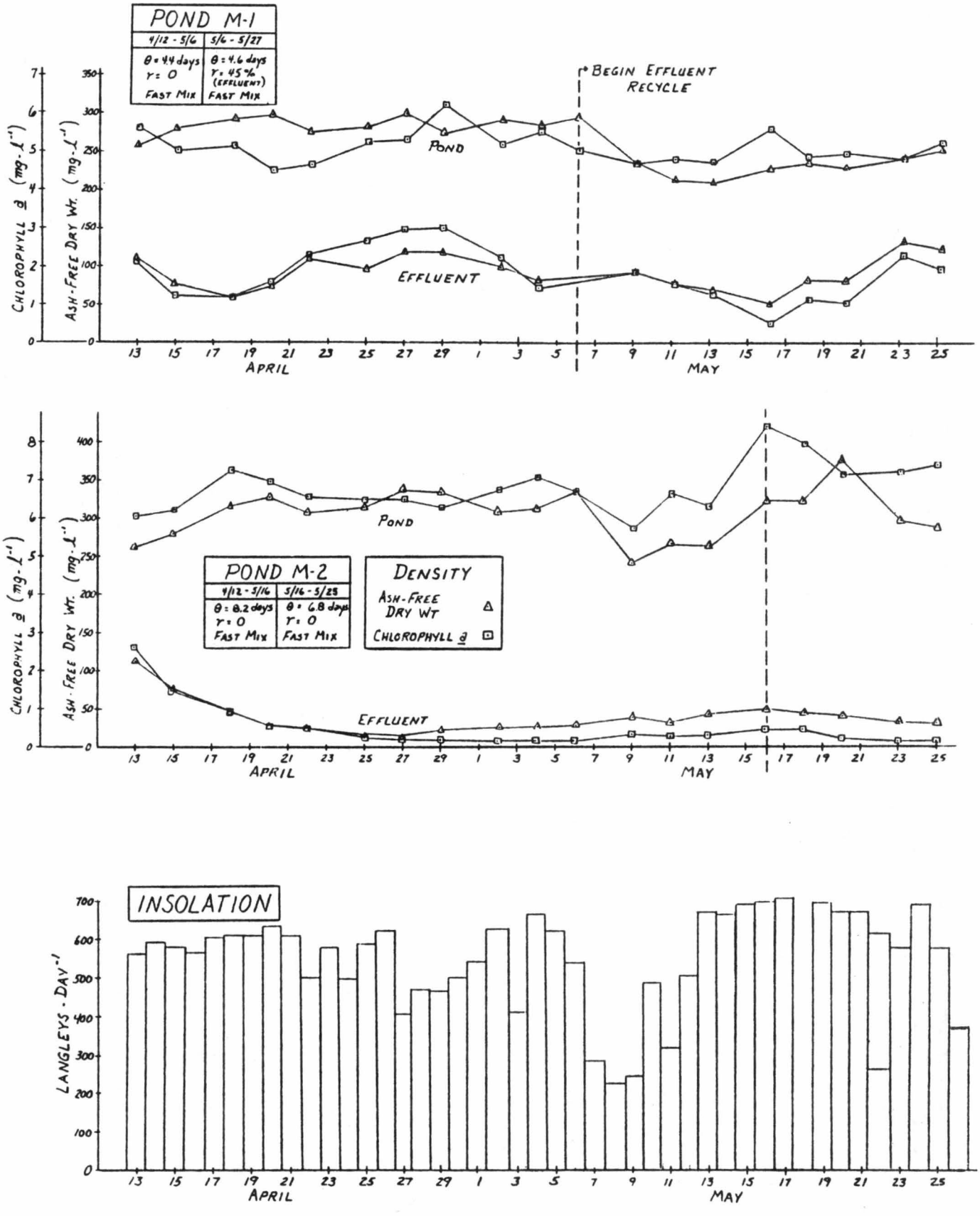


FIGURE IV-18. POND AND MICROSTRAINER EFFLUENT DENSITY, INSOLATION: EXPERIMENT 4

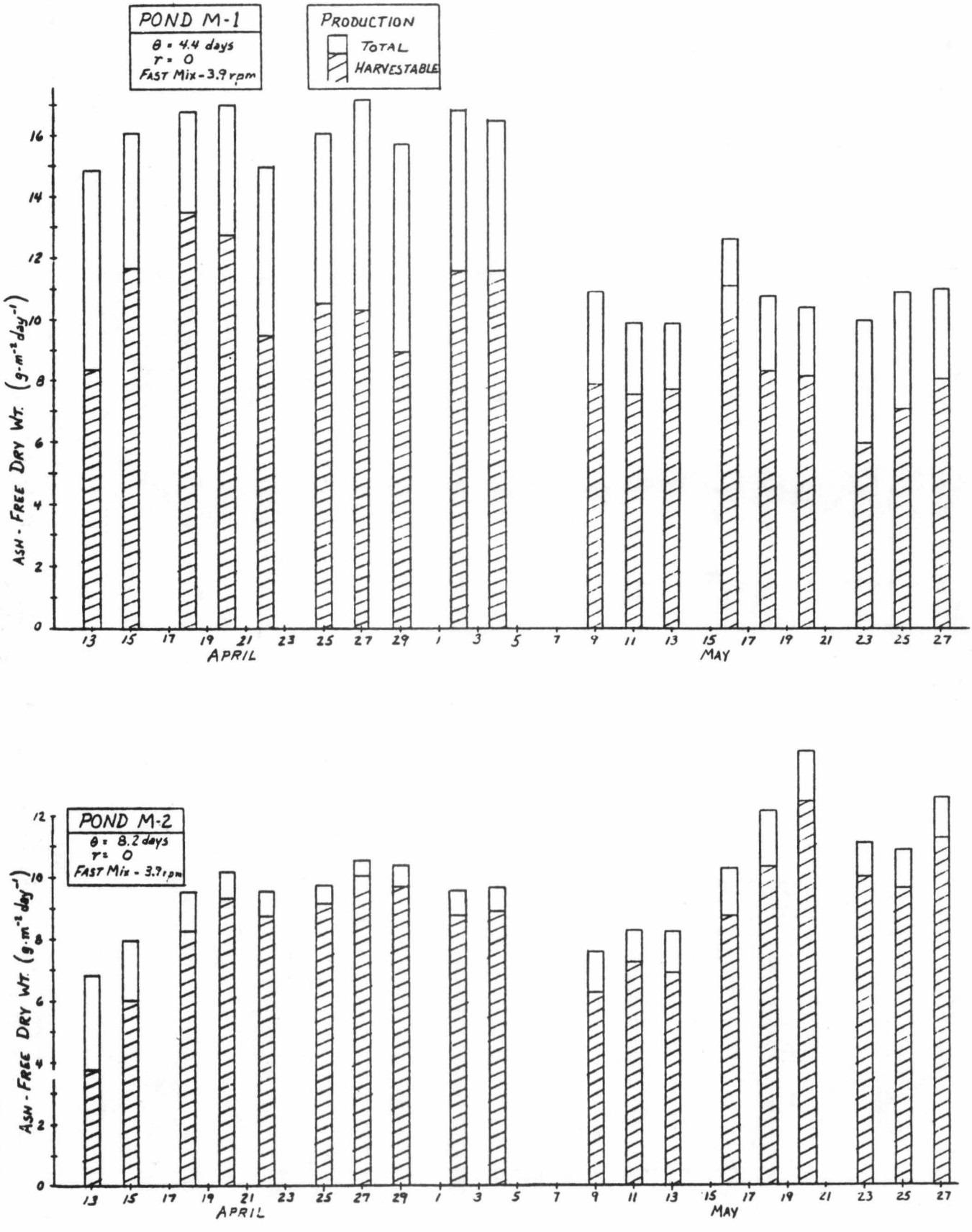


FIGURE IV-19. PRODUCTIVITY: EXPERIMENT 4

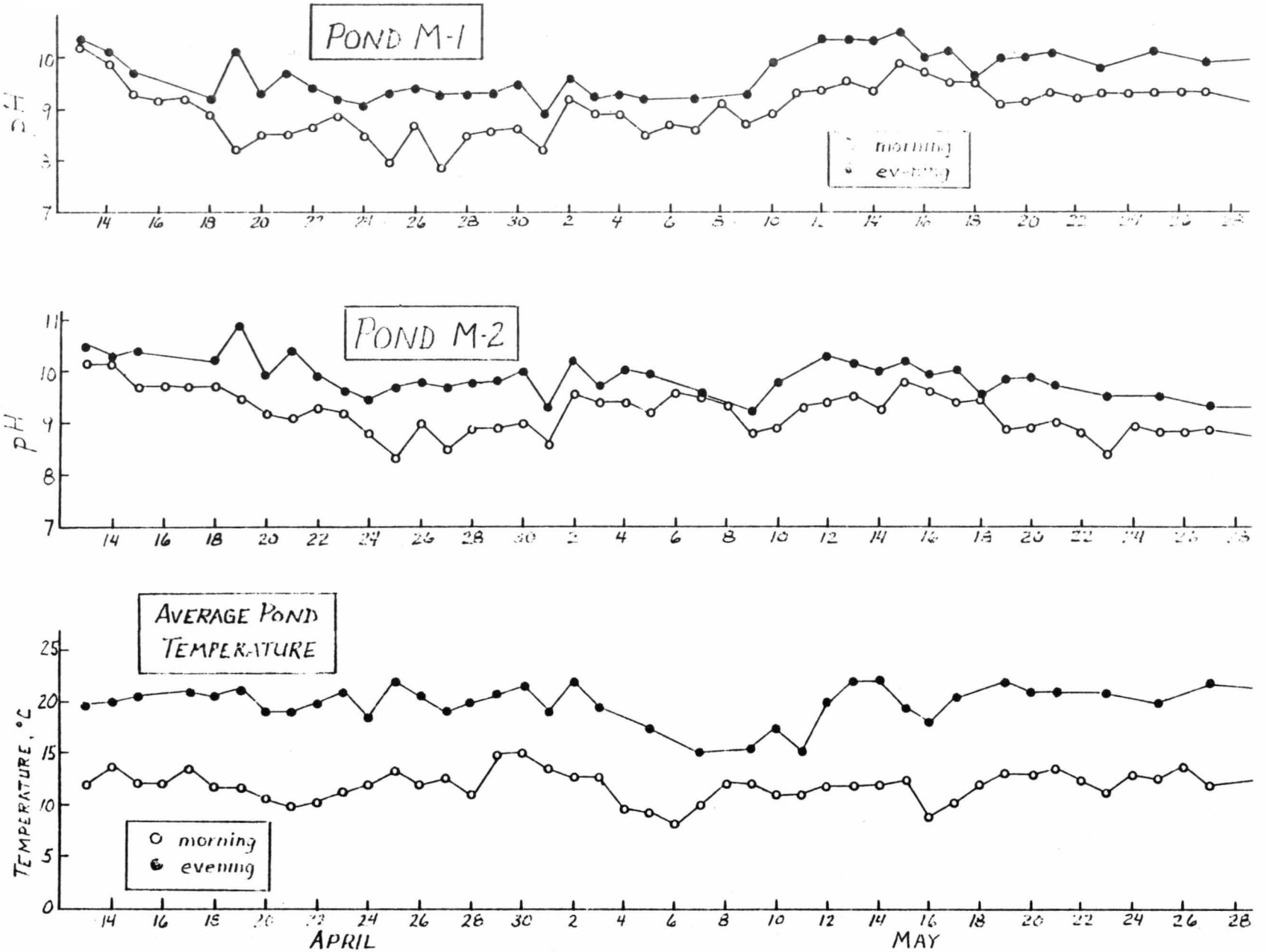


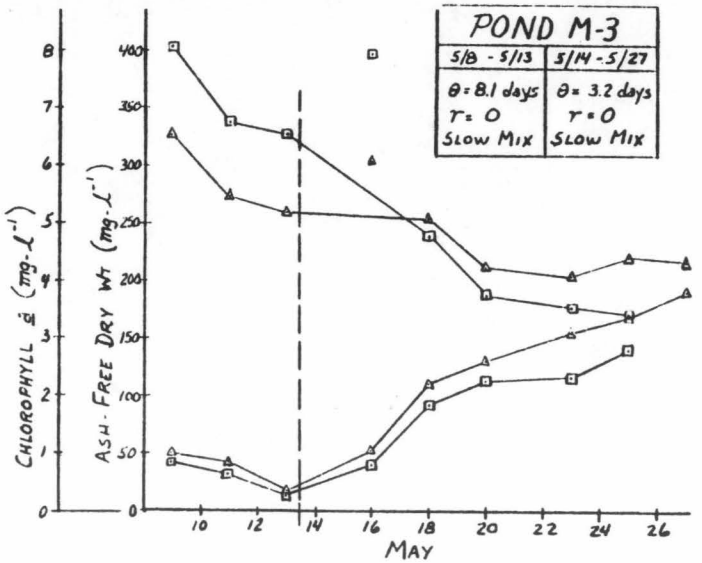
FIGURE IV-20. pH AND AVERAGE POND TEMPERATURE: EXPERIMENT 4

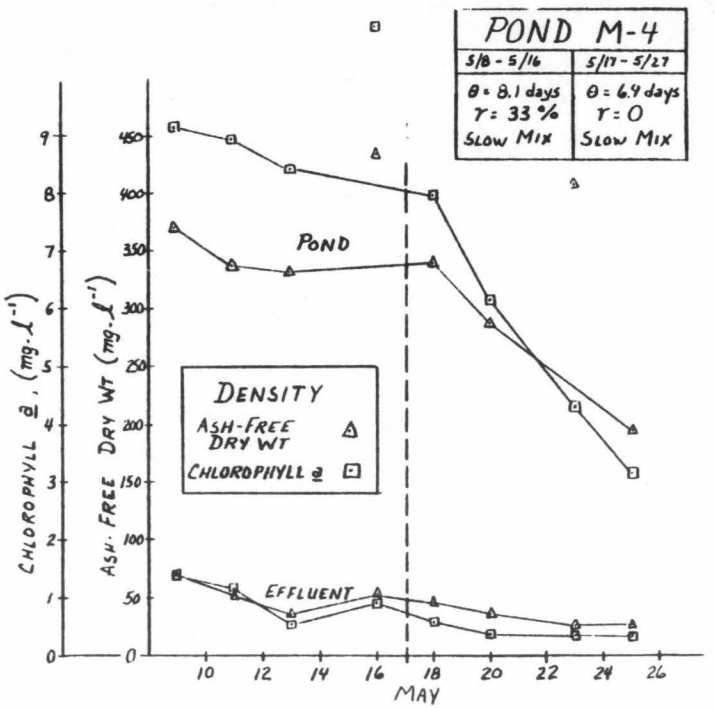
EXPERIMENT 5 RESULTS AND CONCLUSIONS

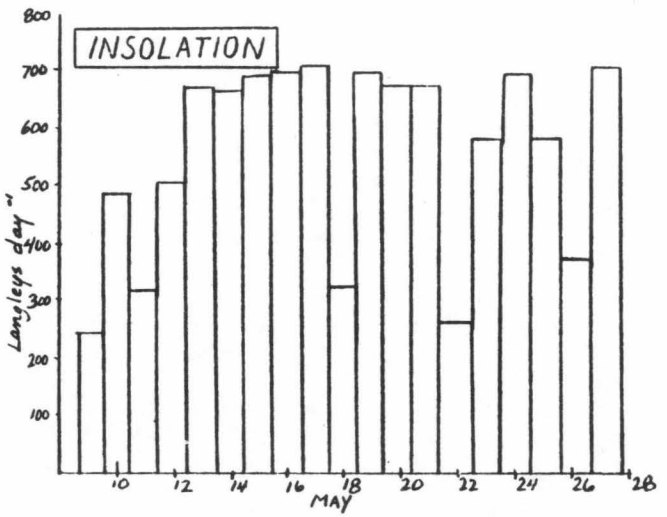
On 6 May M3 and M4 were intermixed. Accumulated sludge was resuspended during this operation. This explains the high initial densities (a 50% increase) of these ponds. Both ponds were put on an 8.1 day detention time and slow mixed. M4 was biomass recycled 33%. Since both remained harvestable, operational changes were made on 16 May. The detention time of M3 was shortened to 3.2 days while that of M4 was shortened to 6.9 days. Recycling of M4 was terminated. The harvestability in M3 decreased dramatically. In less than one detention time, the harvestability went from 90% to 40%. It became virtually zero within five detention times. The abrupt change in harvestability is another indication that short sewage detention times caused colony breakdown. Algae counting also revealed a major change in colony size distribution. On 6 May and 16 May, all algae were in large colonies. On 20 May, only about two-thirds of the colonies were large and on 25 May less than 30% were large. All algal types followed this pattern with Micractinium breaking up fastest. Micractinium also became the dominant alga (comprising 90% of the biovolume on 25 May), but this was already evident before the detention time was shortened. Grazers were first noticed, in rather large numbers, on 26 May. The next day the effluent chlorophyll was markedly lower. Operations were terminated on 5 June when recovery of the pond seemed in doubt.

The effluent from M4 remained low in suspended solids and chlorophyll throughout the experiment. The pond density, however, declined after the change in detention time. Rotifers were first observed on 23 May and increased rapidly. The pond density declined steadily until 29 May when the pond was drained. Effluent densities became very low. On six May through 16 May, almost all of the colonies were large. Ankistrodesmus were dominant (48-49%), Micractinium were subdominant (16-38%), and Scenedesmus were also sub-

dominant (6-13%). On 20 May, all of the colonies were large, and Micractinium had increased to 80%. Scenedesmus started to increase by 25 May when they comprised 25% of the biovolume, with Micractinium comprising 65%.







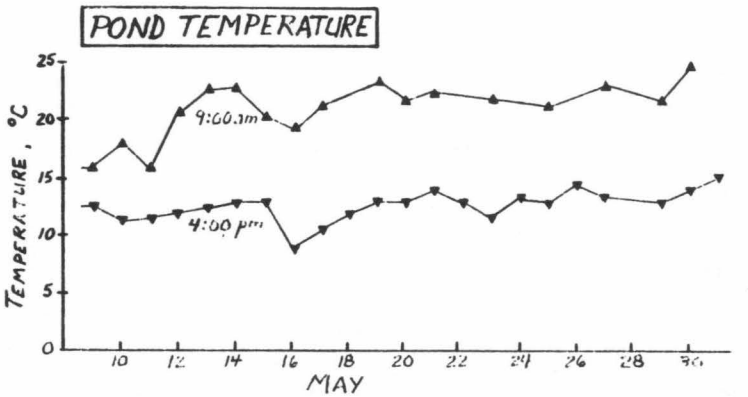


FIGURE IV-21. POND AND MICROSTRAINER EFFLUENT DENSITY, AVERAGE POND TEMPERATURE, INSOLATION: EXPERIMENT 5

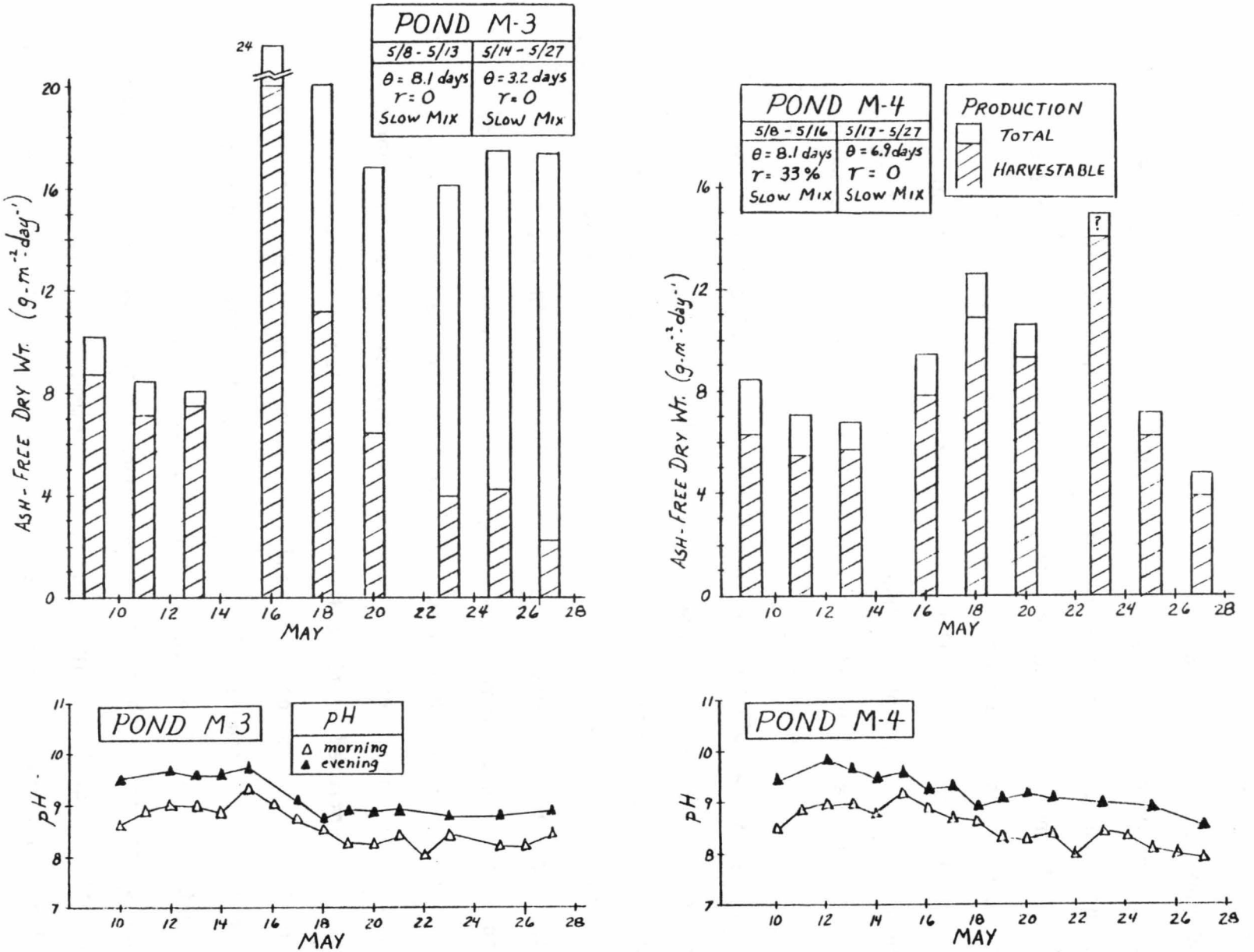


FIGURE IV-22. PRODUCTIVITY AND pH: EXPERIMENT 5

EXPERIMENT 6 RESULTS

At the end of May all of the 12 m^2 high-rate ponds became infested with rotifers. M4 was drained and cleaned on 31 May and refilled with sewage and microstrained effluents from M1 and M2. These effluents were free of rotifers and their eggs. M3 was drained and cleaned on 5 June and refilled with sewage and and microstrained effluents from M2 and M4. On 13 June M1 was drained and cleaned. The entire contents of M2 was microstrained and intermixed with M3 and M4. Sewage was added to fill four ponds. Soon the ponds again showed signs of rotifers. The water between the (mixing) flocs became substantially transparent, although rotifers were not seen in large numbers. In one pond, M3 , pink, daphnea-like crustaceans bloomed. On 22 June all of the ponds were emptied and cleaned and refilled with harvestable algae from the 0.6-acre high-rate pond. This pond did not show any signs of herbivores. Every day two of the 12 m^2 ponds were recirculated through a 350 μ mesh screen to remove crustaceans and large rotifers.

Three operational parameters had been tested so far: detention time, biomass recycling, and mixing. The first was emerging as the most important in maintaining harvestable populations of algae. Cell recycling had not been shown to substantially improve the harvestability of unharvestable ponds. The instability of the small ponds which resulted from herbivore blooms mitigated against doing cell recycle experiments at this time since herbivores are efficiently concentrated by microstraining. So, to further document the effects of detention time on harvestability, and to obtain a productivity-detention time curve, the four 12 m^2 ponds were operated in parallel but at different detention times. The intent was to run one pond at a short enough detention time to render it unharvestable, and thus get some measure of how much productivity must be sacrificed, in a single-pond system, to maintain microstrainable algal populations. Since it was not known whether the 350 μ screen would

control grazing, the results might reveal the effects of dentention time on grazing pressure.

A moderately fast mixing speed was chosen (2.4 rpm, which is equivalent to a linear velocity of 10 cm/sec at the average immersion radius of the paddle wheels) since earlier experiments had shown an increase in productivity (significant on a VSS basis) with faster mixing. Ponds were run at the 10-inch depth. As mentioned above, the experiment was initiated on 22 June by filling all of the ponds with the culture from the 0.6 acre high-rate pond, which was harvestable and did not contain grazers. This explains the high initial pH's of all of the ponds (Figures IV-27, IV-28). Detention times were set at 3.7, 4.6, 6.0 and 7.5 days for M1, M2, M3 and M4 respectively with the intention of shortening these if all ponds stayed harvestable.

In Figures IV-23, IV-24 it is shown that this indeed occurred, so on 30 June the detention times were shortened to 2.3, 3.3, 4.6 and 6 days. After two detention periods at θ=2.3 days, M1 decreased significantly in harvestability while the other ponds remained harvestable. Comparison of Figure IV-23 and Figure IV-29 reveals that the decrease in harvestability and the dramatic shift in species composition (from colonial Scenedesmus to colonial Micractinium) coincided with the change in detention time. The pond harvestability recovered after the detention time was lengthened to 5.2 days on 4 August. No change in species dominance occurred at this time. M2 harvested very well with θ=3.3 days until 25 July. At this same time Micractinium became predominant and the pond density began to decline due to grazing. Micractinium became predominant increasingly more slowly in ponds run at longer detention times. Enough so that it appeared that Scenedesmus was more competetive at longer detention times (see HRP Chapter V). The 4.6 day detention time used for M3 resulted in good harvestability and low effluent densities (Figures IV-24 and IV-26) though the first week in

August. After a rotifer bloom decimated the algal population the pond was drained (12 July) and refilled using M1 unharvested effluents. This provided a direct comparison between two ponds with the same algal types but operated at 2.3 vs. 4.6-day detention times. M3 became very harvestable within one detention period whereas M1 was poorly harvestable through the end of July (Figures IV-23 and IV-24). Micractinium was predominant in M1 during the inoculation of M3. It remained predominant in both ponds thereafter. (It's proportion was increasing sharply in M3 during the rotifer bloom (Figure IV-29)). Thus, a non-harvestable population was made harvestable by lengthening the sewage and cell detention time. The longer detention time correlated with harvestable Micractinium populations, while the shorter detention time correlated with non-harvestable Micractinium. This correlation was also found to hold in winter and spring experiments, no matter what alga was predominant.

Effluent densities from M4 were consistently low, and removals consistently good, when the pond was operated with a long detention time (Figure IV-24). A rotifer bloom resulted in a crash of algal populations without affecting harvestability around 16-17 July. The pond was drained on 21 July. It was refilled with unharvested effluent from M2, the detention time was shortened to 3.3 days, and the paddle wheel rotation was slowed to 1.3 rpm. This allowed a direct, short-term (24 July to 3 August) comparison between M2 and M4 with respect to mixing speed since both were run similarly in all other respects. Neither pond harvested particularly well and no significant differences in effluent quality were observed (Figure IV-23 and IV-24).

M3 was decimated by rotifers again at the end of July when Micractinium was the overwhelmingly dominant alga. The 350 μ screen did not effectively remove rotifers. In order to put a smaller screen in use (150 μ openings) the paddle wheel rotation speed for M3 was reduced from 2.4 to 1.3 rpm on

4 August. This reduced the size of algal-bacterial flocs which otherwise would have been removed by the 150 μ screen. Suspended flocs have been characteristic of all of the fast and moderately fast mixed ponds. The change to slow mixing had no effect on effluent densities from M3 (Figure IV-24). The recirculation through the 150 μ screen, initiated on 10 August, increased both pond and effluent densities. The chlorophyll to VSS ratio in the pond also increased at this time (Figure IV-23). The effluent density soon dropped down again. In M1 the mixing speed was reduced on 12 August and recirculated through a 150 μ screen begun on 15 August. The same changes occurred as in M3 except that the effluent density did not show an initial increase. In September both of these ponds experienced crashes in density which coincided with rotifer blooms. Thus the 150 μ screen also was not effective in controlling grazing. The density crashes started in the middle of September in both ponds. However, M3 had already become unharvestable by this time whereas M1 became unharvestable a week or so later.

Since pond densities changed greatly throughout the summer due to many factors, a productivity comparison between the ponds is only meaningful for those periods of time when populations were relatively stable (see Table IV-5). During these periods grazer populations were presumably lowest. In July the production data followed the expected trend, increasing with decreasing detention time (Figures IV-25 and IV-26). The total production on a VSS basis was approximately inversely proportional to detention time, i.e. the pond densities were similar on a VSS basis. This was not true when algal biomass (chlorophyll density) was considered. The 50% increase in total VSS production of M1 (32 gm/m^2/day, θ=2.3) over M2 (23 gm/m^2/day, θ=3.3)was not matched in total chlorophyll production. Here the increase was only somewhat greater than 25%. Even this is an overestimate if grazing pressure was greater at the longer detention time. Thus, 25% of the algal

biomass production and less than 50% of the total solids production was sacrificed in maintaining harvestable algae.

The probability that CO_2 also limited productivity cannot be ignored given the pH of the ponds. Longer detention time ponds would have been more severely CO_2 limited.

EXPERIMENT 6 CONCLUSIONS

The ponds proved to be unstable throughout the summer operations. Algal populations were repeatedly grazed upon by zooplankton. Ponds run at two or three-day detention times were never totally decimated by grazers, while ponds run at 4 or more days crashed several times. This was not unexpected since rotifers presumably have lower maximum growth rates than algae. However, it was not demonstrated at all conclusively in this experiment that grazing could be controlled by manipulation of detention time, or that rate of growth was the only factor involved. Selective screening was only effective in controlling populations of crustaceans. Crustaceans were never observed in any significant quantity once screening through the 350 μ screen was begun. Other ponds which were not screened (and were not involved in this experiment) did become infested with crustaceans. It appeared at first that selective removal of grazers by recirculation through a screen with 150 μ openings was effective since pond algal densities increased. However, pond crashes and rotifer blooms in September (when detention times were long) showed that this was not true since the screens were ineffective in preventing this. It can be concluded that the screens used were effective in shortening the detention time of large herbivores to the point where their growth could not keep up with their washout. This opened a niche to

smaller grazers. Although many of the smaller rotifers were also removed by screening, and hence their average detention time was shortened, removal was not complete enough to prevent blooms, except in ponds which were operated at short detention times. The relative importance of grazing on the ponds can also be realized by looking at the changes in M1 and M3 after the mixing speeds were reduced and the 150 μ screen was used (Figures IV-23 and IV-25). The pond ratios of chlorophyll to VSS increased greatly. Both changes would contribute to this. However, pond densities increased and thus production increased. Reduction in mixing speed causes pond densities to decrease significantly due to settling (see Experiments 2 and 3). So the grazing pressure must have been great, and the small screens must have relieved this pressure to a large extent. It is possible that large rotifers present before the screens were put into use were replaced by smaller rotifers afterward.

Productivity was obviously affected by detention time, mixing and grazing. Short detention time increased algal biomass production, but sewage turbidity limited the extent of this increase The moderatly fast mixing speed aggravated this by keeping sewage particles suspended. Grazing reduced productivity but short detention time appeared to relieve the grazing pressure. Harvestability was also influenced by the same three factors. Short detention time ponds were unharvestable. Lengthening the detention time transformed unharvestable populations into harvestable ones but by increasing colony size not by inducing changes in algal types. Pond crashes were always accompanied by excellent or improving harvestability, which indicates that grazers preferentially ate the smaller algae and particles. This was also observed microscopically. Since grazing pressure also correlated with detention time (more pressure at longer detention times) the harvestability of long detention time ponds at certain times of the year and under

certain conditions may be due to grazing. Mixing did not have a large effect as the comparison of a moderately fast mixed and slow mixed pond demonstrated (see Results). Thus fast mixing did not promote microstraining harvestability of productive (short detention time) ponds, and prevented use of small mesh screens for removing grazers. But short detention time, fast-mixed ponds were not subject to heavy grazing. This might prove critical in multi-stage systems where total algae removal from the first stage is not necessary.

The effect of seasonal factors on harvestability can be of overriding importance. In September all ponds became unharvestable without exception. All of the algae present were in small colonies. This same trend was observed last year (reference 31)at about the same time. Harvestability did not recover until January of this year.

Species composition was another dynamic factor in the summer's experiments. Many correlations were evident, but too many other factors were involved to arrive at explanations. Scenedesmus was the predominant algal type at the beginning of the summer. It was replaced by Micractinium in all ponds (Figures IV-29 and IV-30). Several parameters were changed during this period which may have played a part in this species change. Again seasonality played a large role since Micractinium bloomed at the same time last year (ref. 31). Thus, Micractinium increased in M1 after the detention time was shortened from 3.3 to 2.3 days (Figure IV-29), but a subsequent lengthening of the detention time had no effect on species composition. Neither did reducing the mixing speed or recirculating the pond through the herbivore screen. The changeover occurred more slowly (and more slowly than the relative rates of turnover) in M2 (Figure IV-30) after its detention time was shortened, suggesting that Scenedesmus was more competitive at longer hydraulic and cell detention time. In M3 the decline in Scenedesmus and rise in Micractinium coincided with a rotifer bloom and

pond crash suggesting that, at this time of year and at the given detention time, Scenedesmus was preferentially grazed upon. In M4, at this same time, the Scenedesmus population did not give way to Micractinium although it was heavily grazed (the pond crashed due to grazing). The detention time was longer and the population of Micractinium was very small. M3 crashed due to grazing in September when it was predominantly Micractinium. By this time the pond was already unharvestable and full of small colonies. Obviously the effects of seasonal changes and operational varieties are not readily, if at all, separable.

TABLE 9

Dates 7/4-8/3

EXPERIMENT 6 SUMMARY TABLE

Depth (cm) 25

	M-1	M-2	M-3	M-3	M-4
Date	7/4-8/3	7/4-8/3	7/4-7/8	7/20-7/29	7/4-7/15
Detention Time (days)	2.3	3.3	4.6	4.6	6.0
Mixing Speed (paddle wheel, rpm)	2.4	2.4	2.4	2.4	2.4
Pond Density (VSS mg/ℓ)	254	270	288	285	310
Effluent Density (VSS mg/ℓ)	73	30	15	21	12
Harvestability (% VSS removal)	71	89	95	92	96
Total Production (gm VSS/m^2 day)	32	23	18	18	16
Harvestable Production (gm VSS/m^2 day)	23	20	17	16	15
% Chlorophyll (by weight)					
Pond	1.6	1.7	1.5	1.9	1.6
Effluent	1.2	0.8	0.4	0.4	0.4
Conversion Efficiency (%)					
of Total Sunlight	2.8	2.0	1.4	1.6	1.3
of PAR	6.2	4.4	3.2	3.5	2.9
Dominant Algae (beginning)	Scen. 66%	Scen. 92%	Scen. 97%	Mic 87%	Scen. 99%
Sub-Dominant Algae (beginning)	Mic. 32%	Mic. 8%	Mic. 3%	Scen. 7%	—
Dominant Algae (end)	Mic. 88%	Mic. 66%	Scen. 97%	Mic. 82%	Scen. 96%
Sub-Dominant Algae (end)	Scen. 6%	Scen. 32%	Mic. 3%	Scen.17%	Flag. 2%
Effluent NH_3 mg/ℓ/% Removal*	19.1/60	14.0/70	9.2/85	8.7/81	7.7/83
Effluent COD mg/ℓ/% Removal*	207/55	132/71.2	158/65	124/74	87/58

*% of pond influent

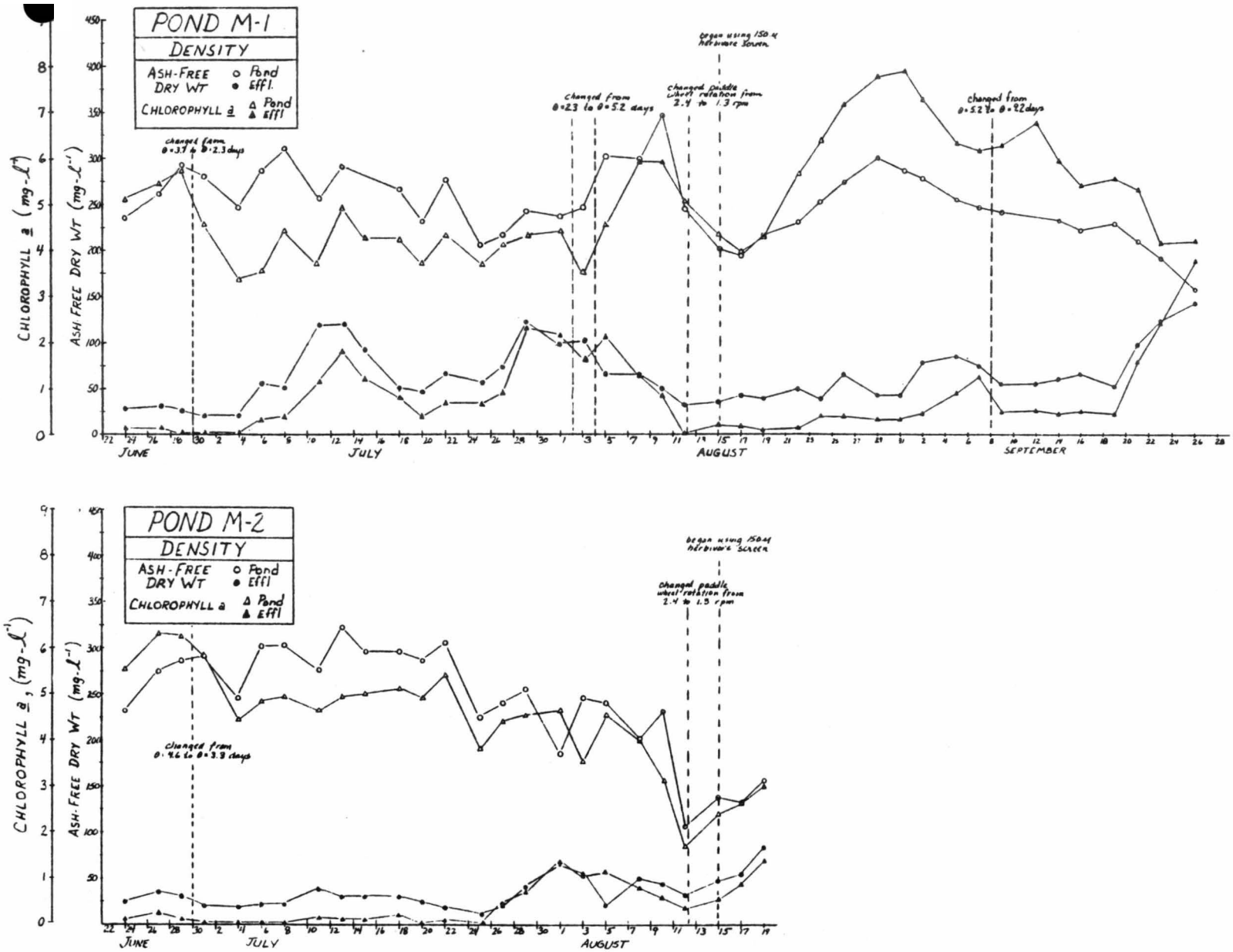
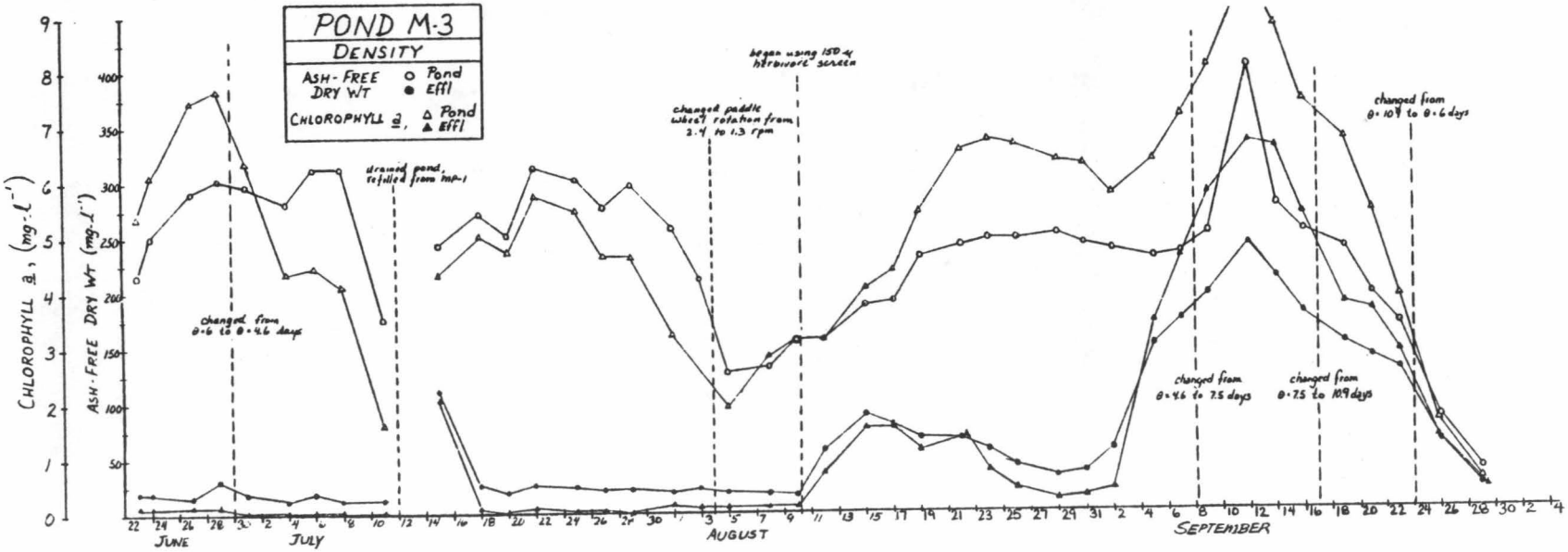


FIGURE IV-23. POND AND MICROSTRAINER EFFLUENT DENSITY : EXPERIMENT 6



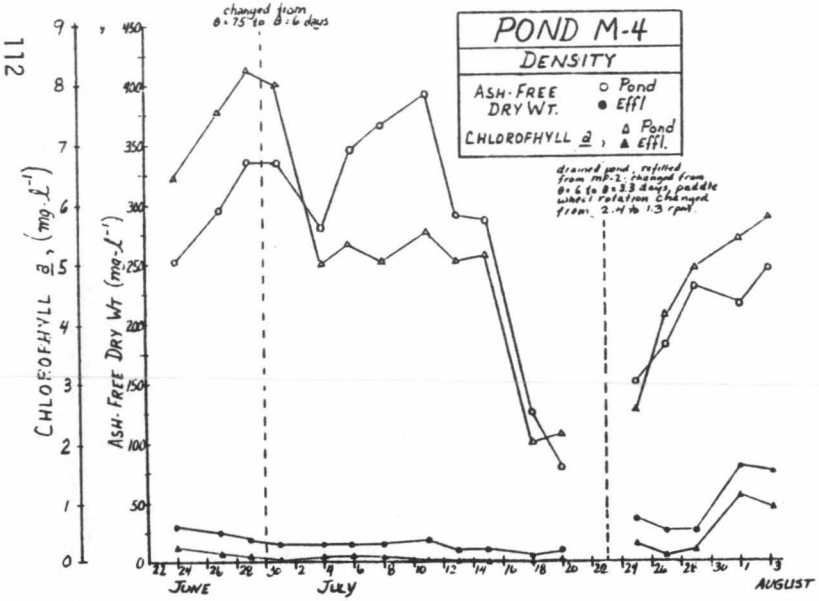


FIGURE IV-24. M-3 AND M-4 POND AND MICROSTRAINER EFFLUENT DENSITY: EXPERIMENT 6

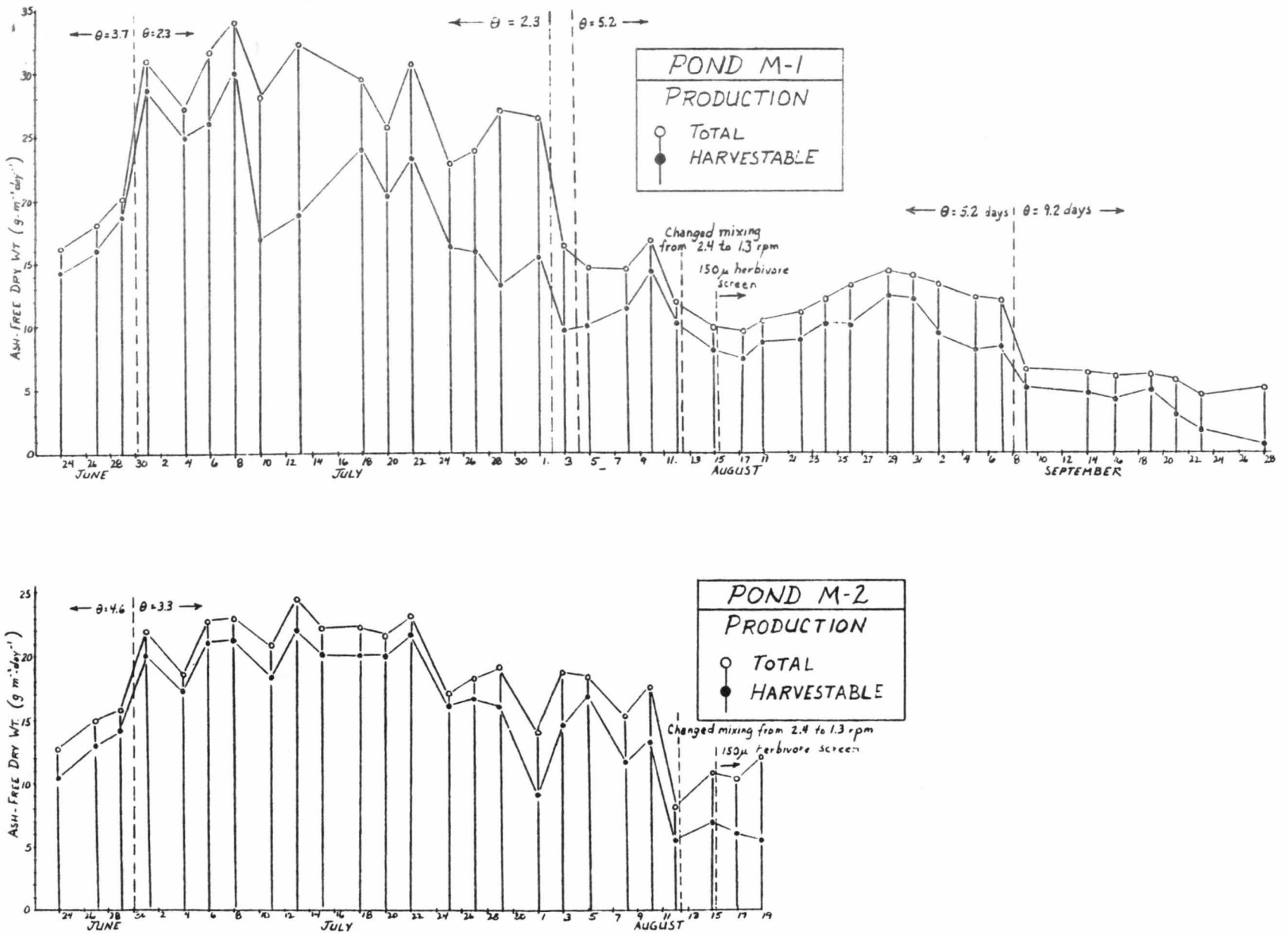
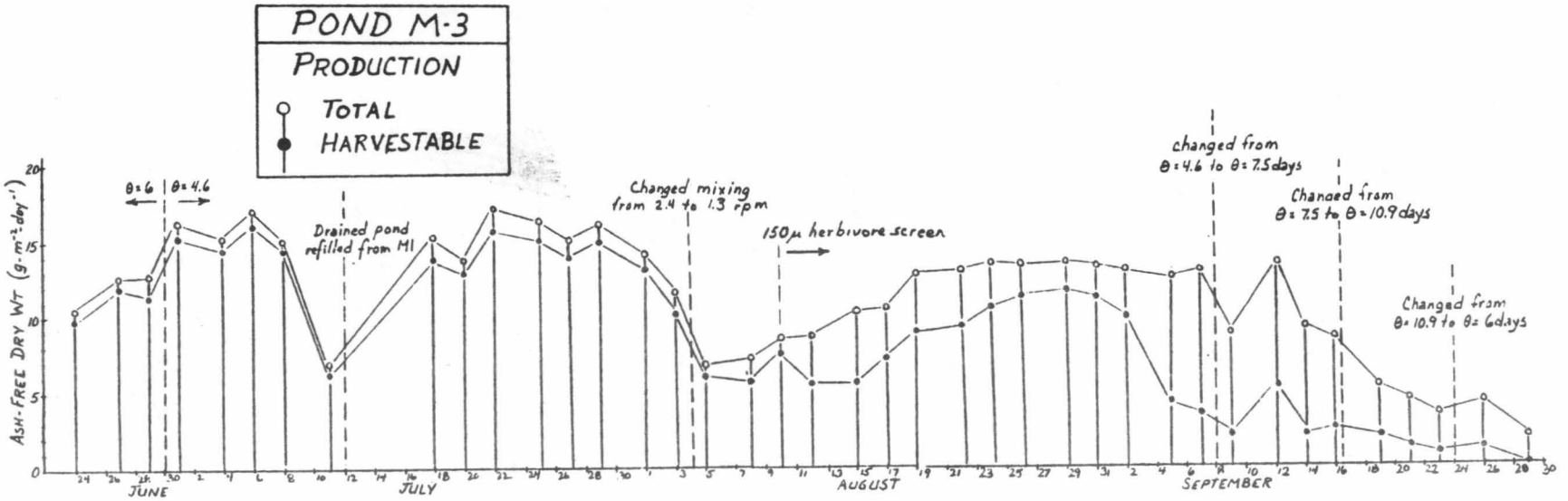
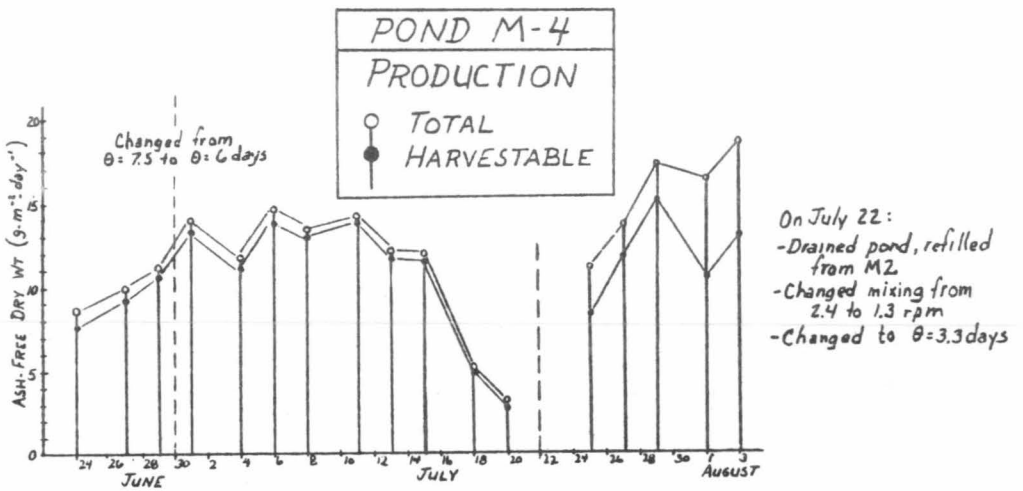


FIGURE IV-25. M-1 AND M-2 PRODUCTIVITY: EXPERIMENT 6





On July 22:
- Drained pond, refilled from M2.
- Changed mixing from 2.4 to 1.3 rpm
- Changed to θ=3.3 days

FIGURE IV-26. M-3 AND M-4 PRODUCTIVITY: EXPERIMENT 6

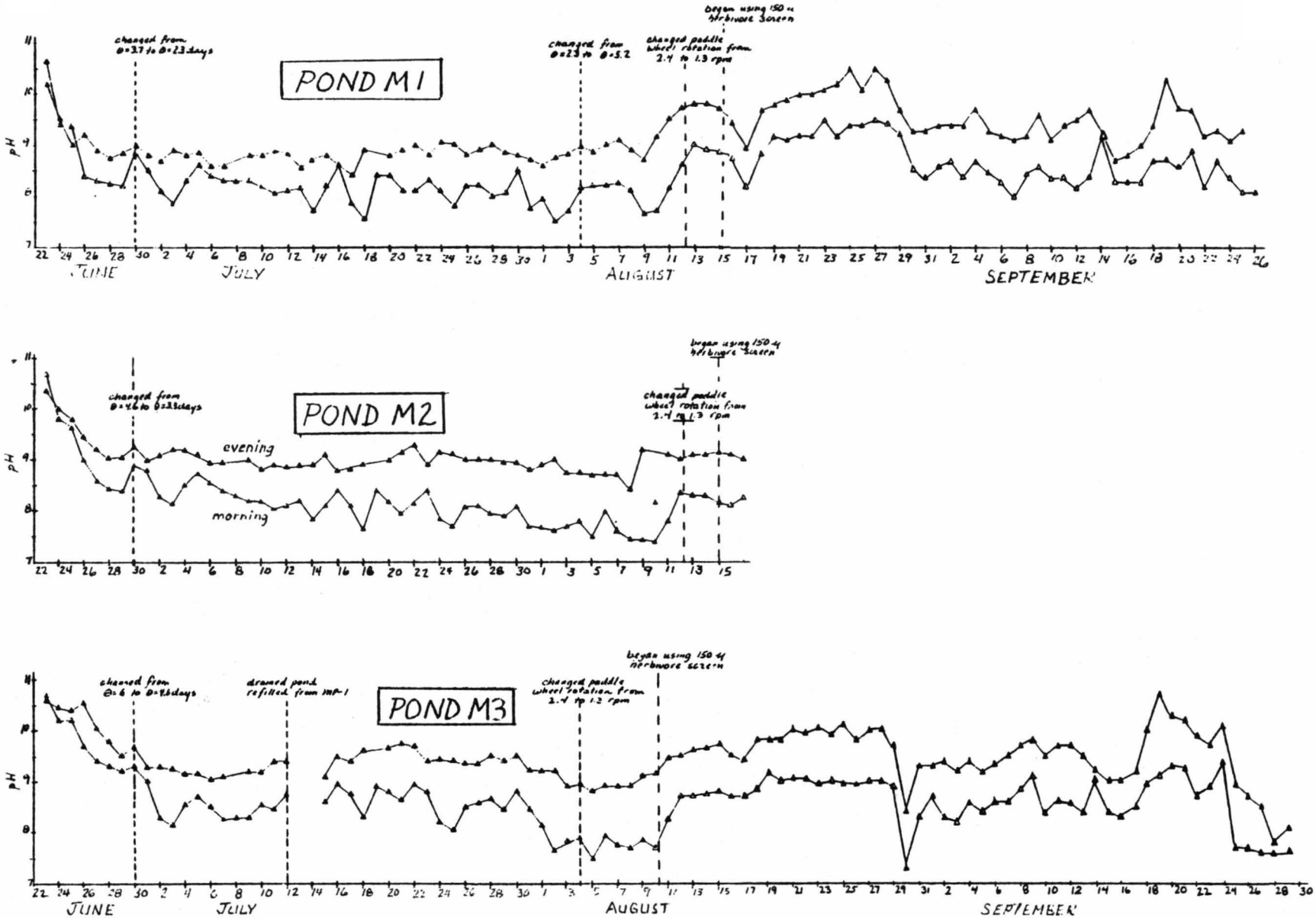


FIGURE IV-27. M-1, M-2, M-3 pH: EXPERIMENT 6

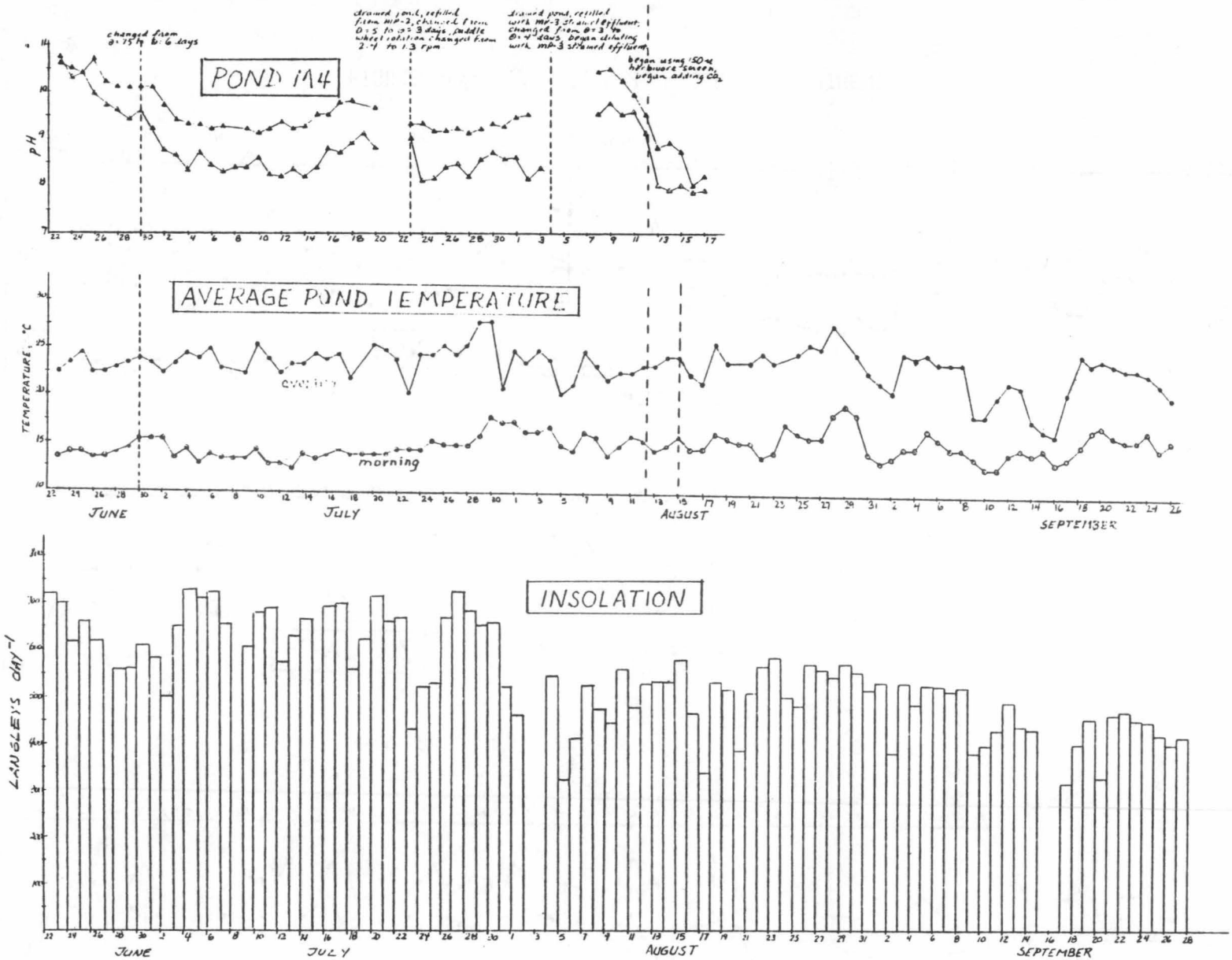


FIGURE IV-28. M-4 pH, AVERAGE POND TEMPERATURE, INSOLATION: EXPERIMENT 6

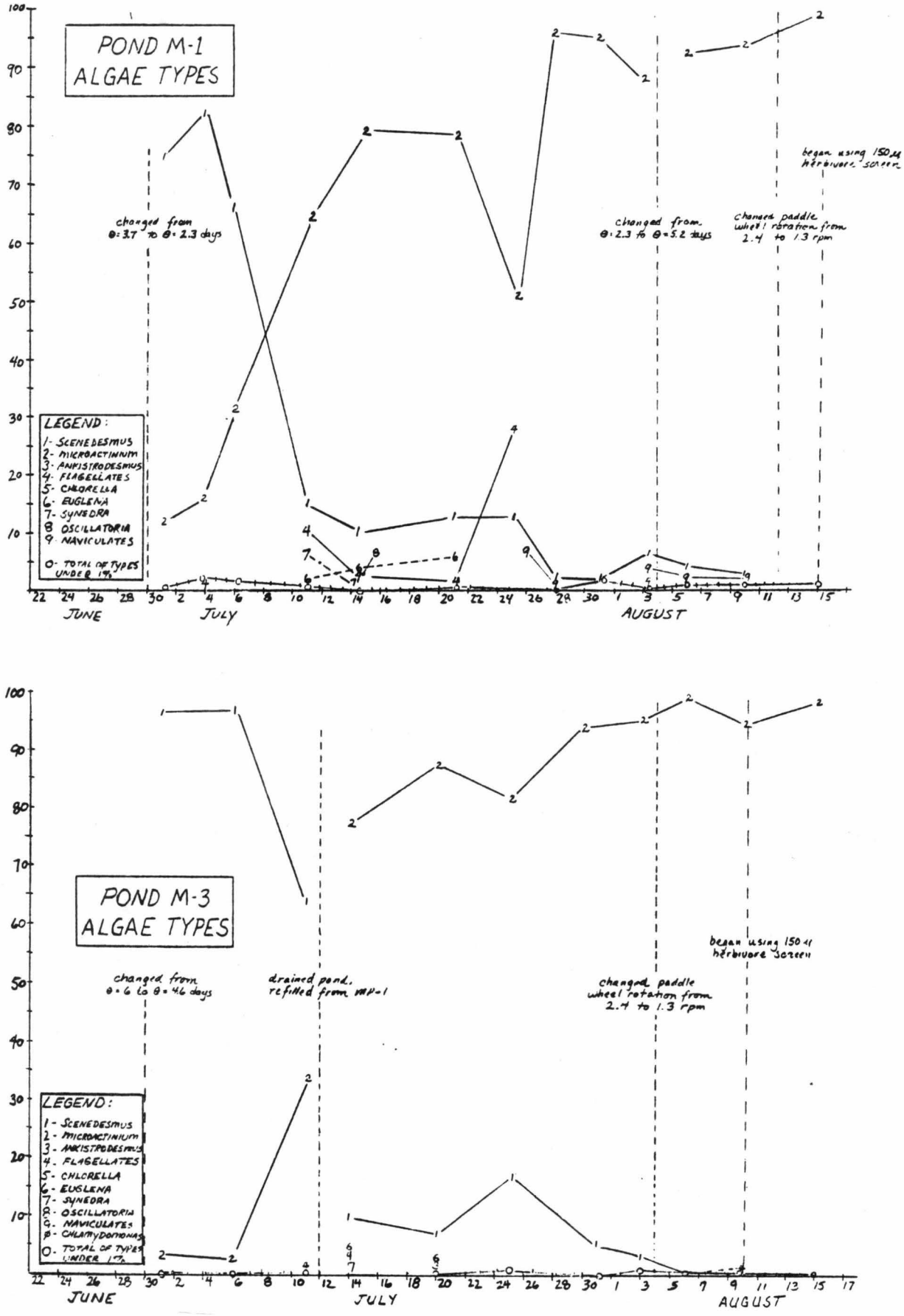
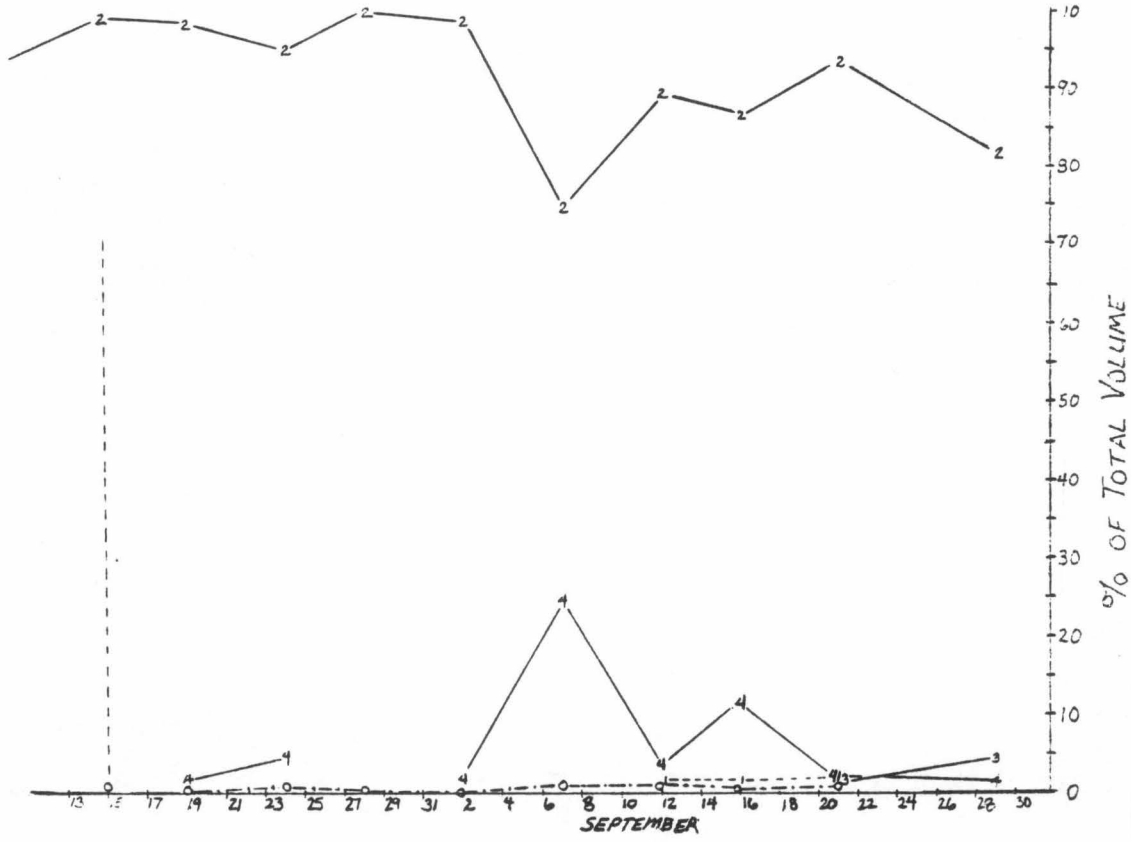


FIGURE IV-29. ALGAL TYPES (% OF TOTAL ALGAL VOLUME) M-1 AND M-3: EXPERIMENT 6



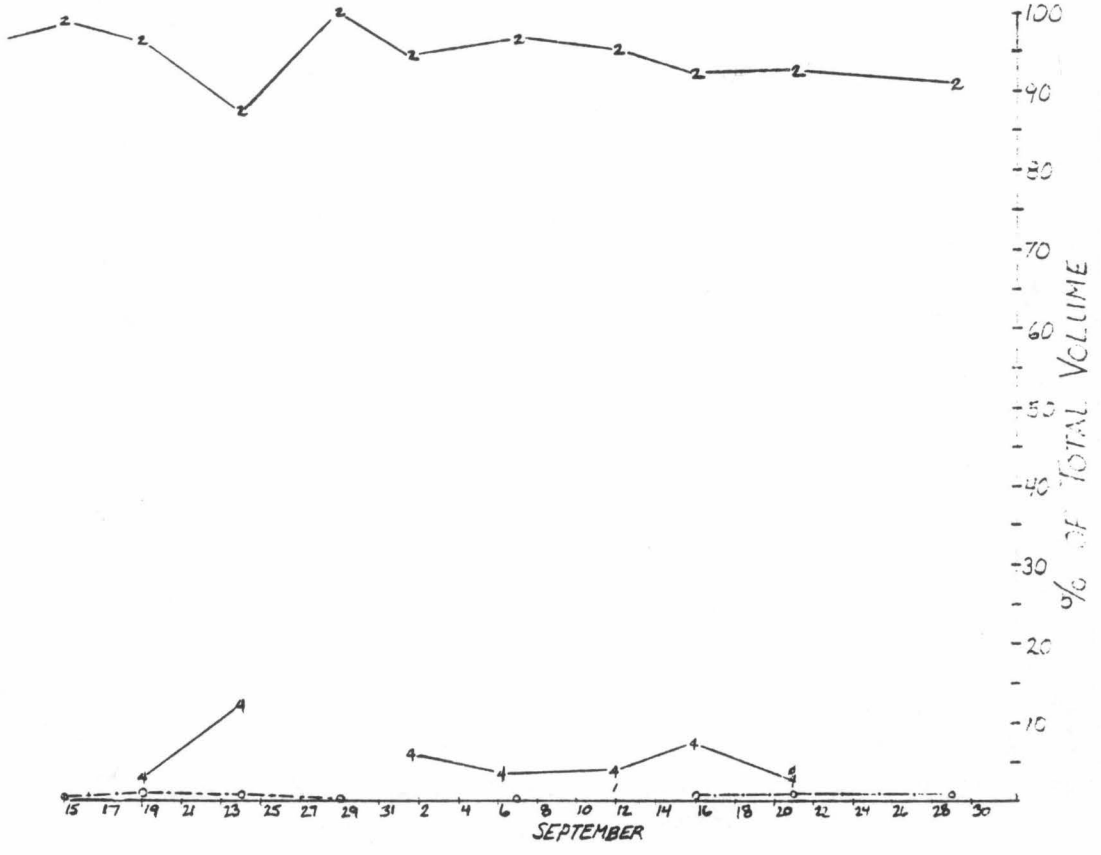
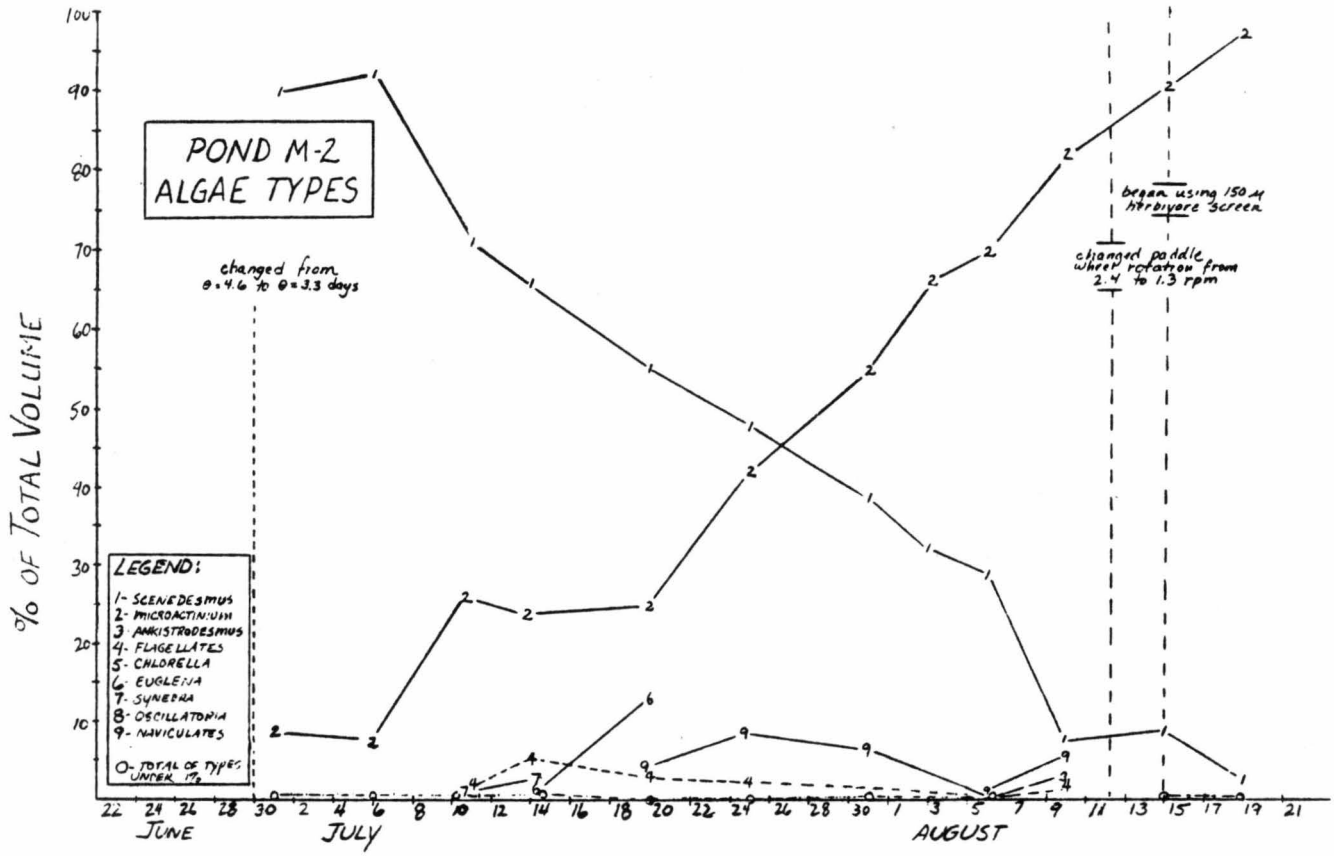


FIGURE IV-29 (continu ed)



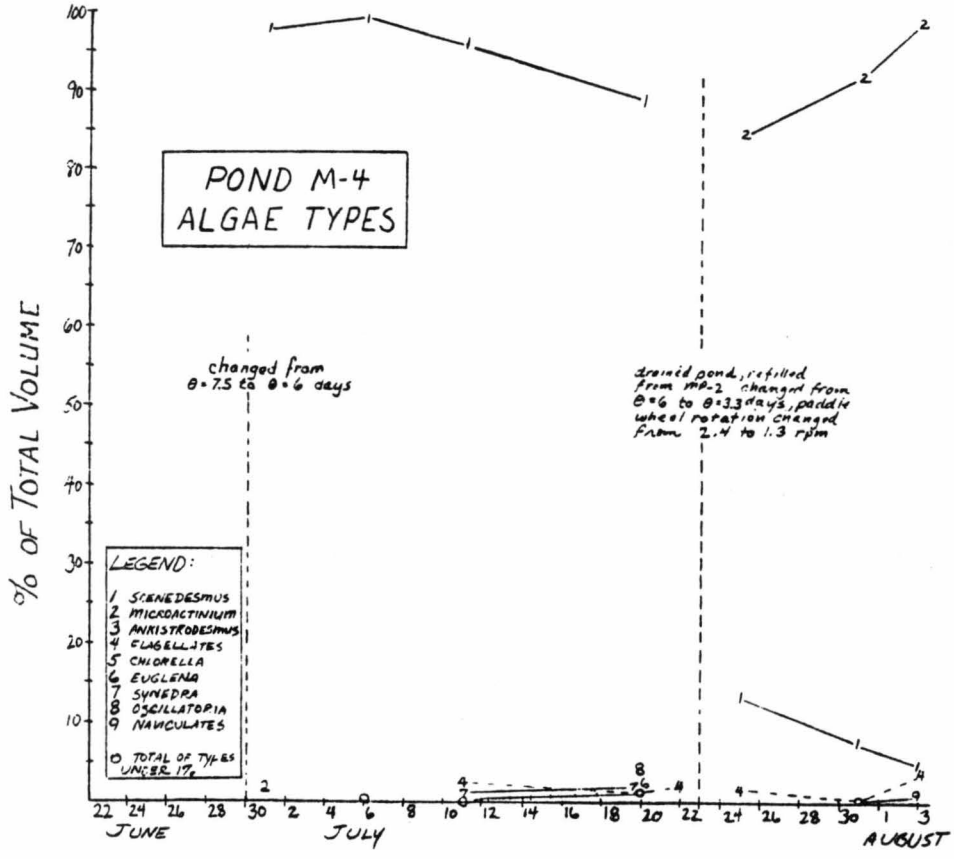


FIGURE IV-30. ALGAL TYPES (% OF TOTAL ALGAL VOLUME) M-2 AND M-4: EXPERIMENT 6

EXPERIMENT 7 RESULTS

Starting in May, algal populations in the 12 m^2 ponds became unstable due to herbivore invasions. This instability poses a serious reliability problem for all ponding applications. In designing an experiment on grazing, two questions were asked. First, could the 150 μ screens effectively prevent herbivore blooms when the ponds were run at a long detention time? The summer's results indicated that they were not effective, but confirmation was sought. A long detention time was chosen because blooms had been most evident under these conditions. Second, could grazing pressure make a pond harvestable by microstraining? This was a possibility. The ponds were all unharvestable at the end of the last experiment as they had been last year at this time (reference 31). The four ponds were divided into two pairs, which were derived from different inocula. M1 and M2 were half-filled with a non-harvestable culture from the 0.6-acre high-rate pond on 28 September and diluted one inch per day with sewage until 3 October when dilution at an eight-day detention time was begun. Grazers were present in this inoculum, but at an extremely low level (< 50 /ml). Starting on 28 September, M1 was recirculated 24 hours a day over the 150 μ herbivore screen. The material retained on the screen was examined and removed 2-3 times daily. M2 was also recirculated 24 hours a day over an identical screen, but the material that was retained was periodically washed back into the pond. This material consisted of paper, algae, sludge but predominantly zooplankton. Therefore grazers were removed from M1 but not from M2.

On 30 September M4 was pumped out and M3 split in two. Sewage was added 1 inch per day to each pond. Dilution at an eight-day detention time was begun on 4 October. The inoculum (M3) had experienced a density crash and coincident grazer bloom (see Figure IV-24, Experiment 6). The grazer population had declined at the time of inoculation. Both ponds were

recirculated all day and all night through 150 μ screens. The catch was examined and removed. In addition, Ortho Malathion-50 (O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate), a cholinesterase inhibitor was added to M4 on the schedule depicted in Figure IV-34. This poison was added to kill grazers, yielding a pond free of their effects.

Although M1 and M3 were operated identically, the histories of the cultures were different since they were inoculated differently. Thus two ponds, M1 and M3, had grazers screened out of them; one pond, M2, did not; and one pond, M4, was screened and poisoned to eliminate grazers. Zooplankton counts were done on the composite pond samples used for dry weight and chlorophyll analyses or simply on 1-liter grab samples from the ponds. Although all zooplankton types were counted, only those types of organisms with at least one dimension averaging over 200 μ are included in the figures. Almost all of these organisms were rotifers of the genus Brachionus, and almost all Brachionus were greater than 200 μ in one dimension (see Table 10).

As shown in Figures IV-31 and IV-32, the pond densities in M1 and M2 both declined from the initial inoculation (and subsequent batch growth) to low points at about the middle of the month, and then rose somewhat. This pattern was much more pronounced in the effluent densities than in the pond densities. This is shown in the figures as an increase, leveling , and decrease in harvestability. The counts of zooplankton greater than 200 μ in one dimension are shown on the same graphs and follow the harvestability curves very closely. The zooplankton count was higher in M2 at the beginning of the experiment because the zooplankton were not removed from this pond as the pond was filling with sewage and grown in batch (from 27 September to 3 October). The maximum zooplankton count was an order of magnitude greater in M2 than M1. The maximum harvestability attained was only slightly higher in M2 and the minimum values for pond and effluent densities were similar.

In M3 and M4 the pond densities initially increased from the low inoculum level (Figures IV-33 and IV-34). Grazers increased in the pond that was screened but not in the pond that was screened and poisoned. Harvestability once again followed the count of large zooplankton very closely. Pond and effluent densities fell about 30% in M3 as the zooplankton count increased. Most of these changes occurred later in M3 than in M1 and M2. The maximum zooplankton count was also much lower in M3. Zooplankton counts and harvestability both remained low and fairly constant in M4, the pond to which Malathion was added. However, a 30% decline in pond and effluent densities, and a subsequent recovery coincided with the commencement and termination of the Malathion addition. Apparently this poison was effective in killing zooplankton and it or its breakdown products were somewhat detrimental to algae growth at the concentration used.

Comparison of the productivity and harvestability data (Figures IV-31-34 and IV-35-38) shows that the decrease of the former was always less than 30%, while the increase of the latter was from 5 to 15 fold. None of the ponds differed significantly in productivity, with 4 $gm/m^2/day$ a typical value.

All of the ponds started with about 80% Micractinium (by volume) and 15% Scenedesmus (see Table 11). The Scenedesmus were found in large and small colonies in M3 and M4 and predominantly in large colonies in M1 and M2. These pairs of ponds were derived from separate sources. By the end of the month the relative proportions of Micractinium and Scenedesmus were reversed in all ponds, whether grazers were present or not. The colony size of the Micractinium remained small throughout the experiment. The Scenedesmus was found more often in larger colonies (>90% large, some very large) when grazers were present than before and after the grazer blooms (when they were 40 to 60% large). In M4, which never had many grazers, not more than 50% of the Scenedesmus colonies were large. The rise of Scenedesmus and concomitant

fall of Micractinium was fastest in M2, which did not have grazer removal, the same in M1 and M4, which had very different levels of grazers, and slowest in M3, which experienced a late grazer bloom.

EXPERIMENT 7 CONCLUSION

The two questions towards which this experiment was directed can be answered. The 150 μ herbivore screens were not effective in preventing the herbivore blooms under the given conditions. The screens only limited the peak number of grazers, but had little other effect. None of the ponds, even M2 which was not screened, were decimated by rotifers as ponds during the summer had been. The effectiveness of the screen can be very roughly estimated by comparing the initial rise of rotifers in M1 and M2 and approximating this rise as linear (the figures are semi-log graphs). This implies a constant specific growth rate. For the most part the mass balance of rotifers was affected only by growth, hydraulic removal (at $D = 1/\theta = .125\ day^{-1}$) and removal by screening. The screening can be described by a rate constant r, where the change in zooplankton concentration, X, due to screening is -rX. Thus, $dX/dt = (\mu-D-r)X$ for M1 and $(\mu-D)X$ for M2. With the data from M2 one can calculate that $\mu = 0.73\ day^{-1}$ which corresponds to an average doubling time of about one day when growth was fastest. This is not the same as length of reproductive cycle, which may be much longer . Using this and the data from M2, r is found to be approximately $.15\ day^{-1}$. Thus the screens removed a low percentage of the grazers over 200 μ. This is born out by the ineffectiveness of the screens. There are several possible explanations for this inefficiency of removal. Many of the grazers whose largest dimension was only about 200 μ may not have been retained initially or were washed through the screen during the time between cleanings. Grazers may have been able to avoid the pump used to pump pond water over the screen. That is, the sampling for recirculation of the pond over the screen may not have been random.

Also, the above analysis ignores the population structure imposed by the reproductive cycle of these organisms. Rotifers are hatched as juveniles or nearly full-sized from eggs that are about 1/3 as large as adults. It is quite possible that these eggs were not removed efficiently. They may have passed through the screen or have settled to the bottom of the pond and thus never were pumped over the screen. Nevertheless, the screening did decrease the average detention time of grazers by an amount that was not insignificant. It may have reduced the average detention time of grazers (over 200 μ) from 8 days in M2 to less than 4 days in M1. At a shorter hydraulic detention time the screen might provide enough extra reduction in grazer detention time to actually control their population.

The data also provides an unequivocal answer to the second question of whether grazing pressure can make ponds harvestable. The correlations between the rise and fall of grazer populations and harvestability certainly indicate that the grazers did improve harvestability. Other factors were obviously involved since two ponds, M1 and M3, which were inoculated differently but operated identically, gave varying results. One became much more harvestable and exhibited a larger rotifer bloom at an earlier time. Despite this, the results make it tempting to postulate controlling the populations of small algae through control of grazers. This might be accomplished at shorter detention times by manipulating the rate of removal of grazers.

An important conclusion from this experiment is that the grazers did not determine the ultimate species change. Initially all of the ponds were predominantly Micractinium. They ended up predominantly Scenedesmus despite the large variation among ponds in grazer concentrations. However, the experiment gave ambiguous results as to whether the grazers determined the rate at which this change occurred. The time course of this change seemed to correlate with the grazing pressure when M1, M2 and M3 are compared

(Table 11). The species change coincided with the grazer blooms in all three ponds. There is no indication as to whether the grazers induced the change or followed it, eating whatever algae were available and edible. On the other hand, M4 changed from Micractinium to Scenedesmus at a rate nearly identical with M1. Yet grazers were absent from M4 and abundant in M1. Although M4 was the only pond which received poison, this comparison indicates that the grazers may have had no effect on the rate of species change.

The greater dip in effluent densities versus pond densities indicate that the small algae, of both types, were preferentially grazed upon. In the case of Scenedesmus, the data in Table 11 is very consistent with this interpretation since the alga was found more often in large colonies when grazers were most abundant. In addition the colony size distribution of Scenedesmus in M4 was similar to the distributions in the other ponds before and after the grazer blooms.

It is difficult to estimate the efficiency of conversion of algae to grazers from the data. It is interesting to calculate this efficiency to see whether the algae consumed could account for the grazers produced, especially since the large differences in the number of grazers in the different ponds were not matched by large differences in algal densities. Of course the ponds were not all operated the same. Also, in any one pond the differences in algal densities when grazers were absent versus present is difficult to determine due to the scatter in the data and because steady densities were not achieved before and after grazer blooms. Nonetheless, calculations can be made from the data from M3, particularly from the differences between dry weight (or chlorophylls, multiplied by an average chlorophyll content equal to about 3%) and grazer densities on 13 October and 17 October. Assuming that the grazers were growing at a maximal rate, the increase from 240/l on 13 October to 2200/l on 17 October corresponds to a production of about

1.5×10^6 grazers over the four-day period (if the effluent pump adequately sampled the pond). Multiplying the average grazer volume of 2×10^6 μm^3 per grazer by an assumed density of 1 g/cm^3 gives about 3×10^3 mg of grazers produced or .06 $gm/m^2/day$. The decrease in algae density from 165 mg/l on 13 October to 125 mg/l on 17 October approximately corresponds to (35 ± 15) g of algae consumed over the four-day period (assuming the density would have remained at 165 mg/l if grazers were absent). This yields an efficiency of about 6-15% for the conversion of algal dry weight to grazer dry weight. From this it is reasonable to assume that the grazers survived on algae alone. However, the order of magnitude difference in the grazer population in M2 versus M1 was not accompanied by a significant difference in the algal densities. Indeed, rough estimates of the grazer productions, including the removal rate due to screening in M1, indicate that about 15 mg/l more grazers were produced in M2 over the course of the experiment (about 25 g/l was produced in M2 totally in 20 days),or about .3 $gm/m^2/day$. Besides suspended algae these grazers must have consumed detritus, settled sludge and/or wall growth.

TABLE 10

NUMBERS AND BIOVOLUMES OF ROTIFERS IN MIDI PONDS
(Mean dimensions = 205 μm x 120 μm)

Date	MP-1		MP-2		MP-3		MP-4	
	No./ liter	$\mu m^3/m\ell$ x 10^6	No./ liter	$\mu m^3/m\ell$ x 10^6	No./ liter	$\mu m^3/m\ell$ x 10^6	No./ liter	$\mu m^3/m\ell$ x 10^6
10/3/77	230	0.481	2200	4.601	120	0.251	220	0.460
10/4					150	0.314	70	0.146
10/6	950	1.987	4600	9.621	280	0.586	110	0.230
10/10	5260	11.002	60300	126.122	140	0.293	70	0.146
10/13	11200	23.426	32400	67.767	240	0.502	30	0.063
10/17	2180	4.560	1840	3.848	2220	4.643	80	0.167
10/20	170	0.356	430	0.899	1580	3.305	90	0.188
10/24	10	0.121	20	0.042	270	0.565	10	0.021
10/27	60	0.125	10	0.021	80	0.167	20	0.042

TABLE 11

ALGAE TYPES

Date	Algae Type	MP-1		MP-2		MP-3		MP-4	
		Pond*	Microstrained Effluent**	Pond*	Microstrained Effluent**	Pond*	Microstrained Effluent**	Pond*	Microstrained Effluent**
9/29	Scenedesmus	12(75)	--	13(75)	--	4(-)	--	--	--
	Micractinium	82(10)	--	81(10)	--	90(0)	--	--	--
10/6	Scenedesmus	14(75)	15(75)-21	30(95)	16(100)-57	9(30)	17(35)-0	20(40)	21(35)-21
	Micractinium	80(0)	73(0)-35	65(0)	80(0)-0	86(0)	77(0)-0	75(0)	74(0)-40
10/10	Scenedesmus	30(95)	--	48(95)	--	--	--	--	--
	Micractinium	66(0)	--	51(0)	--	--	--	--	--
10/13	Scenedesmus	40(95)	18(95)-86	80(95)	32(95)-90	15(90)	11(80)-27	42(60)	25(60)-34
	Micractinium	58(33)	80(10)-58	20(50)	66(40)-30	73(10)	80(10)-0	36(0)	62(0)-0
10/20	Scenedesmus	61(95)	32(80)-70	70(90)	60(60)-56	--	--	67(40)	67(40)-21
	Micractinium	37(0)	64(0)-0	28(5)	40(0)-16	--	--	21(0)	21(0)-22
10/27	Scenedesmus	65(60)	65(40)-15	67(50)	75(50)-0	43(80)	38(70)-16	70(50)	72(50)-2
	Micractinium	30(0)	26(0)-25	29(0)	23(0)-20	49(0)	55(0)-0	18(0)	15(0)-17
11/3	Scenedesmus	72(40)	72(40)-4	66(65)	72(50)-0	75(50)	65(50)-0	73(50)	72(50)-8
	Micractinium	20(0)	17(0)-23	23(0)	19(0)-10	18(0)	27(0)-0	18(0)	22(0)-0

* The first number indicates percentage of total biovolume. Numbers in parentheses indicate the % of that algal type found in large colonies.

** Numbers after dashes show harvestability by counts of pond and effluent.

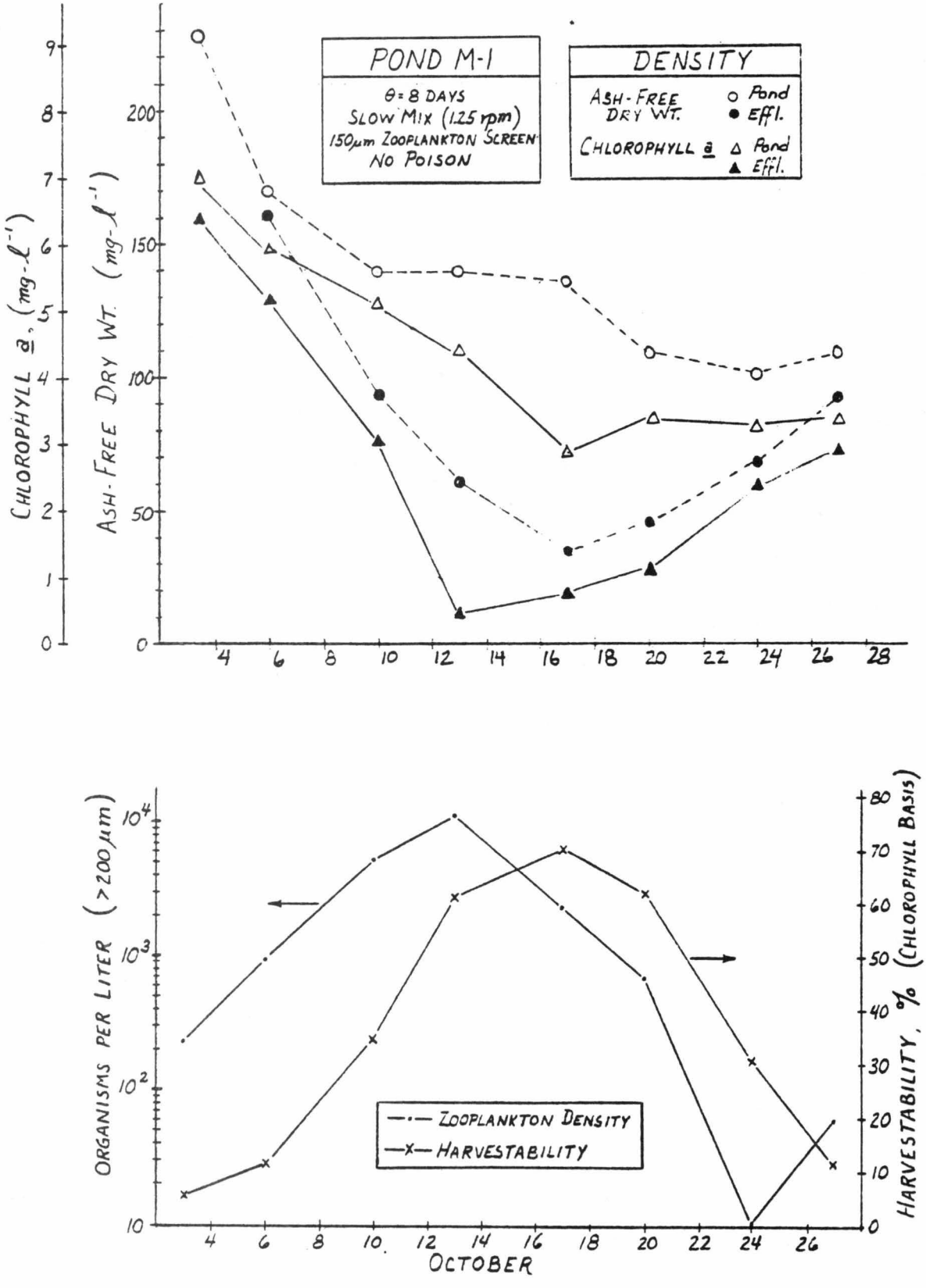


FIGURE IV-31. M-1 POND AND MICROSTRAINER EFFLUENT DENSITY, ZOOPLANKTON DENSITY AND HARVESTABILITY: EXPERIMENT 7

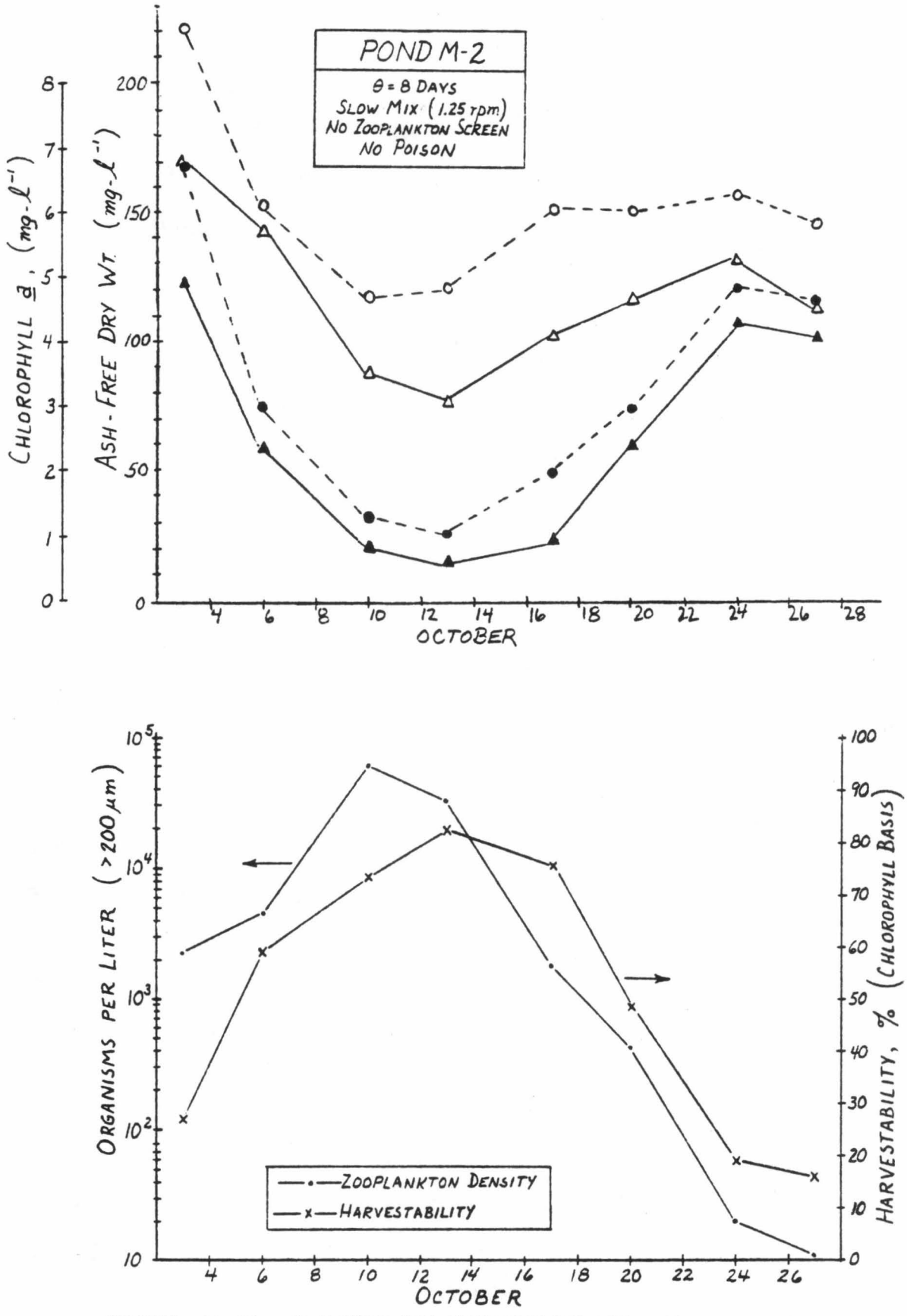


FIGURE IV-32. M-2 POND AND EFFLUENT DENSITY, ZOOPLANKTON DENSITY AND HARVESTABILITY: EXPERIMENT 7

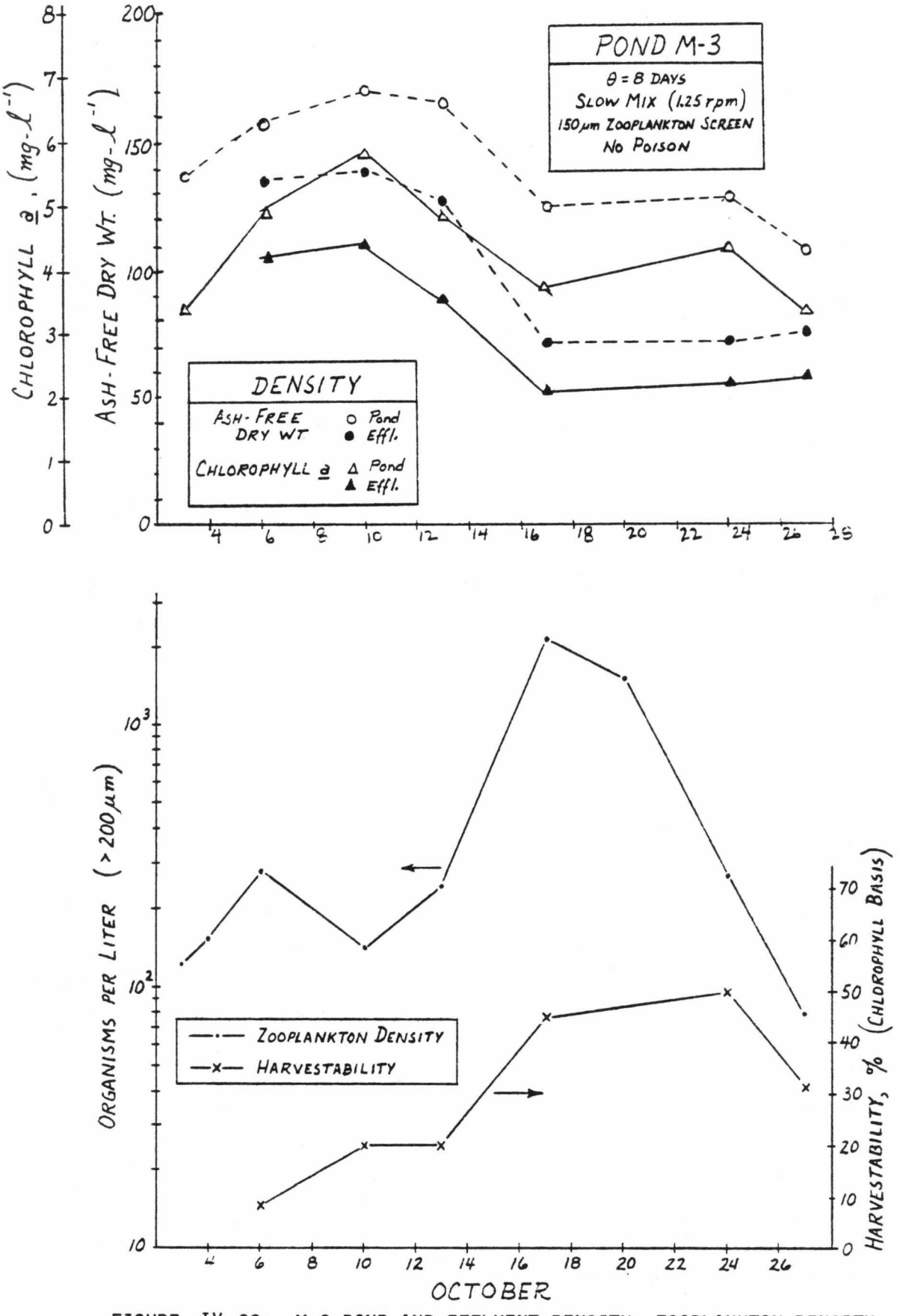
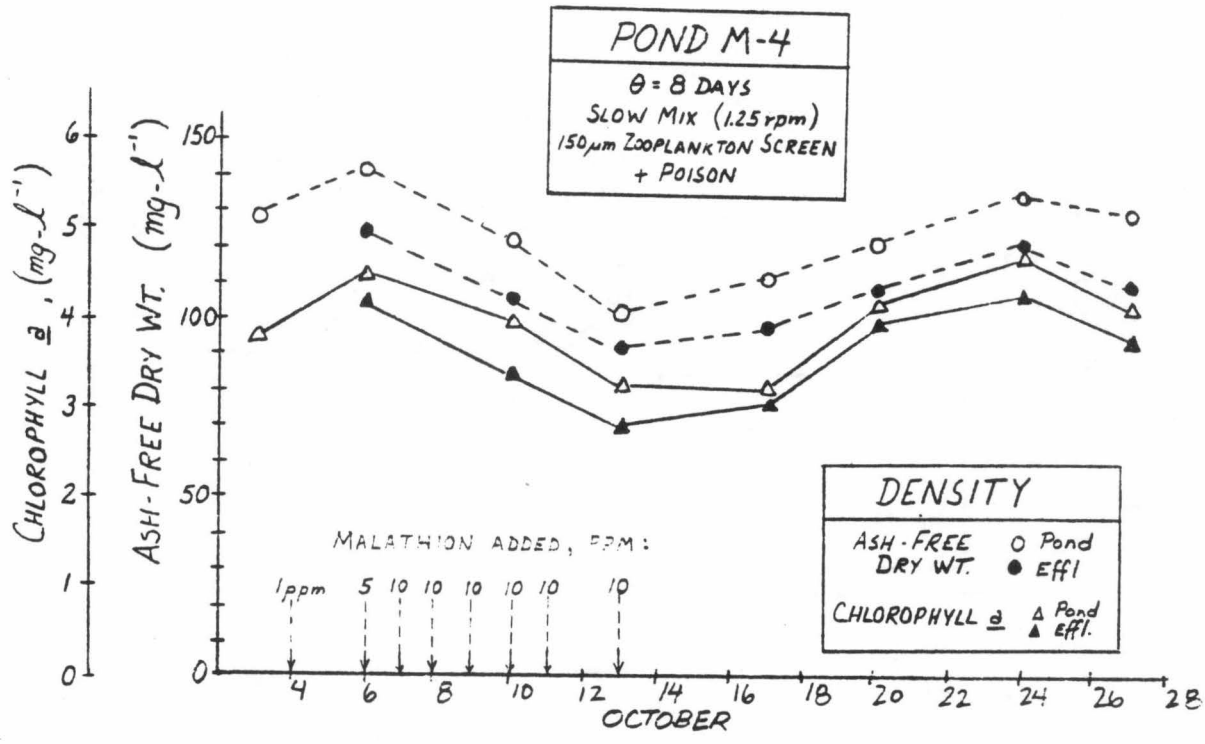


FIGURE IV-33. M-3 POND AND EFFLUENT DENSITY, ZOOPLANKTON DENSITY AND HARVESTABILITY: EXPERIMENT 7



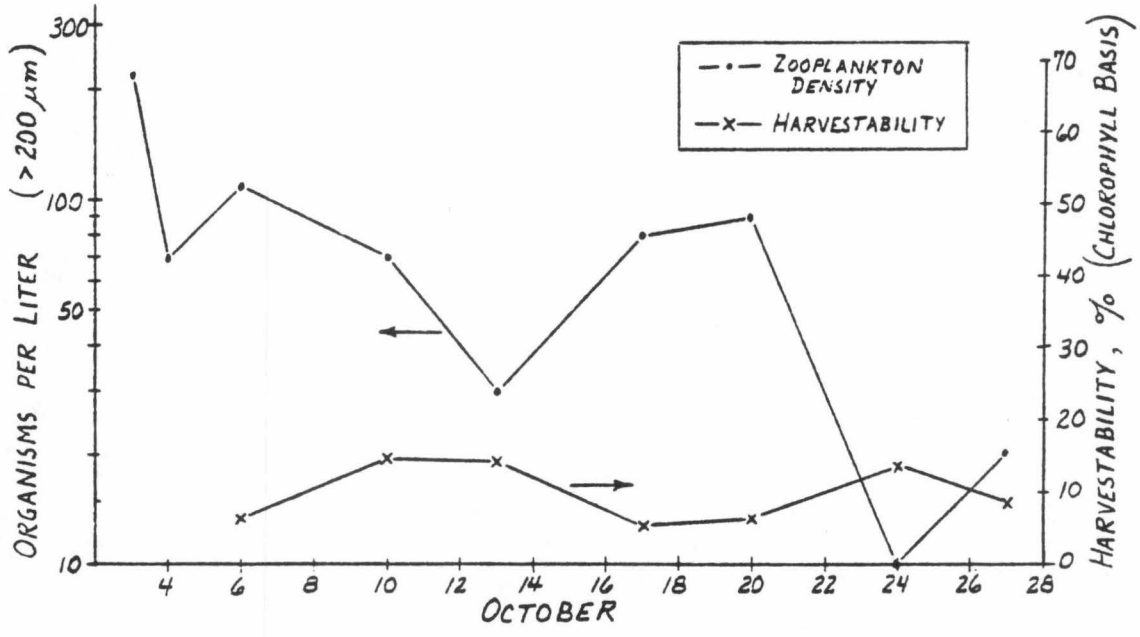


FIGURE IV-34. M-4 POND AND EFFLUENT DENSITY, ZOOPLANKTON DENSITY AND HARVESTABILITY: EXPERIMENT 7

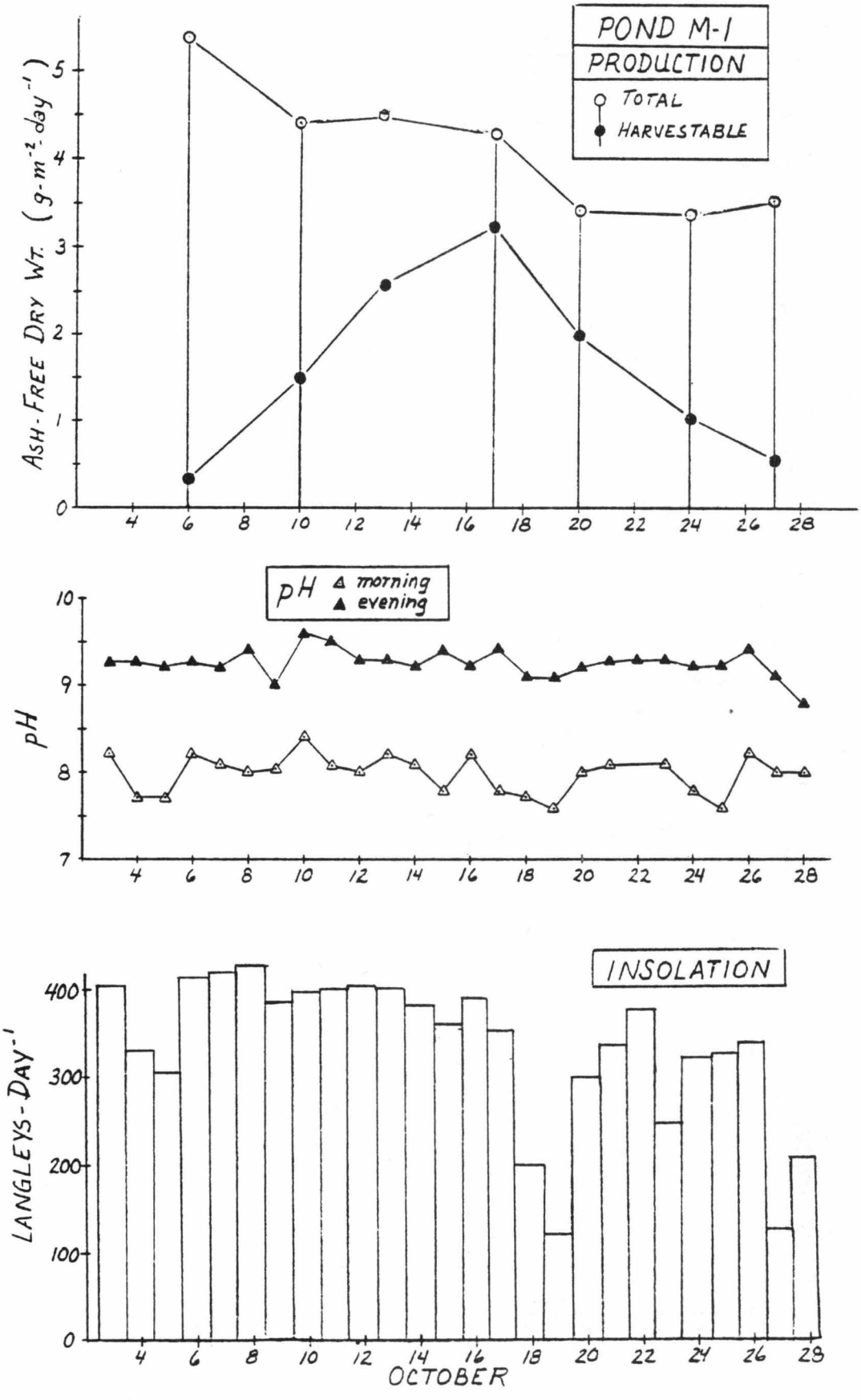


FIGURE IV-35. M-1 PRODUCTION, pH , INSOLATION: EXPERIMENT 7

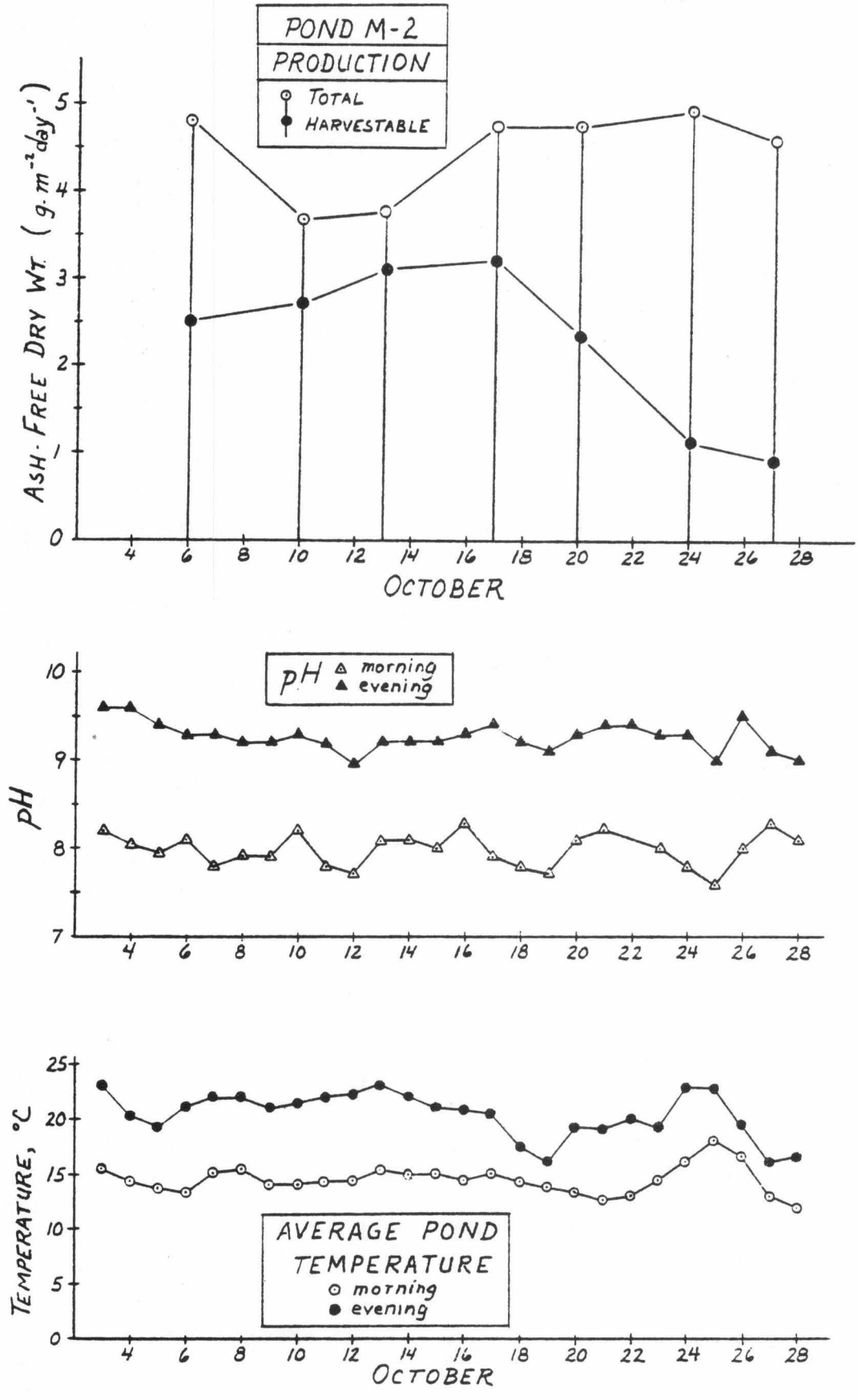


FIGURE IV-36. M-2 PRODUCTION, pH, AVERAGE POND TEMPERATURE: EXPERIMENT 7

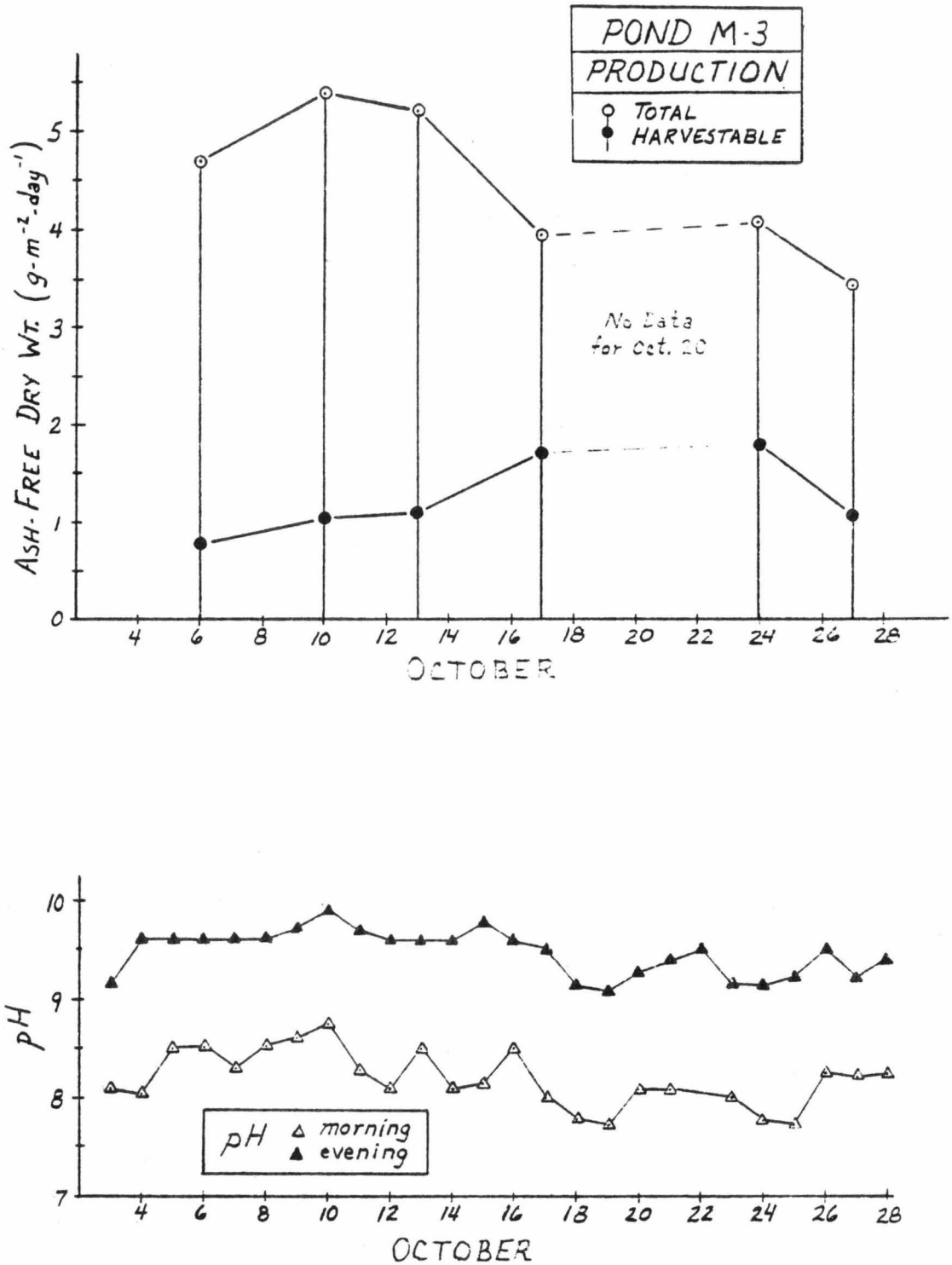


FIGURE IV-37. M-3 PRODUCTION AND pH: EXPERIMENT 7

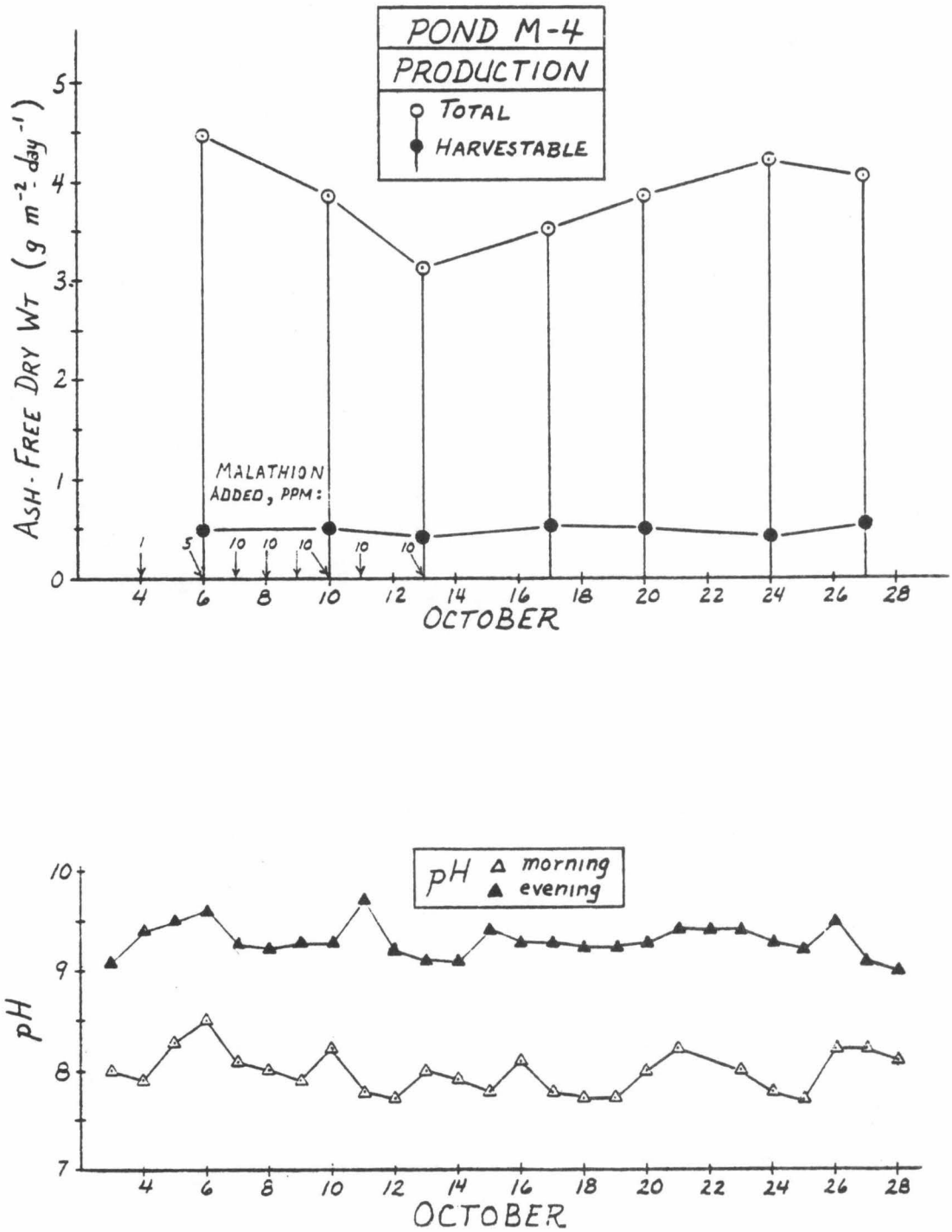


FIGURE IV-38. M-4 PRODUCTION AND pH: EXPERIMENT 7

V. SECONDARY GROWTH AND ISOLATION POND OPERATIONS

The objective of an algal ponding system is to produce the most biomass per unit area per unit time in a recoverable form while efficiently using the incoming nutrients. This is a multi-variable optimization problem which is difficult to solve at this time since the basic science and technology are not well understood or developed. The experiments described in this chapter are preliminary experiments designed to ascertain those kinetic and environmental factors which limit production of algae on a medium with given algal growth potential. The medium used was sewage in which algal yield is usually first limited by carbon, then by nitrogen and finally by phosphorus. The experiments were directed toward scavenging the first two nutrients while developing techniques for removing the biomass produced. In these preliminary experiments we had to be satisfied with measuring productivity, not optimizing it. Since nutrient scavenging is dependent on algae removal, this was our greatest concern. Accordingly, three types of secondary ponds were used. Two were batch processes. Batch growth on effluents from the primary growth ponds occurred whenever the harvestability from the first ponds was efficient. When it wasn't efficient, the process became one of batch isolation. Here the unharvested effluents were left as batch in near stationary phases, and settled at the appropriate time. Continuous second growth ponds were less successful. Nitrogen-fixing blue-green algae were cultivated on batch secondary growth pond effluents (33).

BATCH GROWTH ISOLATION PONDS

Batch ponds were run on effluents from three sources: (1) 12 m^2 high-rate ponds in which a first crop of algae was grown, (2) 12 m^2 high-rate ponds in which a second crop of algae was grown, and (3) other batch ponds. Whether a pond was classified as a growth or isolation pond was decided by the amount of growth which occurred. Normally biomass increases were less than 50% of

the initial inoculum in batch isolation ponds. The amount of growth was usually determined by the density of the initial inoculum and the concentrations of available nitrogen in the influent used. The goal, in both types of batch operations, was to remove the algae and obtain clarified effluents through algae settling.

The data collected is shown in Tables 12 & 14 Ponds were filled on day 0 from the indicated source. To avoid lag times the growth ponds were "seeded" with algae (from microstrainer concentrates of 12 m^2 pond water) if the initial density was very low. When indicated, carbon dioxide was bubbled in using diffusion stones and air pumps. These had limited capacity and so CO_2 was generally one of several limiting factors in all of the growth ponds. Ammonium-nitrogen was determined initially as a measure of nitrogen available for growth and usually as a measure of the suitability of the effluents as media for growth of nitrogen-fixing blue-green algae. These nitrogen-fixing ponds, with added CO_2, could be used to produce biomass to the phosphorus growth potential of the sewage (or other media). Since most of the batch ponds were filled with microstrained effluents from the 12 m^2 ponds, dilution with tap water from the microstrainer backwash was inevitable. The amount of dilution is indicated in Tables 12 and 14 , as is the pond identification, depth, source and whether CO_2 was added or not.

The pattern of growth was similar in all of the batch growth ponds (Figure V-5, page 162).The inocula contained algae with chlorophyll content of a few percent of the dry weight. Both dry weight and chlorophyll increased several fold, with the latter leveling off before the former. Thus, chlorophyll content decreased with time. During the summer months the amount of chlorophyll decreased during the second half of the batch cultivation, resulting in a yellowing of the culture, while dry weight changed very little. The pH usually rose to about 10 and the ammonium nitrogen levels usually fell to less than a few hundred ppb as time went on. NH_4^+-N usually dropped below 1 ppm by the

third day as batch. All of this was accompanied by clumping and autoflocculation of the algae. When the mixing was stopped, the algae usually settled efficiently within a few hours. Settling within a week or so was generally more reliable during the summer than during the fall. Grazers were not present at the beginning of the batch since the media was microstrained. Often the batch growth ponds became infested with crustaceons or rotifers. Most ponds contained only low levels of zooplankton, but some were heavily infested. These latter ponds yielded a brown colored supernatant after settling. The ammonium-nitrogen levels of the final supernatant were also generally a little higher when this occurred. Most of the ponds probably could have been settled several days sooner.

Table 13 shows yields, yield factors, removal efficiencies and production of the batch growth ponds. The maximum yields (maximum algal density minus the inoculum density) ranged between 70 and 210 mg/l. Most ponds yielded about 150 mg/l which is about 50 to 100% of the yields from the first growth continuous ponds. Thus, generally 300-500 mg/l of algae were grown on the available nitrogen in the incoming sewage. The yield factor for ammonium-nitrogen to biomass varied between 8 and 75. This wide variation can be ascribed to the different histories of inocula. Depending on the growth conditions in the first ponds, the nitrogen available for second growth entered the batch ponds extracellularly in the medium or intracellularly in the algae. The yield factor above is based solely on the use of extracellular ammonium-nitrogen. Total Kjeldahl nitrogen ranged from 2 to 10 times the concentration of ammonium in the influents. Stripping of ammonium was very efficient in all of the ponds. Settling efficiencies, on a VSS and chlorophyll basis, were also very high during the summer, but not very high during the fall (CO_2 was generally not added at this time). Since growth ponds became isolation ponds when the stationary phase was reached, it appeared that, in the fall, a longer period of isolation was necessary.

Two productivity values are listed for some of the ponds. One indicates the productivity from inoculation to stationary phase, the other from inoculation to harvest time. The former values were between 9 and 17 gm/m^2/day, the latter between 2 and 8 gm/m^2/day. The difference, of course, is a measure of the loss of productivity during the isolation stage. This stage was necessary to settle the algae.

Table 14 contains the data from the batch isolation ponds. Most of these ponds were not bubbled with CO_2 since little utilization of carbon was expected. Initial densities of these ponds were higher than in the batch growth ponds. Initial chlorophyll content was lower and generally between 1-2% of the dry weight. The initial extracellular levels of ammonium-nitrogen were very low, usually less than 1 ppm. During the isolation period the chlorophyll content decreased to .5-1%, the level of suspended solids decreased and flocculation occurred In many ponds rotifers bloomed. Thus, algae were settling out and being consumed. As with the growth ponds, heavy rotifer infestations were characterized by brown supernatents and higher ammonium-nitrogen levels. The pH dropped in the ponds that were run for longer periods of time.

As shown in Table 15 , the VSS and chlorophyll concentrations of the final effluents were always low. However, the VSS varied considerably. Chlorophyll removal efficiency was always high, whereas some of the ponds run in the fall did not settle out the suspended solids as well as the summer ponds.

Conclusions

During the summer and most of the fall effluents low in suspended solids, chlorophyll and available nitrogen were produced reliably in batch growth and isolation ponds. The reliability factor was at least 80% higher in the summer

than in the fall. Nitrogen was removed primarily through uptake by the algae. The algae were removed by settling and zooplankton. However, only with the former process could algal biomass be recovered. The low N, clarified effluents were used to support the growth of nitrogen-fixing blue-green algae (see ref. 33). Green algae never proliferated on these effluents without prior or coincident growth of nitrogen-fixers. These bioassays verified that the nitrogen growth potential of the sewage had been reached in the batch ponds.

It is not known what minimum conditions were necessary to remove the algae through settling and/or grazing. Nitrogen chlorosis accompanied the process, particularly during the summer months when second crops of algae were grown in the batch ponds. Autoflocculation and mixing-facilitated flocculation were evident. The connection between the nitrogen starvation and the flocculation is not understood. The effect of the history of the cultures is also not clear. Presumably, the condition of the algae as they left the primary growth pond affected the isolation process. That is, it may be possible to operate these ponds in ways which promote settling in the isolation ponds. In the same way, it is not known whether settling during the isolation phase of batch growth ponds was caused by the same factors as settling in purely isolation ponds.

Total yield of algal biomass from primary continuous growth ponds plus secondary batch growth ponds was between 300-500 mg/l. The yield appeared to be somewhat lower when the second pond was a batch isolation pond, perhaps because the cells were not so nitrogen-starved. The maximum production of algal biomass per square meter per day in the batch growth ponds was less than the production in the primary continuous ponds. Of course productivity from the continuous ponds depends on detention times as well as environmental conditions. Total production at θ=3 days, during the summer was about 20 gm/m^2/day in these ponds. Maximum algae

production, during the summer, from batch ponds occurred after about three days and was approximately 15 gm/m^2/day. The continuous pond may possibly have produced more algae at a shorter detention time. Both types would have been more productive with CO_2 additions, but higher pH of the batch ponds indicated more severe CO_2 limitation. During the fall, when detention times of the continuous ponds were increased to eight days, ponds run as batch for 7-9 days produced very nearly as well as the continuous ponds. Maximal continuous productivity could have been attained at shorter detention times. It is important to realize that the production of recoverable biomass from batch runs was only 25% of the maximal productivities because settling was inefficient at the beginning of the stationary phase.

CONTINUOUS SECONDARY GROWTH PONDS

Results

During August and September two ponds were diluted with microstrained effluents from primary growth ponds. On 3 August, M4 was terminated as a primary growth pond and filled with M3 microstrained effluent until 9 August. Continuous operation was begun on 10 August at a four day detention time. The pond and effluent densities for the two months of operation are shown in Figure V-1 along with the density of the microstrained primary effluent used as the feed. Table 16 lists the ammonium-nitrogen levels of the feed and the pond.

The pond density fell immediately. Algae settled out and were consumed by rotifers. Since microstraining very efficiently removed rotifers from the feed, they must have increased in number as the pond was being filled. On 12 August, CO_2 sparging was begun. Prior to this the pH was between 9 and 10.5 (Figure V-4). The algal density recovered, reaching a high of over 200 mg/l on 19 August. During this time the influent densities were about 75 mg/l. Thus, a maximum productivity of about 10 gm/m^2/day was achieved (influent densities subtracted out)along with a harvestability of 90%.

An herbivore screen (150 μ) was used for at least 2 hours per day until September when this was increased to 4 hours a day and all night. The pond always contained grazers, most of which were rotifers. On 24 August, the number of grazers increased greatly. Small, flagellated green algae bloomed on 22 August through 24 August. At the same time harvestability, especially chlorophyll harvestability, decreased. In an attempt to control this flagellate bloom, on 24 August the detention time was decreased to 3 days and biomass recycle (50% nominal) was initiated. The recycled (microstrained) concentrate was first put through the herbivore screen to remove grazers. The number of rotifers increased further, and the pond density fell.

During August the influent (microstrained primary pond effluent) density was low so that M4 was actually a secondary growth unit. The nitrogen algal growth potential of the influent (based on influent ammonium-nitrogen and a yield factor of 10-15) was about 100 mg/l. Sometimes this potential was attained, even with a substantial rotifer population. The chlorophyll content of the algae ranged between 1-2% while, at the same time, the chlorophyll content of the algae in the primary growth pond (θ=4.6 days) was greater than 3%. The ammonium-nitrogen level in the secondary pond averaged below 500 ppb.

Another secondary pond (M2) was filled with M1 microstrained effluent from 20 August to 23 August. Operation was begun at θ=6 days on 26 August (Figures V-2 , V-4). This pond was also sparged with CO_2 and screened to remove grazers. Starting on 24 August both M2 and M4 received half of their influent from each of the primary ponds after microstraining.

There were few rotifers in M2 on 24 August but many by 27 August. The pond density fell and did not recover until the influent density became high due to poor harvestability of one of the primary ponds. This primary pond (M3) harvested poorly throughout September, while the other (M1) produced low density effluents (after microstraining) until 19 September. Starting

on 16 September, M2 was diluted with M1 effluent only and M4 was diluted with M3 effluents which were allowed to settle overnight to lower the algae content. These, as well as other operational changes, were of little consequence as the pond densities crashed at the end of the month. Rotifers were observed in large numbers in both ponds at this time. In M4, from 18 September to 25 September, these grazers consumed all of the algae contained in the influent, producing clear secondary effluents.

During September when the influent densities were high, the net productivities were negative, about 1 to 2 $gm/m^2/day$. As before, the chlorophyll content of the algae in the secondary ponds was between 1 and 2%, while it was 3% in the primary ponds. Secondary ponds appeared yellowish compared to the green primary ponds. The nitrogen growth potential was not fulfilled in September, although NH_4^+ - N removal was extremely good (>95%) as secondary pond NH_4^+ $\pm$ N levels were usually below 200 ppb.

Micractinium was the dominant algal type (comprising 70-90% of the biovolume) in both of the continuous secondary ponds. Scenedesmus was found in much smaller proportions, less than 10%. Small, flagellated algae bloomed at times (see above), but for the most part, these comprised 10% or less of the total biovolume.

Conclusions

In operating the secondary growth ponds we encountered several problems. With the nitrogen growth potential of the feed only 100 mg/l, nitrogen limited the rate at which algae could be grown and produced. Visual observations of pond color, low levels of NH_4^+ - N, and the low chlorophyll a content of the algae all indicated that nitrogen was growth limiting. High algal densities in the pond influents would be expected to make nitrogen limitation less severe, especially in comparison to light limitation, because the incoming algae presumably would bring some nitrogen into the pond intracellularly

while the resulting increased pond densities would make light more limiting. The poor response of pond densities (pond density was not sufficiently higher than feed density to achieve positive productivity) to this increase in feed density may be an indication that nitrogen and light limited growth synergistically. This is reasonable since scavenging and utilizing N is a more energy consuming process at low N levels, while the harvesting of light is highly dependent on protein-rich structures. Significant amounts of algae settled out in the secondary ponds. Indeed, when influents became high in algal content, the secondary ponds became isolation ponds which were difficult to manage. Substantial harvestability from the primary ponds was thus a prerequisite for operating continuous secondary ponds as growth units under these severely limiting conditions.

Net algal production was further reduced by grazing which was substantial and uncontrollable in these experiments. However, the data obtained from M4 at the end of September leaves room for optimism regarding algae removal in secondary grazing ponds. This possibility, which is dependent on grazer control in the primary ponds, will be tested in future experiments.

Increased carbonation, nitrogenous additions, and grazer control are all necessary to increase the effectiveness of continuous secondary ponds for algal production. It is evident, however, that if low nitrogen effluents are to be obtained, the algae must spend some amount of time in the medium when the N content is low. Although the NH_4^+ - N removals were high in the continuous secondary ponds, the batch secondary ponds achieved higher removals while producing more algae and clearer effluents. Batch cultivation is especially effective compared to completely mixed continuous cultivation when the feed levels of nitrogen are as low as they were in this experiment. The batches can grow faster reaching higher densities (and thus light limitation) because the algae can continue to grow using intracellular sources of N. In continuous cultivation the algae are continuously growing in low N media.

At this point, it is not possible to decide on a ponding system that best utilizes incident solar energy while reliably producing effluents low in solids and available nitrogen. Much depends on how low the NH_4^+-N levels must be for growth of nitrogen-fixing blue-green algae. For very low NH_4^+-N levels sufficient reduction cannot be attained in one pond only, even at very long detention times (or substantial effluent recycling). The low levels of N in such ponds would decrease productivity substantially and might well support only unhealthy, starved cultures. Thus, some kind of secondary pond, for N removal and buffering of the system, is necessary. Batch cultivation seems suited to this task, especially if low density effluents can be obtained from primary ponds so that these batches are for growth as well as isolation. Algae removal is of course the central problem, with microstraining or isolation (flocculation and settling) being the cheapest techniques.

The removal of algae by grazers may find applications in waste treatment through aquaculture, however, it is of little interest to this biomass project.

The development of cultivation techniques for algae growth to the N growth potential will require better control over pH and nutrient levels. Whether batch or continuous cultures may prove best in the long range is not yet established. Current knowledge of the regulation of nitrogen fixation in blue-green algae (33) suggests that significant levels of ammonia and fixed N may be present in tertiary nitrogen-fixing pond influents. Therefore, a tight control over ammonia concentrations in the effluents of secondary ponds is not necessary in development of integrated biomass production systems based on advanced waste treatment concepts. Thus the optimization of productivity and algae removal should be possible. In conclusion, Figure V-6 shows a conceptual representation of a multi-stage algae biomass-waste treatment system being proposed on the basis of these experiments as well as the cultivation of nitrogen-fixing filamentous blue-green algae described elsewhere (33).

TABLE 12

BATCH GROWTH PONDS

1. C-2; d=10"; filled with M-2 (θ=3.3 days) 1° effluent + 8 M-2 microstrainer concentrate, 10% tap water dilution; CO_2

Day	Date	pH	VSS, mg/ℓ	Chlor. *a*, mg/ℓ	NH_3-N, mg/ℓ
0	7/26	--	40	.55	17
1	7/27	7.9 9.2	--	--	9
3	7/29	10.2 10.8	136	2.01	--
6	8/1	9.6 9.2	186/5.3*	1.18/.03	.04

2. C-2; d=7"; filled with M-3 (θ=4.5 days) 1° effluent + 12 M-3 microstrainer concentrate, 15% tap water dilution; CO_2

0	8/3	8.2 9.0	40	.41	9
1	8/4	8.1 10.3	--	--	.8
2	8/5	9.2 9.0	101	.89	--
5	8/8	8.0 8.7	102	--	.1
7	8/10	8.1 --	109/3.8	.80/.01	--

* Numbers after slashes refer to supernatent after settling.

TABLE 12 (cont.)

BATCH GROWTH PONDS

3. C-3; d=7"; filled with M-3 1° effluent (θ=4.6 days) + 16 M-2 microstrainer concentrate, 15% tap water dilution; CO_2

Day	Date	pH	VSS, mg/ℓ	Chlor. a, mg/ℓ	NH_3-N, mg/ℓ
0	8/7	8.2 8.6	20.3	.15	10
1	8/8	8.1 9.2	27.6	.63	5
3	8/10	9.8 10.8	208	1.32	--
4	4/11	10.1 11.0	--	--	.6
5	8/12	10.0	190	.61	--
8	8/15	10.2 7.8	-/4.4	-/.004	.15

4. C-2; d=8"; filled with M-1 1° effluent (θ=5.2 days); 30% tap water dilution; CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ
0	8/10	--	--	42	.71	4.4
1	8/11	15/26	8.8/10.6			
2	8/12	16/26	9.6/11.0	126	1.47	
3	8/13	15/27	10.0/10.9			
4	8/14	15/27.5	9.9/10.9			
5	8/15	15/28	10.3/10.9	212	1.39	
6	8/16	15/26	9.6/10.8			
7	8/17	15/24	9.8/10.5	169	.84	
8	8/18	16/28	8.9/9.5			.6
9	8/19	15/26	8.4/9.5	174/32	.60/.12	

TABLE 12 (cont.)

BATCH GROWTH PONDS

5. C-3; d=10"; filled with M-2 1° effluent (θ=3.3 days); 10% tap water dilution; CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3 mg/ℓ	Comments
0	8/15	--/25	--/9.2	38	.45		
1	8/16	15/24	8.5/10.0				
2	8/17	15/23	9.65/11.0	134	2.62		
3	8/18	16/27	10.1/11.0			.1	Added another CO_2 line
4	8/19	15.5/25	9.8/11.0	250	2.78		Harvestability tested poor
5	8/20	15/23	9.0/10.4				
6	8/21	15/25	8.4/9.1				Rotifers present
7	8/22	13/27	7.3/7.5				Pond brown
8	8/23	14/26	7.6/--			1.5	Final supernatent brown; rotifers

6. C-3; d=7"; filled with M-3 1° effluent(θ=9 days) + M-4 2° effluent - 4:3; 15% tap water dilution; no CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/26	--/24.5	--/8.9	25.6	.52	3.4	
1	9/27	17.5/24.5	8.6/10.4				
2	9/28	18.5/21.5	9.7/10.4				
3	9/29	16.7/--	10.0/10.6			.04	
4	9/30	13/24.5	9.9/10.3				
5	10/1	16/--	9.8/--				
6	10/2	15/26.5	9.7/9.9	--/13.7	--/.04	.3	

TABLE 12 (cont.)

BATCH GROWTH PONDS

7. C-3; d=4"; filled with M-2 1° effluent (θ=8 days); 10% tap water dilution; no CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/3	25.0	10.45	144 .	4.10	3	
1	10/4	13.5/24	9.6/10.8				
2	10/5	14/22.5	9.7/11.0				
3	10/6	12/25	10.2/10.6	234	5.45	.14	
4	10/7	12/26	9.7/10.7				
5	10/8	12/25	9.6/10.8				
6	10/9	12/24	9.7/10.8				
7	10/10	10/--	10/--	289/198	3.62/2.13	.06	Supernatant to #23

8. C-4; d=4"; filled with M-2 1° effluent (θ=8 days); 10% tap water dilution; no CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/6	--/23.0	--/10.0	64	2.04	6.5	
1	10/7	11/24	9.5/10.8				
2	10/8	11/24	10.3/10.9				
3	10/9	12/24	10.3/10.8				
4	10/10	10/--	10.6/--	194/149	3.30/1.76	.05	Supernatant to #23

TABLE 12 (cont.)

BATCH GROWTH PONDS

9. A-1; d=6"; filled with M-2 1° effluent (θ=8 days); 10% tap water dilution; No CO_2

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/8	--	--	~50	~1.5	6.5	
1	10/9	12/20	9/10.4				
2	10/10	12/21	10.1/11.0	83/68*	2.1/1.24*	.06	Test settling
3	10/11	12/--	10.1/10.8				
4	10/12	12/22	10.0/10.8				
5	10/13	12/21	10.4/10.8	168/--	2.12/--	.2	Test settled poorly
6	10/14	12/20	10.3/10.8				
7	10/15	13/18	10.0/10.8				
8	10/16	12/20	10.1/10.5				
9	10/17	12/--	9.5/--	198/--	1.2/--	.03	

10. C-4; d=6"; tap water dilution test: filled to 1½" with M-1 effluent; filled to 4" with M-2 effluent; filled to 6" with M-2 effluent; 10/11; No CO_2; 10% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/10	--	--	47	1.5	6.7	
1	10/11	10/24	9.3/10.5				
2	10/12	12/24	9.9/10.8				
3	10/13	12/24	10.5/10.9	145/112	2.78/1.73	.04	Test settled 3½ hrs.
4	10/14	12/22	10.5/10.9				
5	10/15	13/22	10.3/10.8				
6	10/16	12/22	10.5/10.9				
7	10/17	12/--	10.1/--	187/112	2.30/1.17	.03	Settled 5 hrs., supernatant to #24 Combined with C-3 in C-4

TABLE 12 (cont.)

BATCH GROWTH PONDS

11. C-3; d=6" tap water dilution test: filled to 2" with M-1 effluent, filled to 4" with H_2O + 7 mg NH_4Cl; filled to 6" with M-2 effluent on 10/11; no CO_2; 50% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/10	--	--	40	1.4	7.0	
1	10/11	10/24.5	9.3/10.3				
2	10/12	12/24	9.8/10.7				
3	10/13	12/24	10.4/10.8	120/92	2.54/1.69	.04	Test settled 3½ hrs
4	10/14	12/23	10.5/10.8				
5	10/15	13/23	10.2/10.7				
6	10/16	13/23	10.4/10.8				
7	10/17	12/--	9.8/--	121/76	1.36/.73	.03	Settled 5 hrs; supernatant to #24

TABLE 13

YIELDS, YIELD FACTORS, REMOVALS, AND PRODUCTION FROM BATCH GROWTH PONDS

BATCH	ΔVSS Max mg/ℓ	ΔVSS Max/ΔNH_3-N	NH_3-N Removal %	VSS Removal %	Chlor. a Removal %	Production gm/m^2/day at Onset of Stationary Phase/at Harvest Time
1	146	8.6	99.8	97.3	97.4	7.8/7.8
2	70	7.6	>99	96.3	98.8	2.3/2.3
3	190	18	98.5	97.9	100	14.2/4.8
4	170	45	86	80	75	8.7/3.8
5	210	15	90	--	--	17/--
6	--	--	90	--	--	--
7	145	60	80	31	41	2.7/2.7
8	130	21	>99	23	47	4.3/4.3
9	150	23	>99	<40	<60	--/4.2
10	150	22	>99	40	50	6.7/3.9
11	80	11	>99	37	46	15.4/6.6

TABLE 14

BATCH ISOLATION PONDS

12. A-1; d=10"; diluted at θ=3 days with M-4 effluent from 7/29-8/1; CO_2; 10% tap water dilution

Day	Date	pH	VSS, mg/ℓ	Chlor. *a*, mg/ℓ	NH_3-N, mg/ℓ	Comments
0	7/29	8.5 9.4	96	1.73	--	
3	8/1	9.2 10.1	110/64	2.17/1.13	.5	
5	8/3	9.8 10.6	139	2.06	--	
6	8/4	10.0 10.7	--	--	--	
7	8/5	9.8 10.0	129/--	1.14/--	--	Rotifers present

13. A-2; d=10"; diluted at θ=6 days with M-4 effluent from 7/29-8/1; CO_2; 10% tap water dilution

Day	Date	pH	VSS, mg/ℓ	Chlor. *a*, mg/ℓ	NH_3-N, mg/ℓ	Comments
0	7/29	8.3 9.6	118	2.38	--	
3	8/1	9.5 10.3	146/59	2.85/1.01	.5	
5	5/3	9.8 10.6	173	2.48	--	
6	8/4	9.9 10.7	--	--	.08	
7	8/5	9.9 --	198/22.6	.92/.04	--	Rotifers present

TABLE 14 (cont.)

BATCH ISOLATION PONDS

14. C-3; d=7½"; filled with M-4 2° effluent; no CO_2; 15% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/4	--	--	95	1.8	.09	
1	9/5	16/27.5	9.7/10.3				
2	9/6	16.5/25.5	10.1/10.35				
3	9/7	13/--	100/--	142/--	1.0/--	.04	

15. C-1; d=7"; filled with M-2 2° effluent, no CO_2; 15% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/ 7	--/--	--/10.0	53.9	.95	.07	
1	9/8	13/26	9.7/10.3				
2	9/9	12.5/--	--/--				
3	9/10	--/--	--/10.2				
4	9/11	13/--	10.0/--	17.6/--	.09/--	.08	Mostly settled; remainder settled in 30 min.

TABLE 14 (cont.)

BATCH ISOLATION PONDS

16. C-1; d=7"; filled with M-2 2° effluent, no CO_2; 15% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/1	--/24.5	--/10.5	83	.96	.06	
1	9/20	16.5/23.5	9.9/10.3				
2	9/21	14.5/--	9.8/--	22.4	.07	.04	

17. C-3; d=8"; filled with M-2 + M-4 2° effluent; no CO_2; 25% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/15	--/18	--/9.4	82	1.12		
1	9/16	12.5/16	9.2/9.8				
2	9/17	14.5/21	9.5/10.0				
3	9/18	15.5/25	9.9/10.2				
4	9/19	17/25.5	9.85/10.4	113/28	.49/.1	.04	settled 3 hrs; pumped supernatent back into pond
6	9/21	16/--	9.3/--	--/.12	--/.03	.3	

TABLE 14 (cont.)

BATCH ISOLATION PONDS

18. C-3; d=8"; filled with M-2 + M-4 2° effluent; no CO_2; 25% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/8	--	--				
1	9/9	14/--	--/--	80	.84		
2	9/10	--/--	9.9/10.2				
3	9/11	13/22	9.9/10.2				
4	9/12	13/--	10.0/--	118/--	.74/.14	.04	Settled well in 2 hrs

19. C-1; d=8½"; filled with M-2 + M-4 2° effluent; no CO_2; no mixing; 25% tap water dilution

Day	Date	T	pH	VSS mg/	Chlor. a mg/	NH_3-N mg/	Comments
0	9/4	--/23	--/9.7	103	1.3	.09	Overnight settling, no mixing
1	9/5			20.1	.16	.05	

TABLE 14 (cont.)

BATCH ISOLATION PONDS

20. C-3; d=8½"; filled with M-2 + M-4 2° effluent; no CO_2; no mixing; 25% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/21	--/240	--/9.5	61	.68	.06	no mixing
1	9/20	18.5/26	9.8/9.6				no mixing
2	9/23	15/--	9.4--	14	.05	--	no mixing

21. C-3; d=7"; filled with M-2 + M-4 2° effluent, no CO_2; no mixing; 25% tap water dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	9/23	--	--	38.5	.39	--	no mixing
1	9/24	--	8.9/9.2				no mixing
2	9/25						no mixing
3	9/26	15/--	8.8/--	12.6	.04	.03	no mixing

TABLE 14 (cont.)

BATCH ISOLATION PONDS

22. A-2; d=8"; filled with #7 + #8 supernatent; no CO_2; no dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/10	--/26	--/10.9	175	1.95	.06	
1	10/11	12/--	9.7/104				
2	10/12	12/20	9.3/10				
3	10/13	13/19	9.1/9.5	174	1.45	.1	settling test: poor
4	10/14	12/19	8.0/8.5				rotifers present; brownish color
5	10/15	13/19	7.5/7.6				rotifers present; brownish color
6	10/16	13/18	7.8/8.0				rotifers present; brownish color
7	10/17	12/--	7.6/--	103/8	0.43/0.01		rotifers present; final supernatent brown

23. C-4; d=8"; filled with #10 + #11 supernatent; no CO_2; no dilution

Day	Date	T	pH	VSS mg/ℓ	Chlor. a mg/ℓ	NH_3-N mg/ℓ	Comments
0	10/17	--/22	--/10.8	94	.95	.03	
1	10/18	--/--	9.9/10.5				
2	10/19	12/14	9.6/10.1				
3	10/20			117/49	.66/.36	.04	test settled 3 hrs.
4	10/21						
5	10/22						
6	10/23						
7	10/24			--/3.7	--/.006		rotifers present; settled in 45 min.

TABLE 15

YIELD AND REMOVALS FROM BATCH ISOLATION PONDS

Batch	ΔVSS Max.	VSS Removal %	Chlor. a Removal %	VSS of Final Effluent mg/ℓ	Chlor.a of Final Effluent mg/ℓ
12	44	--	--	--	--
13	80	90	>99	23	.04
14	45	--	--	--	--
15	--	--	--	--	--
16	-60	--	--	--	--
17	30	90	99	11.5	.03
18	40	--	81	--	.14
19	--	80	90	20	.16
20	--	75	90	14	.05
21	--	67	90	13	.04
22	-70	90	95	8	.014
23	23	--	--	4	.006

TABLE 16

CONTINUOUS SECONDARY GROWTH PONDS - INFLUENT AND EFFLUENT NH_4^+-N CONCENTRATION, mg/ℓ

Date	M2		M4	
	Influent	Effluent	Influent	Effluent
8/4	--	--	10.1	--
8/8	--	--	12.3	--
8/11	--	--	6.8	0.7
8/18	--	--	11.4	0.3
8/22	7.4	--	7.3	0.15
8/25	2.6	1.0	2.6	0.08
8/30	6.6	--	6.6	0.1
8/31	5.9	0.07	5.9	0.8
9/5	7.1	0.08	7.1	0.1
9/7	9.5	0.1	9.5	0.02
9/12	5.6	0.2	5.6	0.1
9/14	6.3	0.15	6.3	0.1
9/19	5.5	0.1	5.5	0.04
9/21	2.9	0.04	2.9	0.15
9/27	7.0	0.05	7.0	0.3

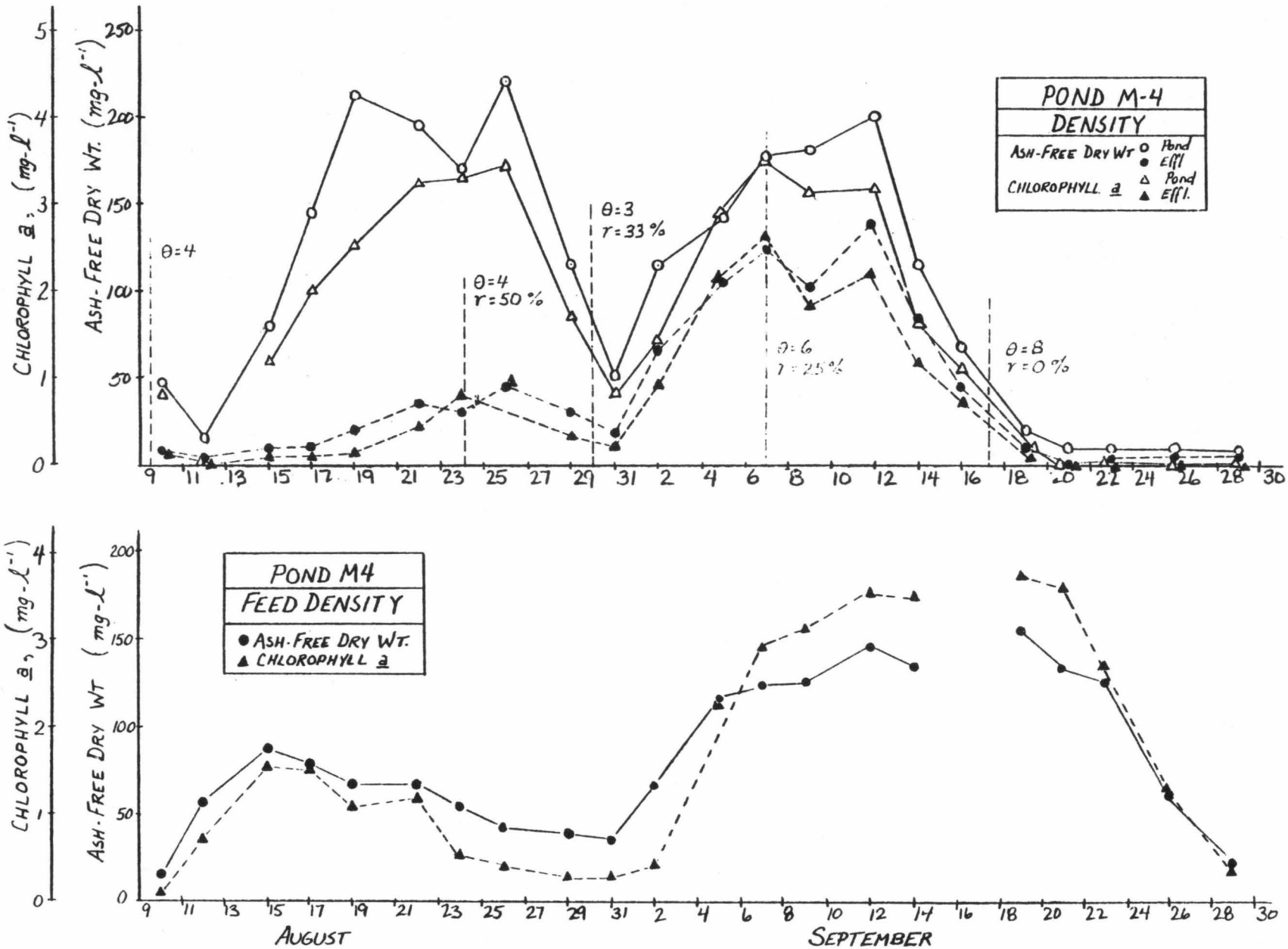


FIGURE V-1. CONTINUOUS SECOND GROWTH PONDS:M-4 POND AND MICROSTRAINER EFFLUENT AND FEED DENSITY

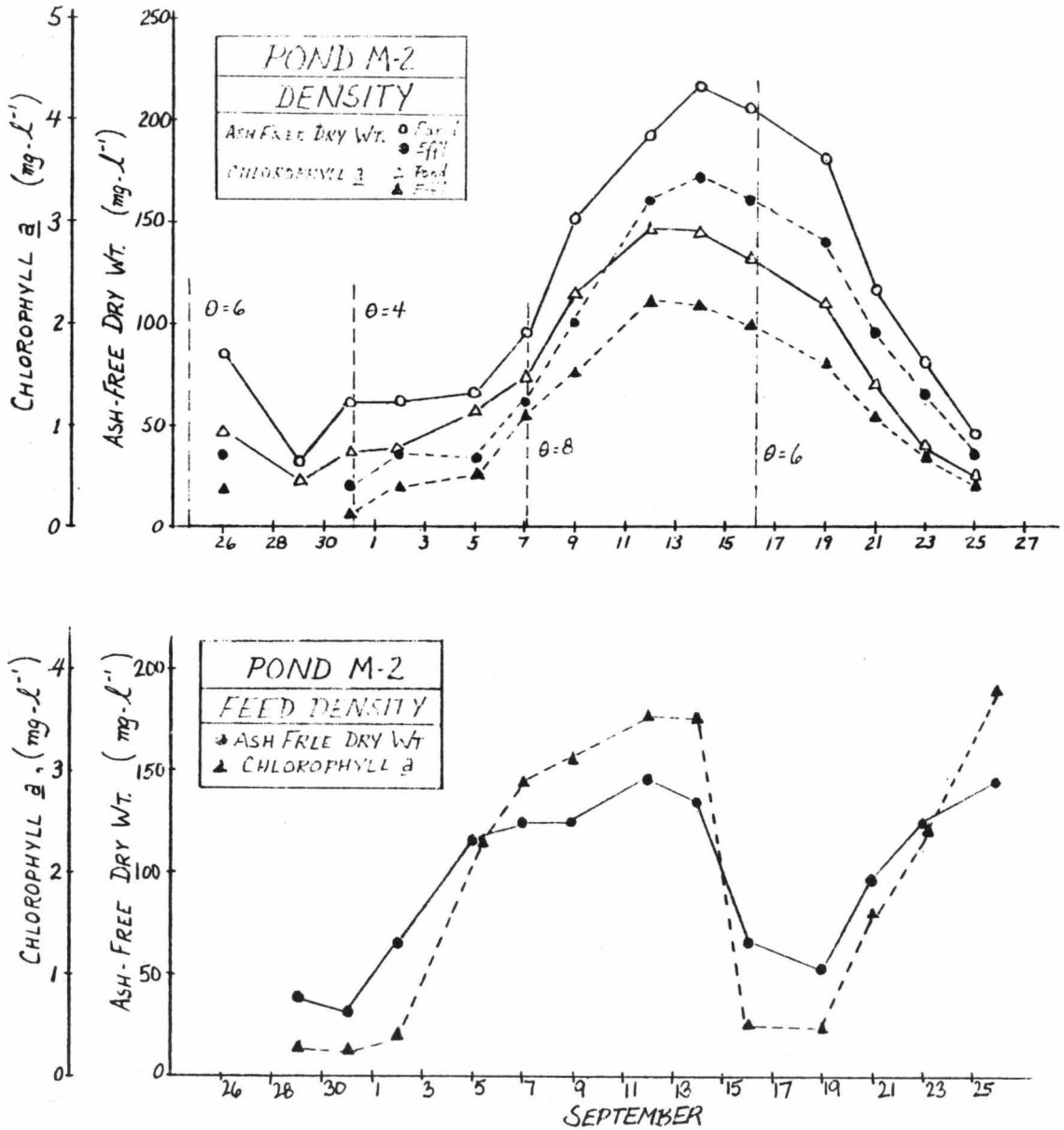


FIGURE V-2. CONTINUOUS SECONDARY GROWTH PONDS : M-2 POND AND MICROSTRAINER EFFLUENT AND FEED DENSITY

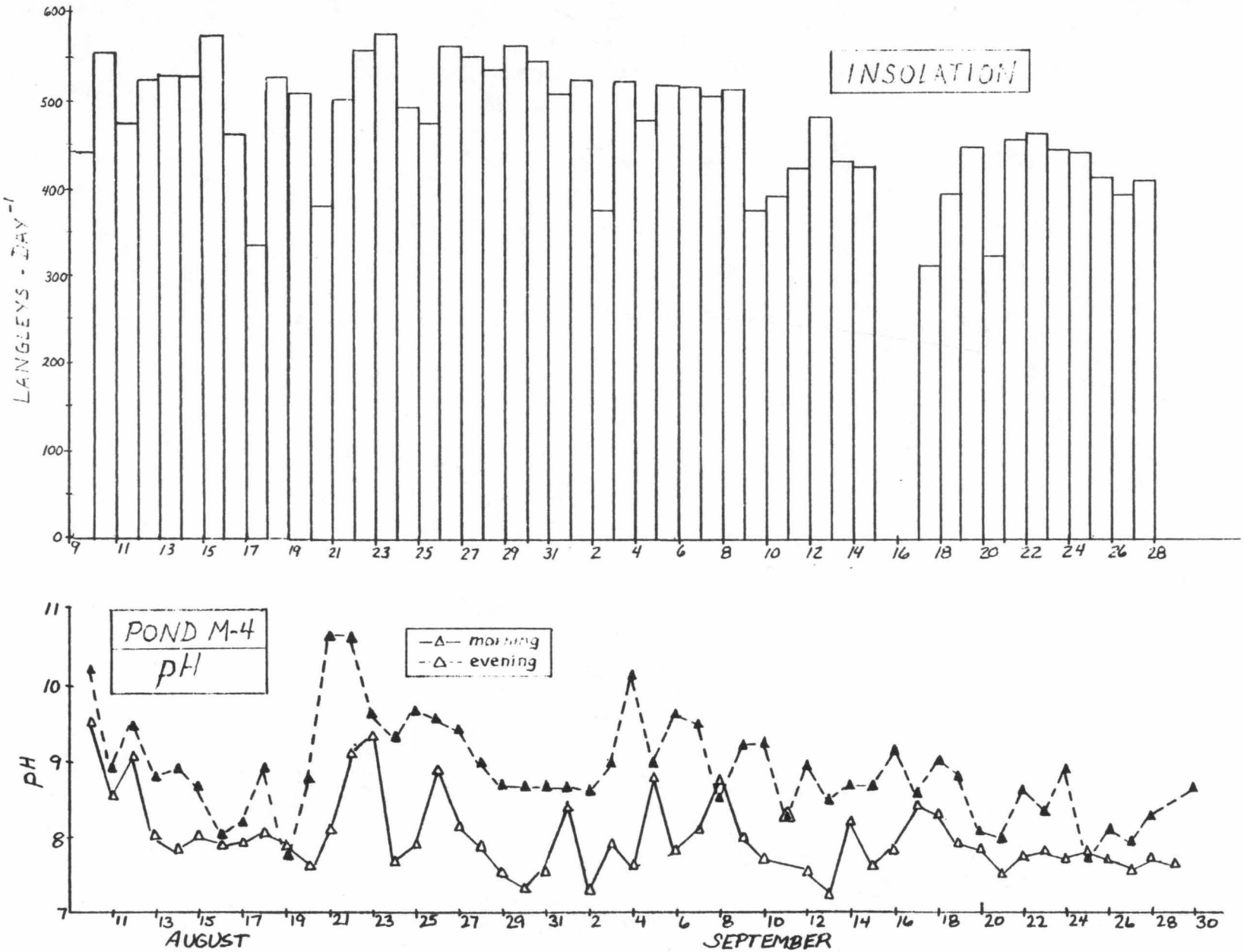
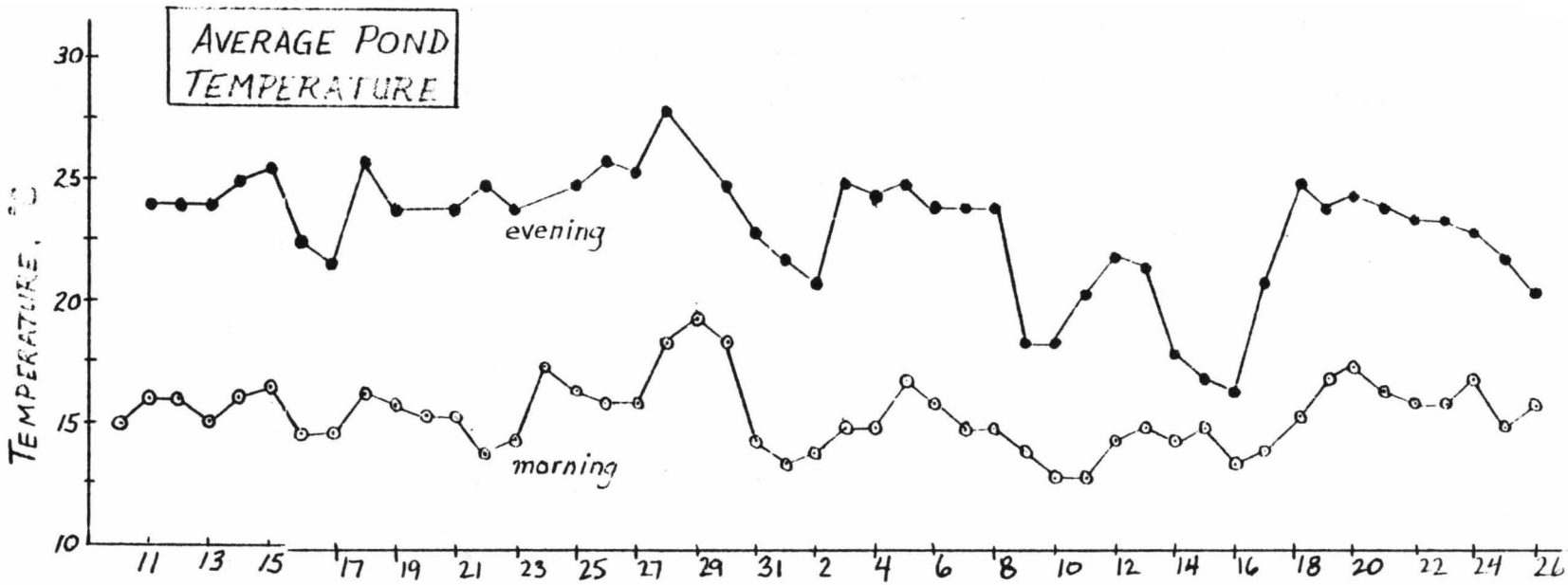


FIGURE V-3. CONTINUOUS SECOND GROWTH PONDS: INSOLATION, M-4 pH



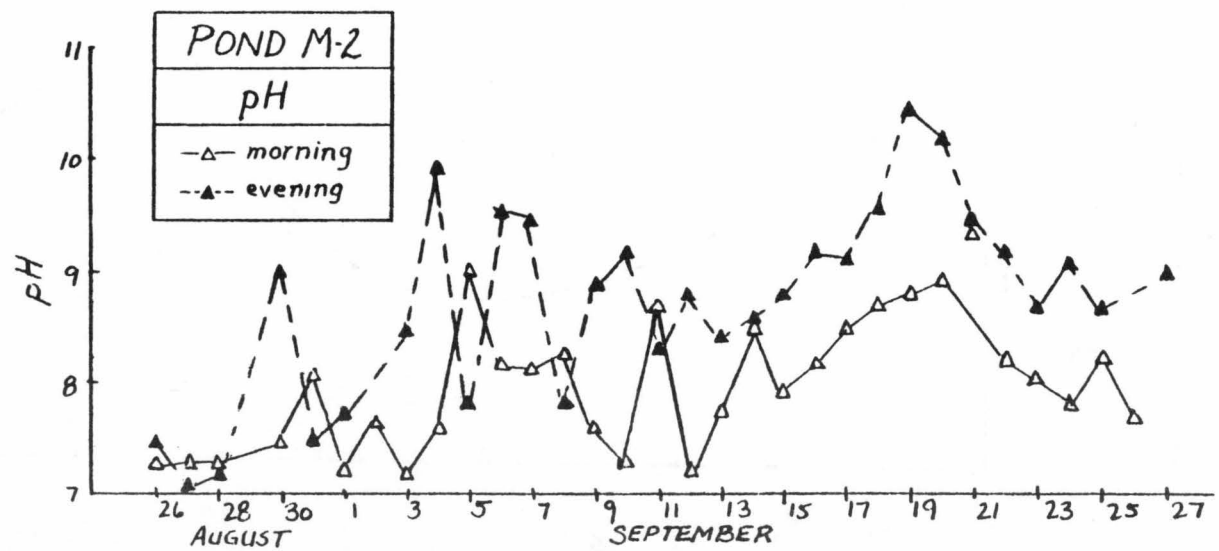


FIGURE V-4. CONTINUOUS SECOND GROWTH PONDS: AVERAGE POND TEMPERATURE, M-2 pH

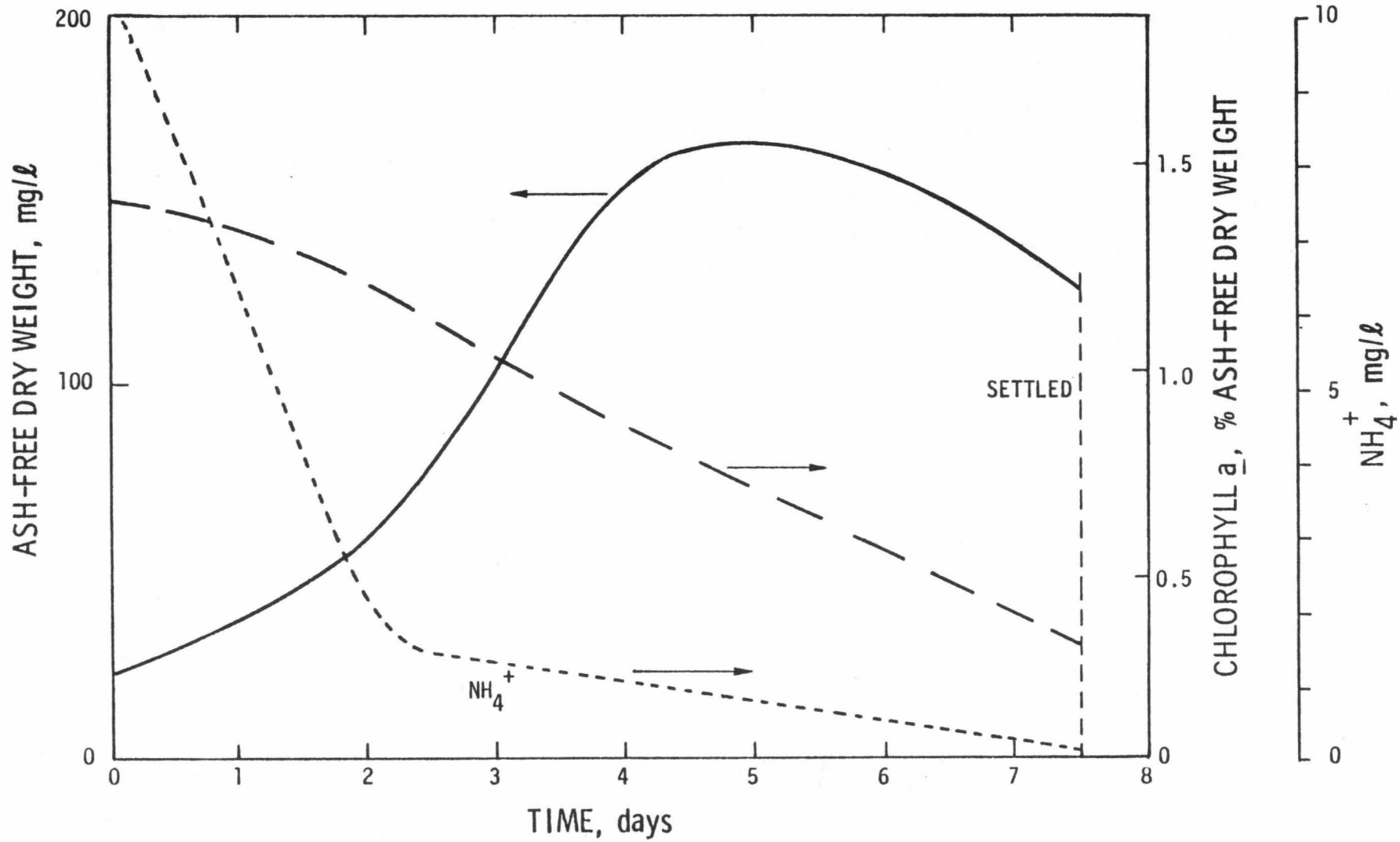


FIGURE V-5. SKETCH OF TYPICAL CHANGES IN DENSITY, CHLOROPHYLL a CONTENT AND NH_4^+-N CONCENTRATION DURING BATCH GROWTH IN SECOND-STAGE PONDS

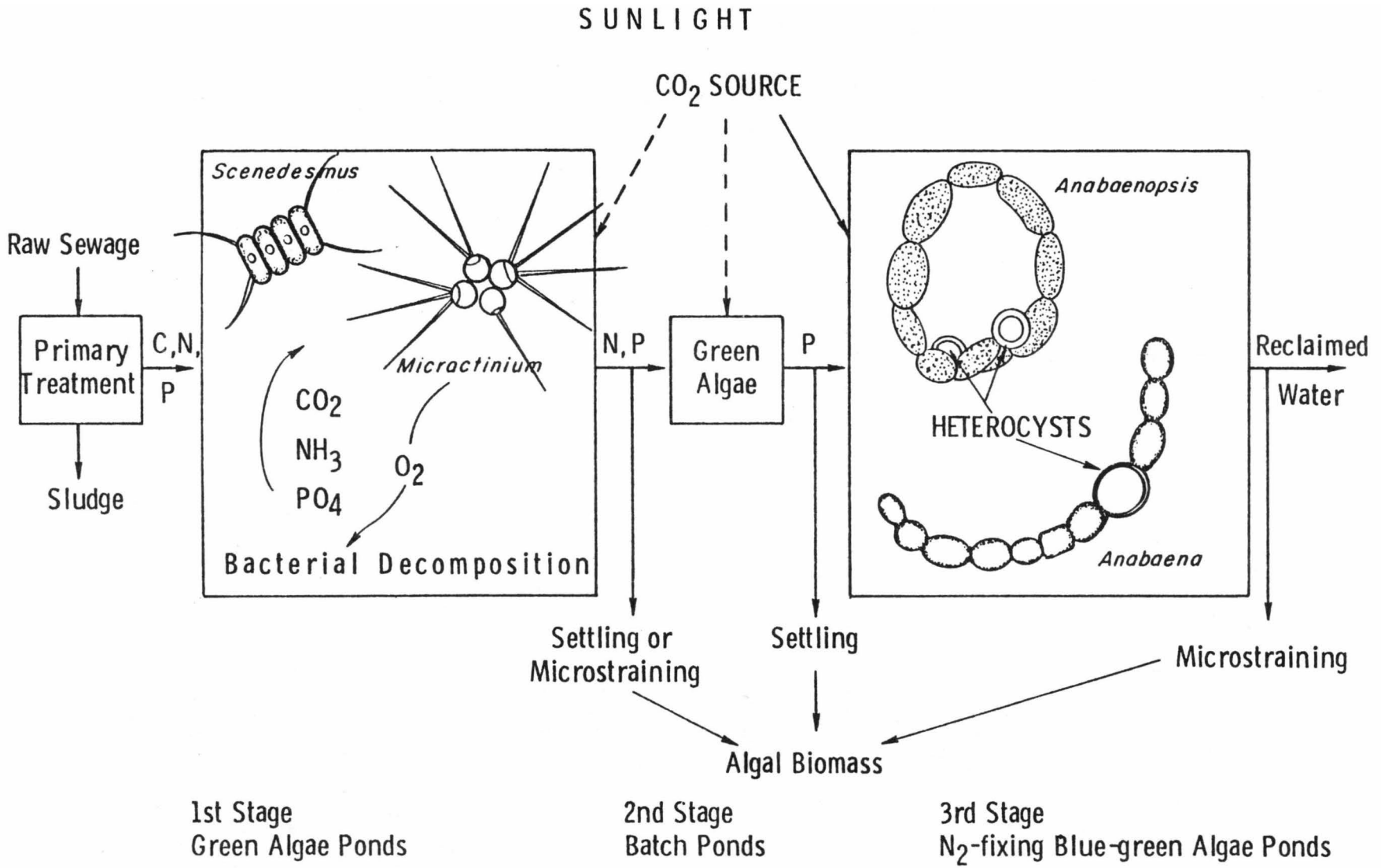


FIGURE V-6. PROPOSED ADVANCED WASTEWATER TREATMENT SYSTEM USING A MULTI-STAGE PONDING SYSTEM
Relative sizes of ponds are not accurately represented.

VI. HIGH-RATE POND

INTRODUCTION

The objectives of the operation of the 0.25 hectare high-rate pond were: (1) to demonstrate that a large-scale algal growth pond can be operated in such a manner as to be consistantly harvestable by microstraining, and (2) to demonstrate that the results obtained in 12 m^2 ponds can be reproduced in larger ponds if the operational parameters (i.e mixing, loading, detention time, depth) are the same.

POND OPERATION AND RESULTS

The 0.25 ha high-rate pond was operated throughout the winter while work continued on the sewage supply and flow metering systems. The pond and effluent densities observed during February and March are shown in Figure VI-1 During most of this period the detention time averaged 20 days. Loading was increased in March bringing the detention time down to about 10 days. Mixing was minimal (< 3 cm/sec). It was provided by a small recirculation pump operating continuously. This was supplemented by an occasional 1-hour fast mix (about once a week).

The algae was partially microstrainer-harvestable during February. Pond density was quite low in February and increased in March with a concurrent loss of harvestability. The changes observed in March appeared to be related to the increase in loading. It was evident that the pond was nutrient-limited, however loading could not be increased due to the small capacity of the sewage supply system.

In April the pond was drained, weeded, and cleaned. The mixing pumps were rebuilt (insufficient funds were available for installation of paddle wheels which could duplicate the mixing system in the 12 m^2 ponds). The flumes and platforms for the mixing pumps were also rebuilt. The sewage supply system was modified to provide a larger and more consistent flow of settled,

screened sewage. Sewage flow was calibrated by wier box depth, and work was started on a more accurate flow metering system which was not operational until the beginning of July.

The pond was refilled slowly over a period of about 14 days beginning on May 15. During this time, it was inoculated (without mixing) daily with all of the harvestable algae (predominantly Scenedesmus) produced in the 4 12 m^2 ponds. The pond turned pale green after 3 days and deep green at about the sixth day of inoculation. The initial algal population consisted predominantly of small Scenedesmus which were not harvestable.

By 29 May the pond had reached its operating depth of 10" and a regular operational schedule was established. The sewage loading during the first month was 88,500 liters/day (estimated by periodic measurements of wier-box depth). This loading corresponds to a 7-day detention time. Sewage was added to the pond over a 13-hour period, from 0700 to 2000. A 7-day detention time is longer than optimal for algal growth during the summer months, but the results of small experiments had indicated a strong positive correlation of detention time with microstrainer harvestability. It was felt that the long detention time would assure that the Scenedesmus culture would be harvestable. Harvestability of the culture was indeed very high for the first half of this experimental run (Figure VI-2).

Mixing was minimal during June. A 5 h.p. submersible pump lifted about 40 GPM over the main pump station and back into the first channel, directing approximately 30 GPM through a fine mesh DSM screen. This flow provided some circulation of pond water, but was insufficient to cause a noticeable mixing velocity. The recirculation pump was run continuously. The large pumps were not used at first because of the possibility that shear forces within the pumps would have an adverse effect on the formation of large colonies. As the pond density increased however, the afternoon pH began to reach nearly

11, and autoflocculation caused almost all of the algae to settle out each afternoon (Figure VI-2). This phenomenon was first noticed on June 25 and continued through the 27th when a 1/2-hour noon fast-mix was begun. The mixing resulted in a slight lowering of pH and also in mechanical re-suspension of the settled algae. In spite of this short period of fast-mix, the pond density decreased and considerable settling remained evident. Therefore it was decided on 2 July to run one of the fast-mix pumps continuously. This provided a faster mixing velocity, but even this mixing was not effective throughout the pond. The continuous mixing caused a brief rise in pond density accompanied by a slight, temporary decrease in afternoon pH. This was followed by a continuation of the decline in density that did not reverse itself until after the detention time was shortened.

Zooplankton did not appear to be responsible for this density decline. Ennumeration of zooplankton was not attempted but no obvious bloom of zooplankton was observed. The whole water column was green in the mornings indicating partial resuspension of the algae overnight. Settling occurred during the late morning and early afternoon. By mid-afternoon, the water was virtually clear. The density values given in the figures are composites, and so the decline represents both a decline in the morning density and faster, more extensive settling.

By July the small pond experiments had shown that Scenedesmus could remain harvestable at much shorter detention times, and it was decided to increase the sewage flow to the high-rate pond. Starting on 11 July, the detention time was decreased to an average of 5.2 days. This caused a decline in pH followed by a sharp rise in pond density.

The predominant alga throughout the month of June was Scenedesmus. The pond had been inoculated with only harvested algae colonies, yet it remained almost totally non-harvestable during the start-up period. By 31 May the pond contained a reasonably dense culture of Scenedesmus (160 mg/l VSS)

which was only 3% harvestable (by chlorophyll a). With the establishment of a regular operating regime,however, the harvestability increased dramatically, reaching 98% removal on 18 June and remaining over 95% through 11 July.

Micractinium began to appear in the pond at the end of June and by 28 July it had displaced 50% of the Scenedesmus (by volume). The timing of this transition in algae type was virtually identical to that observed in the 12 m^2 pond, M-2 (which was run at 3-day detention [see Figure IV-30]). In the large pond, however, the complete transition took much longer than in the M-3. The two algae types each remained between 40% and 60% of the algae volume for at least a month in the large pond as opposed to less than 10 days in M-2. In M-1 (2-day detention) the entire transition took only 14 days, and the period between 60% and 40% was less than 5 days (see Figure IV-29). No small pond was run at a longer detention time through this transition period without being disturbed (pumped out and reinoculated), but from the above observations it seems likely that both long detention time and heterogeneous conditions caused by suboptimal mixing allowed Scenedesmus to compete more successfully in the large pond than in the smaller ones.

Figures IV-2 and IV-4 show that the rise in proportion of Micractinium coincided with an increase in effluent chlorophyll a and VSS levels and with a sustained rise in pond densities. All of the above followed shortly after the detention time was cut from about 7 days to about 5 days. In the absence of a control pond, the causes and effects here are not easily identified. The fact that the density was 1 1/2 times that of June without significantly higher insolation seems to indicate that the pond was nutrient-limited in June, and that this condition was partially corrected by the increased loading. The timing would indicate that increased loading alleviated the problems of autoflocculation and settling, and that this allowed pond densities to rise. An alternative explanation is that Micractinium settled less readily under the existing conditions that did Scenedesmus, but this seems unlikely since

the culture was still half Scenedesmus when the density reached its highest point in August.

Harvestability, however, was influenced by the change in algae types as well as by the detention time. The effluent densities rose concurrently with the proportion of Micractinium and also leveled off when the Micractinium leveled off. When the Micractinium stabilized at 50% and the detention time was about 5 days the harvestability seemed to stabilize around 80% (by chlorophyll a).

Since the aims of this project were being redirected from development of species control and low cost harvesting technology to determination of potential productivity of microalgae biomass systems, on 20 August a decision was made to attempt to increase productivity by further shortening the detention time. Results of the small pond experiments indicated that the increased loading was very likely to result in an algae culture that was not harvestable by microstrainer, especially in light of the slow but distinct rise in effluent densities that was already occurring. It seemed desirable, however, to determine whether total production in this pond could be brought up to the levels achieved in the 12 m^2 ponds at short detention times. Figure VI-3 shows that the productivities achieved during the 18 days run at short detention time (θ=3.5 days) averaged 18.9 $\frac{gm}{m^2\ day}$ (not counting the first sample point which is an artifact of the change in detention time). This corresponds to a conversion efficiency of 4.7 % of PAR which compares quite favorably with the productivity obtained in ponds M-1 and M-2 during July when they were run for maximum productivity. The chlorophyll content was 2.6%, considerably higher than in the 12 m^2 ponds, indicating that the algae content of the solids were at least equivalent.

Figure VI-2 shows that effluent densities rose dramatically in response to increased loadings accompanied by a decline in harvestability from around

60% to around 20% (chlorophyll a removal) in a period of 2 weeks. Similar loss of harvestability was observed in the 12 m^2 ponds upon equivalent increase in loading.

It was during the period of operation at short detention time that Micractinium achieved complete dominance over Scenedesmus. This observation is consistent with the results of the 12 m^2 experiments (See Figure IV-29 and IV-30) where Micractinium displaced Scenedesmus much more rapidly in the pond with the shortest detention time.

The detention time was lengthened gradually over the next three weeks (7 September to 28 September) in an attempt to reverse the loss of harvestability. The attempt was not successful. It is evident that harvestability can be destroyed rapidly by increasing loading beyond some threshold level. It is also evident that harvestability is not easily restored by simple manipulation of loading. Harvestability remained poor through the end of the month when the experiment was terminated.

In conclusion, these experiments with the 0.25 ha high-rate pond were reasonably consistent with the smaller, 12 m^2 , ponds. Both productivity and harvestability responses to detention time were similar in both types of ponds. Before further analogies can be drawn the 0.25 acre high rate pond must be modified to more closely resember the experimental ponds and split into two ponds to allow controlled experimentation. One of the most hopeful observations was the apparent lack of pond instability caused by zooplankton herbivores. This observation will need to be confirmed in longer range experiments.

TABLE 17

HIGH-RATE POND SUMMARY

	Dates 6/10-7/10	7/15-8/17	8/24-9/7
Average Detention Time (days)	7	5.2	3.6
Pond Density (VSS mg/ℓ)	174	213	242
Pond Density (Chlor. *a* mg/ℓ	3.8	5.2	6.2
Effluent Density (VSS mg/ℓ)	27	80	156
Effluent Density (Chlor. *a* mg/ℓ)	0.2	1.4	3.6
Harvestability (% VSS)	85	62	34
Harvestability (% Chlor. *a*)	95	72	41
Total Production (gm VSS/m^2/day)	7	12	19
Total Production (mg Chlor. *a*/m^2day)	150	283	503
Harvestable Production (gm VSS/m^2/day)	6	7	6
Harvestable Production (mg chlor. *a*/m^2/day)	143	204	190
% Chlor. *a*	2.2	2.4	2.6
Average Total Insolation (Langleys/day)	588	566	512
Solar Conversion Efficiency (% total insolation)	0.6	1.1	2.0
Solar Conversion Efficiency (% photosynthetically active radiation)	1.4	2.5	4.7
Average Effluent NH_3 (mg/ℓ)	2.6	5.0	12.4
Average NH_3 Removal (%)	94	89	74
Average Effluent BOD (mg/ℓ)	36.4	48.0	67.9
Average BOD Removal (%)	88	78	64
Average Effluent COD (mg/ℓ)	185.0	234.0	342.3
Average COD Removal (%)	55	50	24

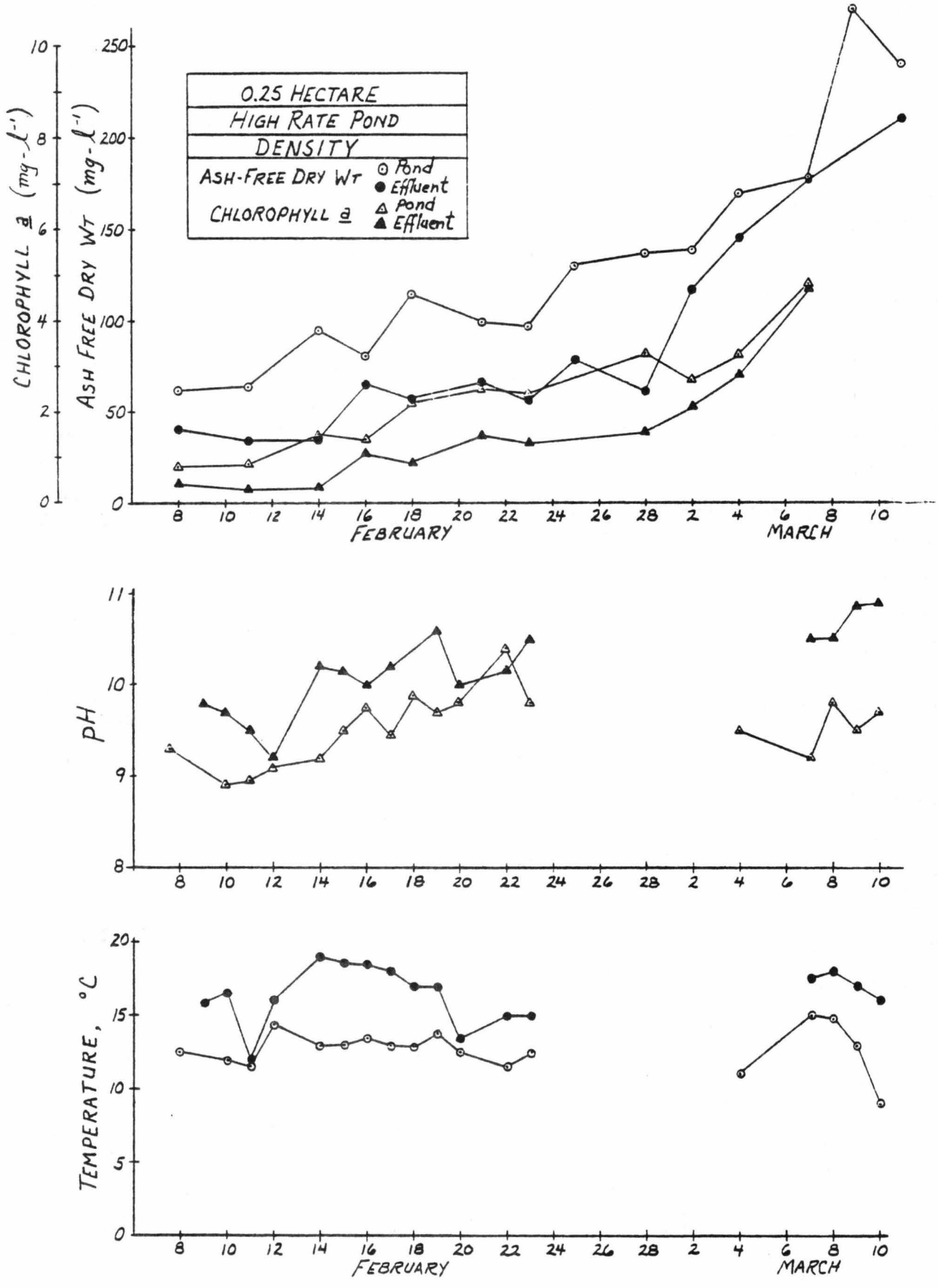


FIGURE VI-1. 0.25 HECTARE POND AND MICROSTRAINER EFFLUENT DENSITY, pH AND TEMPERATURE

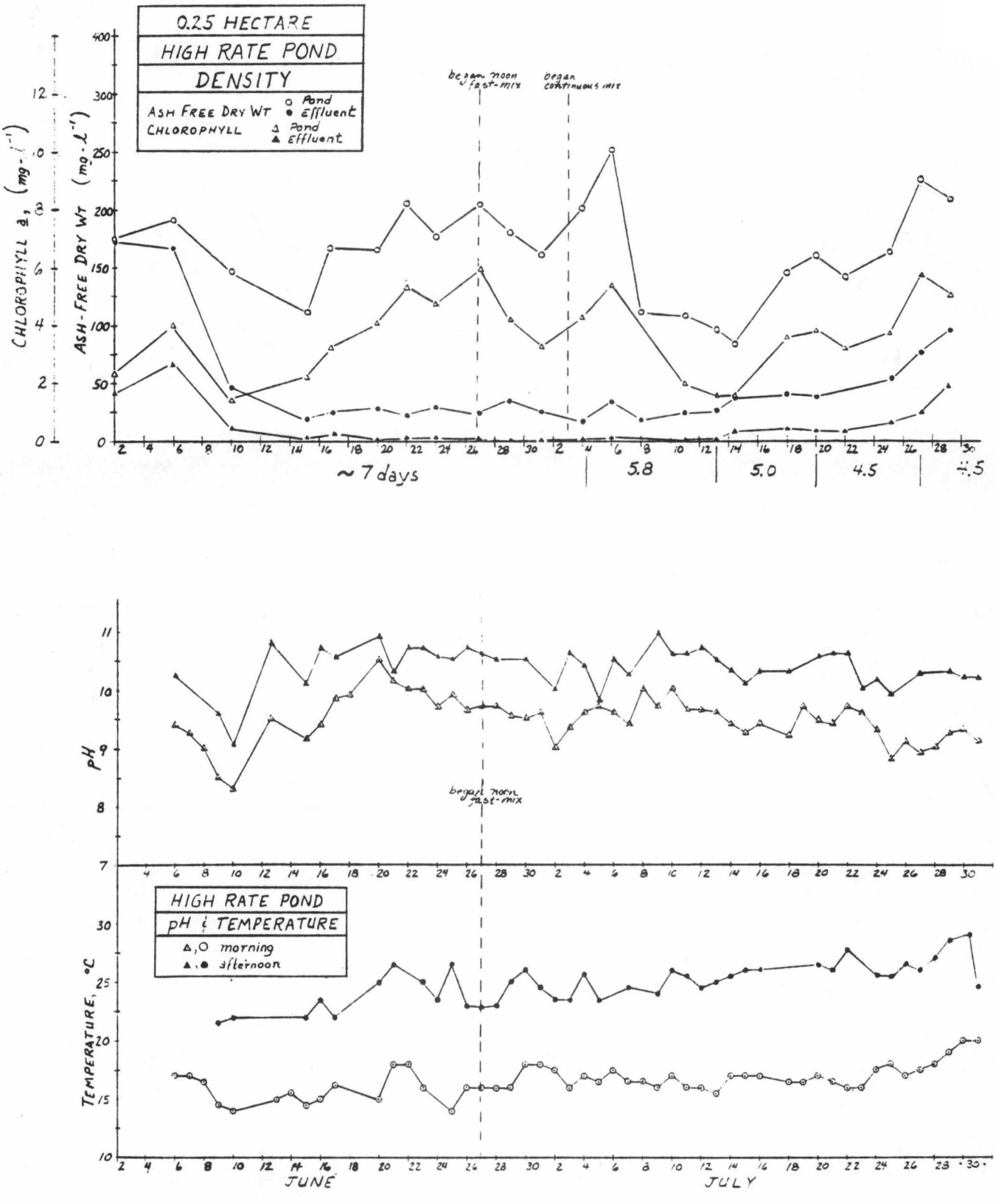


FIGURE VI-2. 0.25 HECTARE POND AND MICROSTRAINER EFFLUENT DENSITY, pH AND TEMPERATURE

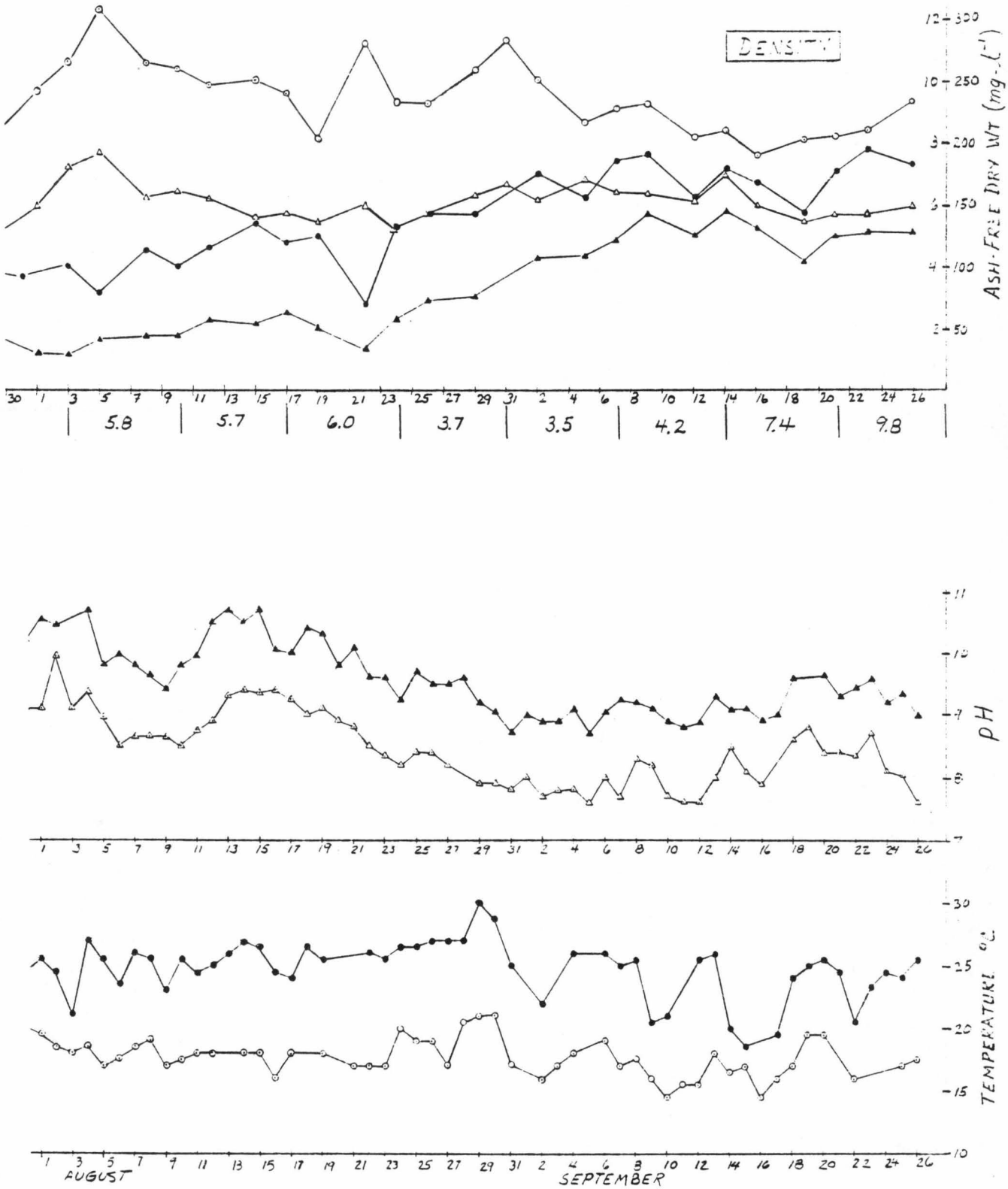
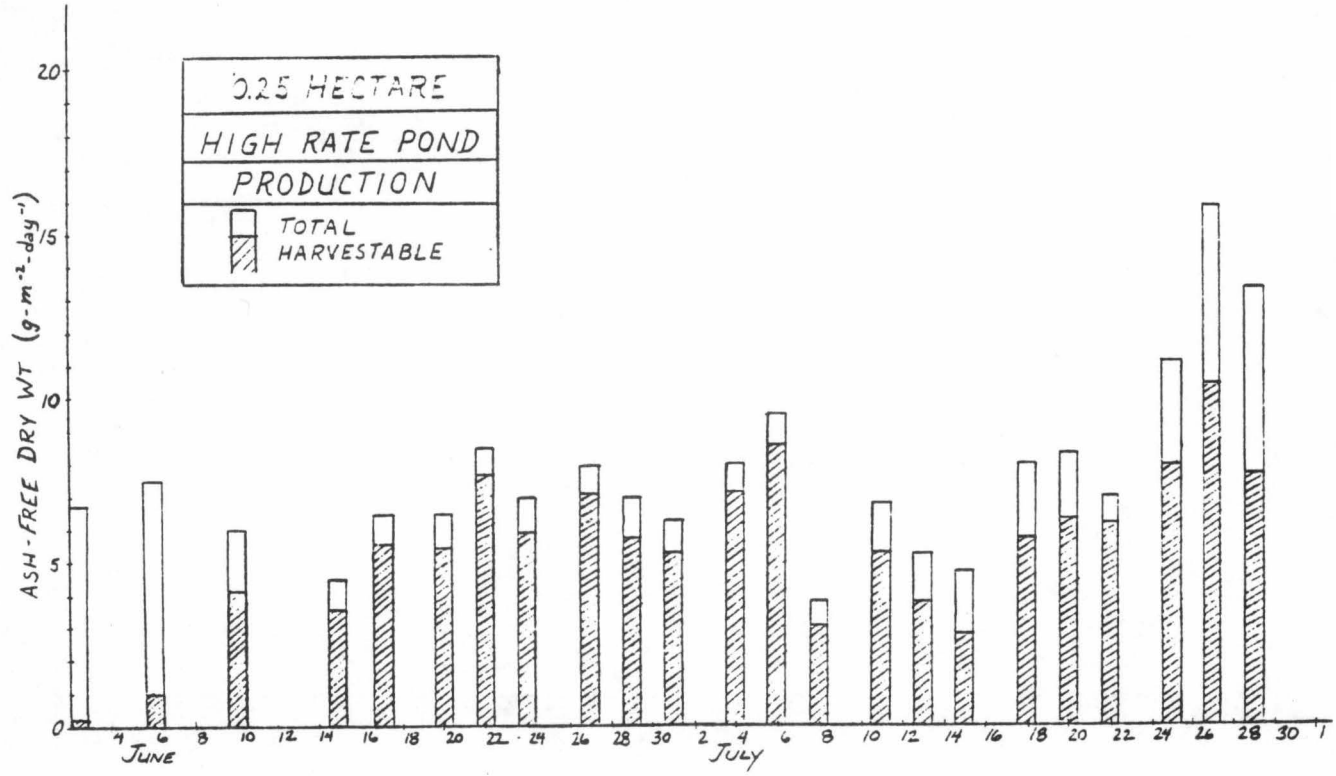


FIGURE VI-2. (cont.)



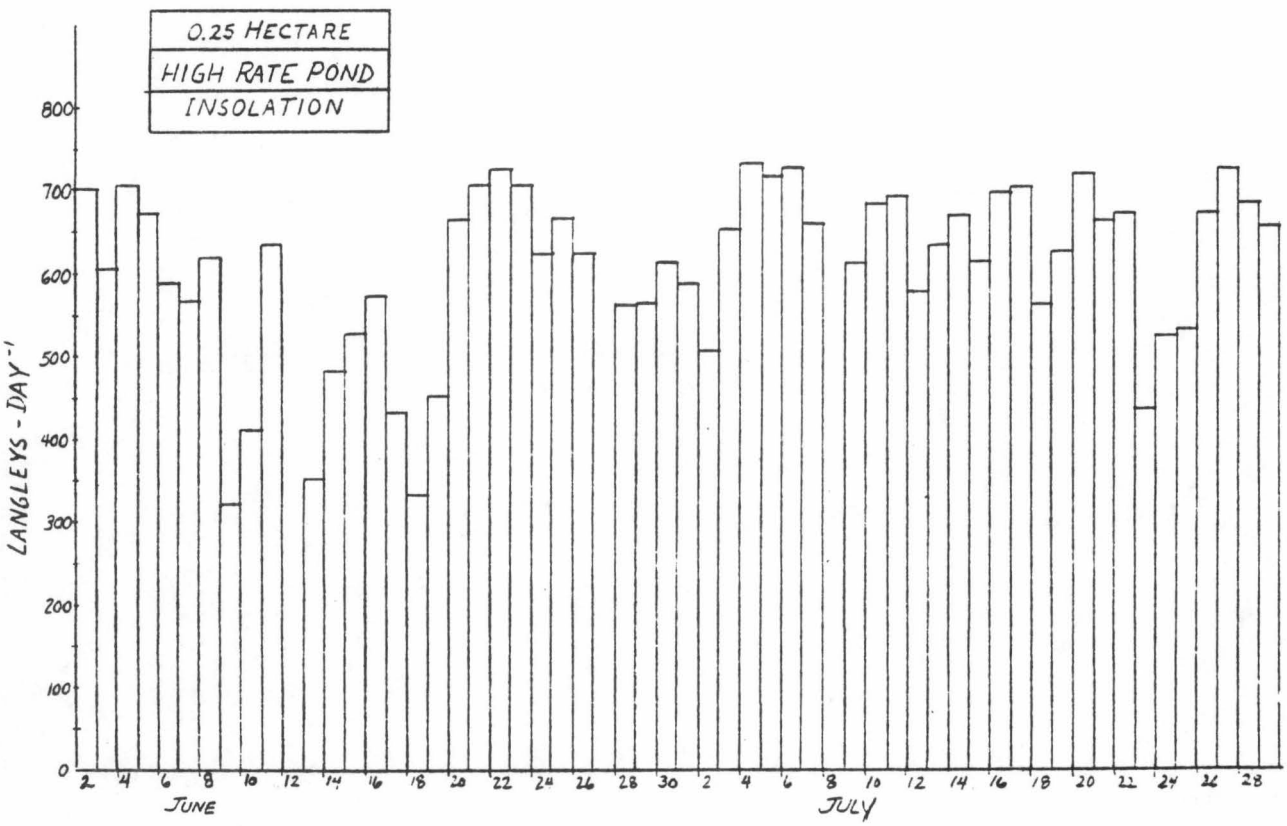
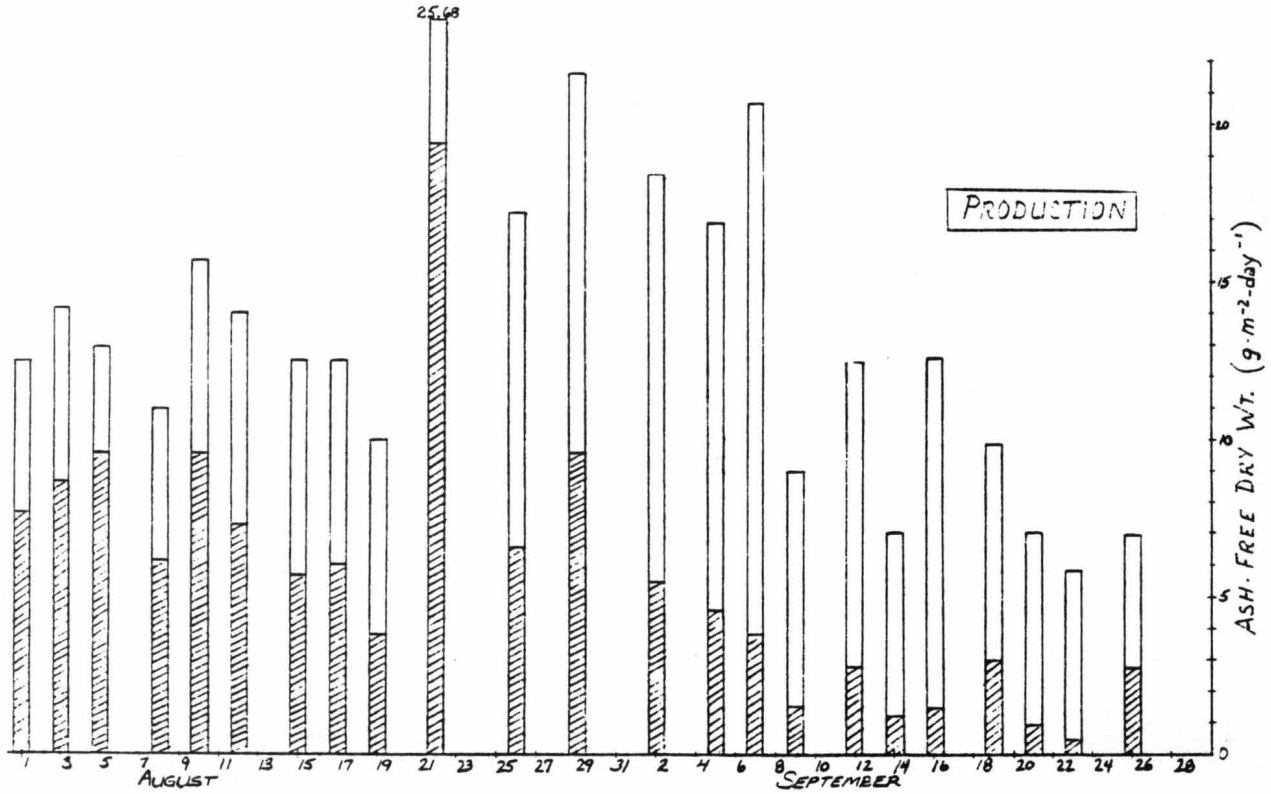


FIGURE VI-3. 0.25 HECTARE POND PRODUCTIVITY, INSOLATION



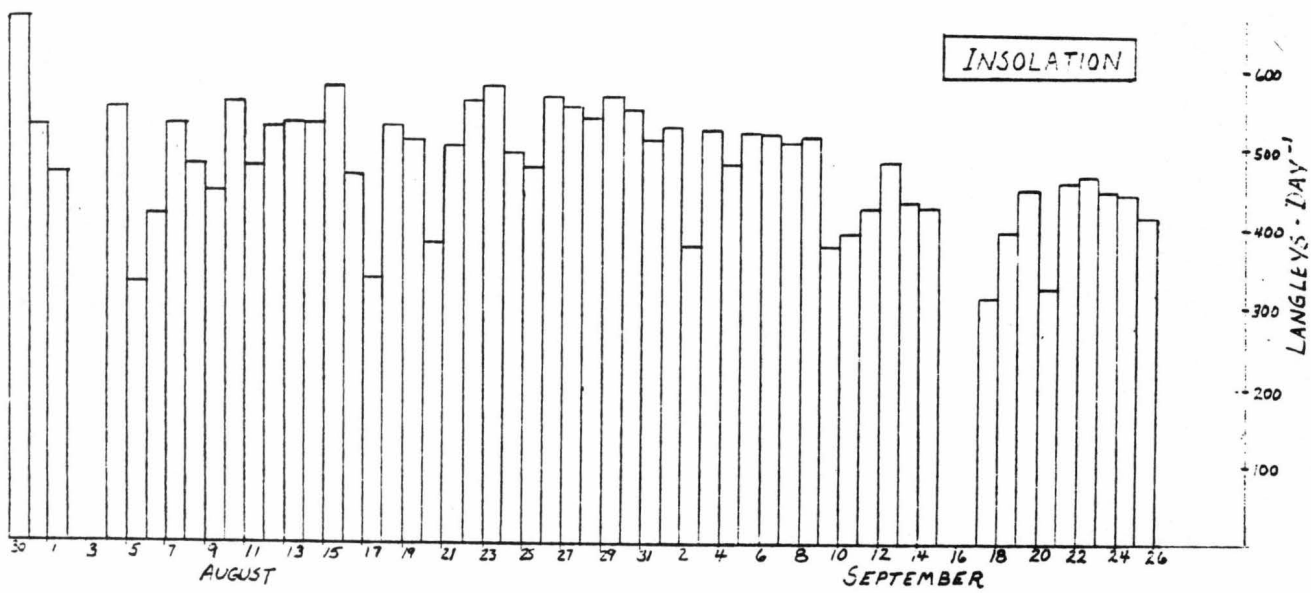


FIGURE VI-3. (cont.)

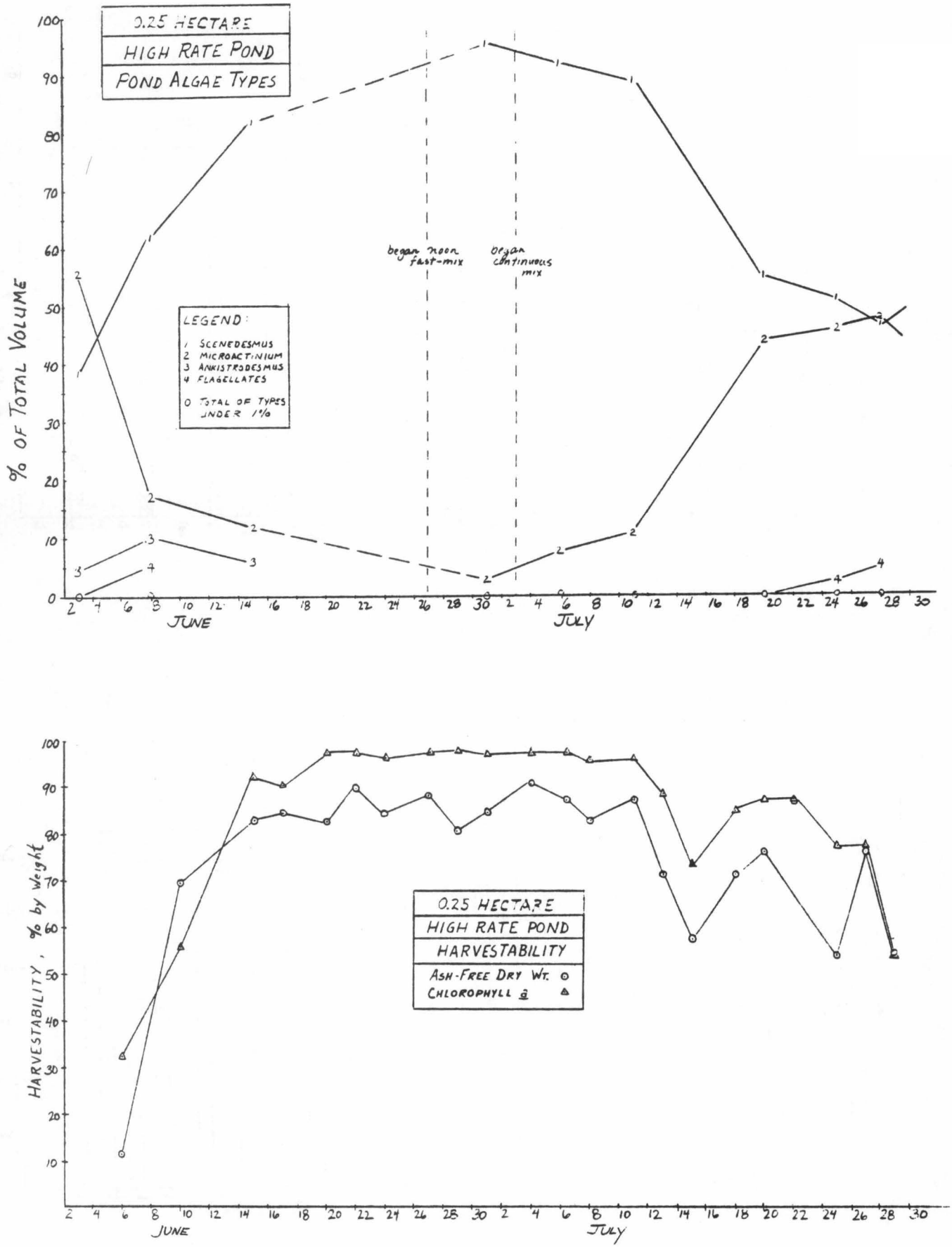
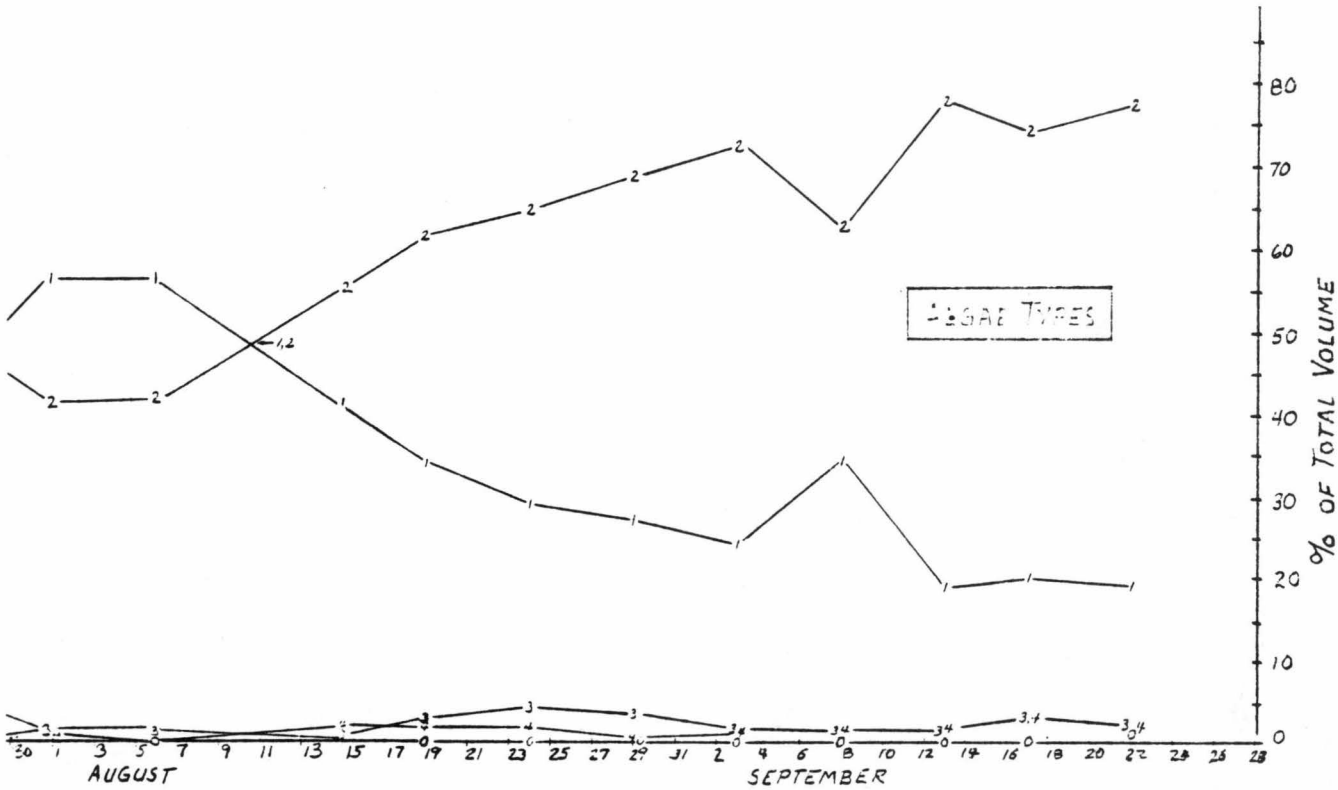


FIGURE VI-4. 0.25 HECTARE POND ALGAE TYPES, HARVESTABILITY



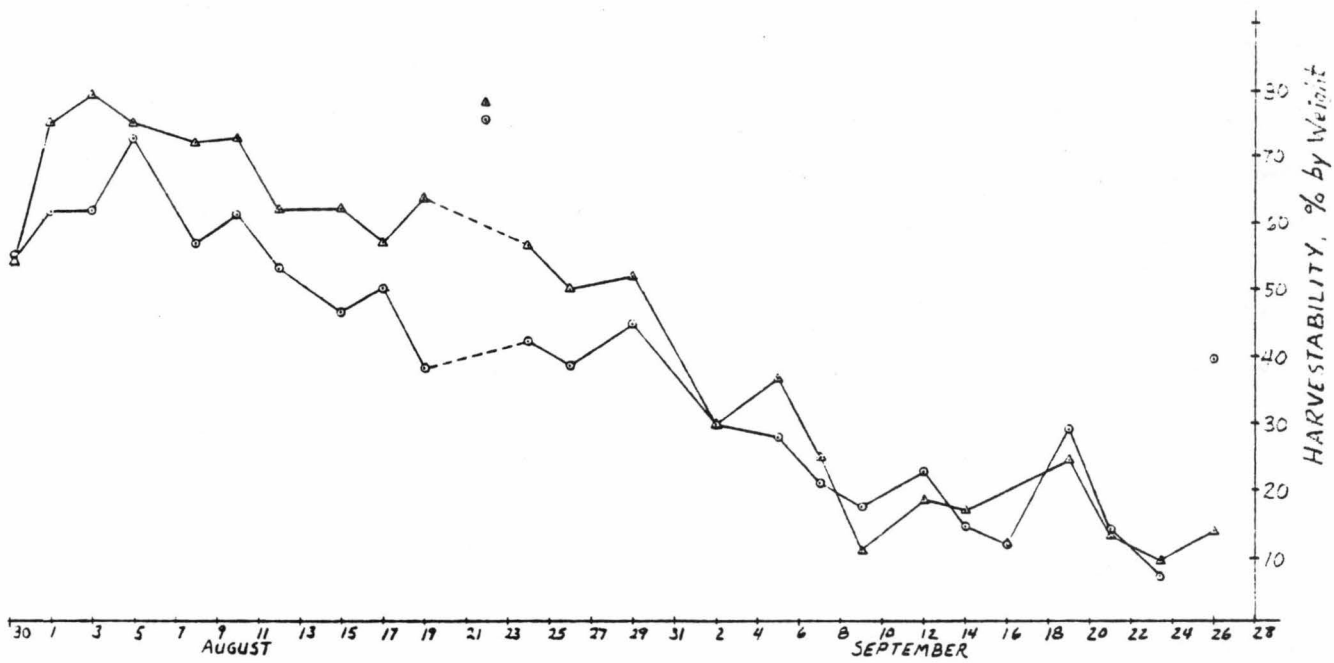


FIGURE VI-4. (cont.)

VII. DISCUSSION

The data presented in this report develops the conceptual and experimental foundation of an integrated waste treatment-biomass production pond system. As this project is one of a series carried out recently at this laboratory (14,31,33,34,35) which are still ongoing (46), the results must be interpreted in the context of the overall effort. Thus an economic feasibility analysis has been carried out (31,34,35) and will not be repeated here. Briefly, it has been demonstrated that algae biomass production ponds can be of low cost both in capital investment and O & M. The presently limiting factor in applying such systems to municipal waste treatment is the cost-reliability of microalgae harvesting processes. Microstraining is a relatively low cost ($50/MG for a 10 MGD system) harvesting method of low reliability when applied to waste treatment ponds. The principal objective of this research was to develop pond operations that increase the effectiveness and reliability of microstraining. This requires control of the algae populations in the ponds such that larger colonial or filamentous types predominate.

Overall the results of the research must be considered preliminary. Although longer detention times proved fairly effective in maintaining a harvestable culture, they lowered biomass production rates. Specific biomass recycle had previously been shown to be somewhat effective in helping induce or maintain a harvestable pond culture (31) and the experimental verification of the theory of such a process could be demonstrated in the laboratory (Appendix II). However, this selection process suffers from several drawbacks (such as lowering productivity) which severely limit its usefulness. Finally, fast mixing speeds are a critical parameter in the formation of algae-bacteria flocs that are readily harvestable by both microstraining or settling. It proved difficult to optimize these three distinct pond operation factors such that successful maintenance of a harvestable culture was obtained under

conditions of high productivity. Thus (Chapter IV, Experiment 3) fast mixing had positive effect on harvestability but could not make a short detention pond reliably harvestable.

Data from the most productive and most harvestable of the 12 m^2 ponds from each experiment are presented in Table 18. This table is a summary of ten months of operation, no data being available for November and December. The most productive ponds averaged 13.4 gm m^{-2} day^{-1} in VSS total production, two-thirds of which was harvestable. This average must be considered a conservative estimate of what is attainable because generally the ponds were not operated to a maximal productivity. Average production from the most harvestable ponds was 8.5 gm m^{-2} day^{-1} with 7.2 gm m^{-2} day^{-1} harvestable. The microstrained effluents from these ponds contained an average of 42 mg l^{-1} volatile suspended solids (45-50 total suspended solids) as compared to 74 mg l^{-1} residual in the effluents from the most productive ponds. On an eight month basis, from January through September, the best effluents average only 32 mg l^{-1} VSS. Thus microstraining is effective in removal of solids for 8 to 10 months in the Richmond environment.

The most severe problem identified during this project was the instability of the experimental scale (12 m^2) algae cultures due to invasion by algae predators (herbivorous zooplankton such as rotifers, copepods, etc.) This was a particularly severe problem under conditions favoring microstrainer harvestable cultures: long detention times or biomass recycle (the latter favoring the recycle of zooplankton). Shorter detention time ponds resulted in more stable cultures, however, harvestability was poor. Various methods for zooplankton control were tested, principally removal by large mesh (150 μ) screens. However, these were only partially successful. This problem will require more investigation.

One of the principal objectives of this project is to develop a multi-stage wastewater ponding system that results in advanced waste treatment

(advanced being defined as nutrient removal; sometimes this level of waste treatment is also called "tertiary" treatment). For this reason a series of experiments was carried out (Chapter V) which investigated the operation of a "secondary pond" which would result in the growth of an additional crop of algae which would effectively strip the sewage of its available ammonia nitrogen. This was accomplished in a series of batch growth pond experiments in which algae were removed either by settling or by microstraining. These ponds are an intermediate stage between the primary oxidation ponds and tertiary nitrogen-fixing blue-green algae ponds designed to remove phosphates. The concept of nitrogen-fixing blue-green algae cultivation and their application in advanced wastewater treatment (Figure V-6) has been presented elsewhere (33).

A final objective of this project was to test the results obtained with small scale experimental ponds on a larger pilot pond scale. For this purpose the 0.25 hectare pilot pond was operated. Harvestability was good until detention times were reduced, at which point a non-harvestable algae culture developed. Most significantly this larger pilot pond did not exhibit the pronounced invasions by zooplankton grazers observed with the smaller scale ponds.

The approach followed by this project has been discontinued for two reasons: 1) the objectives of the Fuels From Biomass Program at DOE were incompatible with the development of integrated wastewater-biomass production systems and 2) algae settling proved to be a much lower cost and possibly a controllable method of algae harvesting. Thus in the continuation of this project (45) the use of algae flocculation (without chemicals) and settling as a method of algae harvesting is emphasized, along with the development of concepts applicable to large-scale biomass systems designed exclusively for energy production.

TABLE 18

SUMMARY OF PEAK POND PRODUCTIVITY AND HARVESTABILITY DATA FOR 12 SQ M PONDS DURING 1977

Date	Most Productive Pond							Most Harvestable Pond						
	Total Productivity $g/m^2/d$	Harvest Productivity $g/m^2/d$	Chlor.a Removal %	Eff. VSS mg/ℓ	0 Days	Mixing Speed cm/sec	Recycle %	Total Productivity $g/m^2/d$	Harvest Productivity $g/m^2/d$	Chlor.a Removal %	Eff. VSS mg/ℓ	0 Days	Mixing Speed cm/sec	Recycle %
1/17-2/21	3.9	1.7	48	44	6.7	5	0	1.9	1.7	85	20	15	5	0
3/4-3/12	8.0	4.0	50	80	4.4	5	0	2.5	1.5	78	50	13.6	5	33
3/16-4/8	13.6	10.9	85	46	4.4	15	0	3.4	2.5	80	34	9.4	5	33
4/12-5/6	16.3	10.0	60	94	4.4	15	0	9.7	8.7	92	32	8.2	15	0
5/8-5/28	16.5	4.0	30	150	3.2	5	0	11.0	9.0	80	35	7.5	15	0
7/4-8/3	32	23	71	73	2.3	10	0	18.0	17.5	94	18	4.6	10	0
8/4-9/8	13	10.5	80	50	5.2	5	0	13	10.5	80	50	5.2	5	0
9/8-9/20	7	5	67	75	9.2	5	0	7	5	67	75	9.2	5	0
10/3-10/27	4	2	50	90	8.0	5	0	4	2	50	90	8.0	5	0
Avg. Over 10 mo.	13.4	8.7	65	74				8.5	7.2	-	42 (32 averaged from Jan.-Sept.)			

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APPENDIX I. SEWAGE TREATMENT DATA

Because the primary objectives of this project were the development of algae biomass production technology, waste treatment aspects of the experiment were not emphasized during the discussion. In this appendix all waste treatment data is collected in tabular form. Conditions of pond operations can be found in Chapter IV.

TABLE I. BIOCHEMICAL OXYGEN DEMANDS OF INFLOWING SETTLED SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS WITH REMOVAL PERCENTAGES

DATE	SEWAGE BOD mg/ℓ	MP-1		MP-2		MP-3		MP-4		HRP	
		BOD mg/ℓ	% R	BOD mg/ℓ	% R	BOD mg/ℓ	% R	BOD mg/1ℓ	% R	BOD mg/1ℓ	% R
7-8-77	308.0	39.6	87.1	28.3	90.8	18.8	93.9	26.3	91.5	36.4	88.2
7-13-77	214.2	53.2	75.2	28.8	86.6	--	--	13.8	93.6	32.5	84.8
7-22-77	212.8	56.5	73.4	18.1	91.5	35.4	83.4	--	--	52.8	75.2
8-3-77	229.4	69.8	69.8	35.6	84.5	34.0	85.2	41.7	81.8	43.2	81.2
8-24-77	190.4	52.1	72.6	28.0	85.3	37.7	80.2	21.8	88.6	67.9	64.3
9-21-77	220.3	49.0	77.8	16.3	92.6	59.0	73.2	7.7	96.5	68.1	69.1
10-7-77	195.5	49.1	74.9	38.7	80.2	33.6	82.8	14.0	92.8	76.8	60.7
10-20-77	251.1	32.6	87.0	37.3	85.1	46.1	81.6	42.8	83.0	69.8	72.2
10-27-77	306.6	40.2	86.9	41.4	86.5	38.1	87.6	42.4	86.2	84.5	72.4

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TABLE II. CHEMICAL OXYGEN DEMANDS OF INFLOWING SETTLED SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS WITH REMOVAL PERCENTAGES.

DATE	SEWAGE COD mg/ℓ	MP-1		MP-2		MP-3		MP-4		HRP	
		COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R
1-28-77	341.9	157.7	53.9	126.5	63.0	152.2	55.5	120.0	64.9	--	
2-9-77	367.1	186.9	49.1	79.5	78.3	194.8	46.9	81.5	77.8	--	
2-16-77	366.1	137.8	62.4	74.8	79.6	126.0	65.6	82.7	77.4	159.4	56.5
2-23-77	416.3	184.1	55.8	92.1	77.9	176.1	57.7	124.1	70.2	186.1	55.3
3-2-77	406.3	--	--	--	--	--	--	--	--	252.7	37.8
3-9-77	360.6	206.3	42.8	113.9	68.4	250.8	30.5	143.6	60.2	404.3	
3-17-77	459.0	--	--	--	--	--	--	134.8	70.6	380.9	17.0
3-24-77	367.7	--	--	--	--	--	--	111.9	69.6	363.8	1.1
3-31-77	402.9	--	--	--	--	--	--	121.5	69.8	478.6	
4-7-77	544.0	119.2	78.1	--	--	405.9	25.4	130.0	76.1	439.3	19.3
4-21-77	558.6	223.4	60.0	154.8	72.3	152.9	72.6	209.7	62.5	--	

TABLE II. CHEMICAL OXYGEN DEMANDS OF INFLOWING SETTLED SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS WITH REMOVAL PERCENTAGES (Cont.)

DATE	SEWAGE COD mg/ℓ	MP-1		MP-2		MP-3		MP-4		HRP	
		COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R
4-28-77	493.9	256.8	48.0	117.6	76.2	219.5	55.6	186.2	62.3	--	--
5-4-77	464.7	186.6	59.9	121.8	73.8	220.0	52.7	192.5	58.6	--	--
5-17-77	455.4	166.3	63.5	174.3	61.7	202.0	55.6	158.4	65.2	--	--
6-15-77	368.5	150.0	59.3	153.7	58.3	148.1	59.8	140.7	61.8	159.3	56.8
7-6-77	453.3	192.8	57.5	151.1	66.7	157.9	65.2	165.0	63.6	210.7	53.5
7-13-77	448.0	250.9	44.0	125.4	72.0	--	--	95.8	78.6	163.8	63.4
7-18-77	511.5	162.6	68.2	131.8	74.2	125.3	75.5	97.2	81.0	210.6	58.8
7-25-77	470.7	174.7	62.9	85.8	81.8	123.6	73.7	155.8	66.9	216.3	54.0
8-3-77	430.4	255.4	40.7	166.1	61.4	144.6	66.4	203.6	52.7	216.1	49.8
8-10-77	505.6	177.2	65.0	151.1	70.1	166.0	67.2	128.7*	74.5*	248.1	50.9
8-15-77	445.2	157.2	64.7	200.6	54.9	241.5	45.8	123.5	72.3	278.8	37.4
8-23-77	469.0	165.5	64.7	--	--	223.8	52.3	142.5	69.6	187.3	60.1

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TABLE II. CHEMICAL OXYGEN DEMANDS OF INFLOWING SETTLED SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS WITH REMOVAL PERCENTAGES (Cont.)

DATE	SEWAGE COD mg/ℓ	MP-1		MP-2		MP-3		MP-4		HRP	
		COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R	COD mg/ℓ	% R
8-29-77	452.7	149.6	67.0	--	--	164.8	63.6	117.4	74.1	314.4	30.6
9-5-77	449.6	205.9	54.2	128.5*	71.4	330.6	26.5	238.0*	47.1	370.2	17.7
9-14-7	323.1	137.0	57.6	231.0*	28.5	305.6	5.4	149.3*	53.8	307.8	4.7
9-20-77	403.9	143.9	64.4	224.4*	44.4	254.1	37.1	82.6*	79.5	278.6	31.0
9-30-77	421.4	382.1	9.3	371.4*	11.9	135.7	67.8	101.8*	75.8	--	--
10-4-77	476.6	398.8	16.3	323.9	32.0	--	--	--	--	443.0	7.0
10-6-77	382.4	322.9	15.6	225.1	41.1	289.6	24.3	266.2	30.4	344.4	9.9
10-10-77	440.9	230.8	47.7	151.0	65.8	268.9	39.0	264.6	40.0	258.0	41.5
10-17-77	489.1	168.1	65.6	168.1	65.6	198.7	59.4	303.8	37.9	366.8	25.0
10-24-77	542.8	210.7	61.2	229.3	57.8	239.2	55.9	296.5	45.4	394.1	27.4
11-7-77	450.2	321.1	28.7	332.9	26.1	334.9	25.6	374.4	16.8	382.3	15.1

*Denotes secondary pond diluted with microstrained effluent.

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TABLE III. AMMONIA CONCENTRATION (mg/ℓ N) OF SETTLED INFLOWING SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS (filtered samples) WITH REMOVAL PERCENTAGES

DATE	SEWAGE NH_3 mg/ℓ	MP-1		MP-2		MP-3		MP-4		.25 HECTARE HRP	
		NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R
1-28-77	23.8	8.4	64.7	3.2	86.5	4.2	82.4	1.9	92.0	--	--
2-9-77	28.8	17.8	38.2	7.0	75.7	11.1	61.5	3.2	88.9	--	--
2-16-77	30.6	14.0	54.3	6.5	78.8	13.6	55.6	4.4	85.6	4.9	84.0
2-23-77	32.3	14.2	56.0	3.3	89.8	11.5	64.4	2.5	92.3	2.7	91.6
3-10-77	38.4	11.7	69.5	5.2	86.5	15.0	60.9	1.3	96.6	1.8	95.3
3-17-77	28.5	--		--		--		1.4	95.1	1.3	95.4
3-24-77	33.6	--		--		--		1.4	95.8	0.9	97.3
3-31-77	21.8	--		--		--		1.3	94.0	0.5	97.7
4-7-77	27.5	11.7	57.5	--		14.6	46.9	1.2	95.6	1.9	93.1
4-14-77	31.8	--		--		--		3.2	89.9	--	
4-28-77	33.0	10.9	67.0	5.8	82.4	4.0	87.9	5.6	83.0		
5-4-77	34.0	12.1	64.0	5.3	84.4	7.2	78.8	8.5	75.0		
5-17-77	40.0	1.9	95.3	3.0	92.5	6.0	85.0	2.1	94.8	--	
5-19-77	37.4	2.6	93.1	3.5	90.6	--		--		--	
5-23-77	43.6	8.1	81.4	10.1	76.8	--		--		--	

TABLE III. AMMONIA CONCENTRATION (mg/ℓ N) OF SETTLED INFLOWING SEWAGE AND OUTFLOWING MICROSTRAINED EFFLUENTS (filtered samples) WITH REMOVAL PERCENTAGES (Cont.)

DATE	SEWAGE NH_3 mg/ℓ	MP-1		MP-2		MP-3		MP-4		.25 HECTARE HRP	
		NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R
5-25-77	51.5	4.9	90.5	9.0	82.5	--		--		--	
6-16-77	37.0	7.0	81.1	6.9	81.4	7.4	80.0	6.1	83.5	3.0	91.9
6-29-77	46.6	10.4	77.8	8.3	82.2	4.4	90.5	2.3	95.1	1.9	95.9
7-6-77	62.2	17.6	71.6	10.8	82.6	9.2	85.1	6.4	89.6	2.8	95.5
7-13-77	39.7	19.6	50.6	14.5	63.5	--	--	9.0	77.3	5.6	85.9
7-21-77	46.6	20.8	55.2	13.6	70.9	6.4	86.4	4.4	90.4	4.5	90.3
7-25-77	45.8	22.3	51.3	17.3	62.2	9.6	79.0	14.1	69.2	6.2	86.5
7-27-77	47.7	21.1	55.8	16.2	66.0	10.0	79.0	13.4	74.6	7.1	85.2
8-1-77	48.5	13.0	73.2	11.5	76.3	8.2	76.3	8.5	83.2	3.2	93.3
8-4-77	42.7	18.8	55.9	13.1	69.3	10.1	76.3	--	--	2.4	94.5
8-8-77	33.3	7.2	78.4	13.7	58.9	12.3	63.1	2.1	93.1*	4.8	85.6
8-11-77	48.7	5.2	89.3	18.4	62.2	6.8	86.0	0.7	98.6*	6.9	85.8
8-18-77	42.0	9.3	77.9	17.3	58.8	11.4	72.9	0.3*	99.3	--	--
8-22-77	48.8	7.4	84.8	--	--	7.3	85.0	0.15*	99.7	5.3	89.1
8-25-77	46.7	1.9	95.9	1.0*	97.9	3.2	93.1	0.08*	99.8	--	--
8-30-77	41.2	5.7	86.7	--	--	7.4	82.6	0.1*	99.8	10.9	74.4

*Denotes second pond diluted with previously microstrained effluent.

APPENDIX I

TABLE III. AMMONIA CONCENTRATION (mg/ℓ N)OF SETTLED INFLOWING SEWAGE AND OUTFLOWING MICROSCTRAINED EFFLUENTS (filtered samples) WITH REMOVAL PERCENTAGES (Cont.)

DATE	SEWAGE NH_3 mg/ℓ	MP-1		MP-2		MP-3		MP-4		.25 HECTARE HRP	
		NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R	NH mg/ℓ	% R
8-31-77	51.2	5.8	88.7	0.07*	99.9	5.9	88.5	0.8*	98.4	14.0	72.7
9-5-77	50.2	6.3	87.5	0.08*	99.8	7.9	84.3	0.1*	99.8	12.2	75.7
9-7-77	49.0	9.5	80.6	0.1*	99.8	9.4	80.8	0.02*	100.0	11.6	76.3
9-12-77	53.3	4.4	91.7	0.2*	99.6	6.7	87.4	0.1*	99.8	11.1	79.2
9-14-77	55.7	4.6	91.7	0.15*	99.7	7.9	85.8	0.1*	99.8	12.3	77.9
9-19-77	43.2	6.7	84.5	0.1*	99.8	4.2	90.3	0.04*	99.9	12.2	71.8
9-21-77	57.6	3.8	93.4	0.04*	99.9	1.9	96.7	0.15*	99.7	8.4	85.4
9-27-77	46.3	4.8	89.6	0.05*	99.9	9.0	80.6	0.3*	99.4	7.6	83.6
9-30-77	35.2	--	--	--	--	17.3	50.9	1.8*	94.9	16.7	52.6
10-3-77	47.4	7.3	84.6	3.6	92.4	--	--	--	--	12.5	73.6
10-6-77	46.7	11.4	75.6	7.5	83.9	7.2	84.6	6.5	86.1	14.2	69.6
10-10-77	39.7	8.1	79.6	7.5	81.1	3.5	91.2	5.4	86.4	12.9	67.5
10-13-77	26.0	6.1	76.5	9.9	61.9	4.7	81.9	7.6	70.8	15.8	39.2
10-17-77	44.1	6.8	84.6	8.6	80.5	6.2	85.9	10.9	75.3	17.4	60.5
10-20-77	36.1	10.4	71.2	9.3	74.2	14.2	60.7	15.6	56.8	20.2	44.0
10-24-77	41.3	7.1	82.8	6.5	84.3	7.3	82.3	7.8	81.1	18.6	55.0
11-3-77	27.2	8.3	69.5	3.6	86.8	2.6	90.4	2.0	92.6	15.2	44.1

*Denotes second pond diluted with previously microstrained effluent.

TABLE IV. TOTAL KJELDAHL NITROGEN (TKN) CONCENTRATIONS OF SETTLED SEWAGE AND 2-STAGE REMOVAL SYSTEM. (a) Un-microstrained effluent nitrogen with % removal from sewage. (b) Microstrained effluent nitrogen with % removal from un-microstrained effluent. (c) Total removal from sewage.

DATE	SEWAGE TKN mg/ℓ	(a) Unmicrostrained									
		MP-1		MP-2		MP-3		MP-4		HRP	
		TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R
4-14-77	45.9							33.6	26.8		
5-19-77	65.8	25.2	61.7	53.5	18.7						
5-26-77	56.9	47.7	16.2	47.0	17.4						
8-8-77	66.9			51.5	23.0	36.7	45.1				
8-30-77	56.0	42.7	23.8			42.0	25.0	29.8	46.8	46.9	16.3
9-21-77	66.5	34.1	48.7						35.4	46.7	
		(b) Microstrained									
4-14-77										10.8	--
5-19-77		20.8	17.5	20.4	61.9						
5-26-77		22.6	52.6	17.6	62.9						
7-27-77		33.3	--	22.4	--	9.2	--	22.8	--	19.9	--
8-8-77				31.6	38.6	30.2	17.7	5.3	--	29.6	--
8-30-77		16.6	61.1			18.7	55.5	6.7	77.5	35.0	25.4
9-21-77		20.4	40.2	8.7	--						
10-24-77		16.3	--	24.8	--	19.2	--	26.8	--	39.4	--

TABLE IV. TOTAL KJELDAHL NITROGEN (TKN) CONCENTRATIONS OF SETTLED SEWAGE AND 2-STAGE REMOVAL SYSTEM. (a) Un-microstrained effluent nitrogen with % removal from sewage. (b) Microstrained effluent nitrogen with % removal from un-microstrained effluent. (c) Total removal from sewage. (Cont.)

DATE	SEWAGE TKN mg/ℓ	(c) Microstrained									
		MP-1		MP-2		MP-3		MP-4		HRP	
		TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R	TKN mg/ℓ	% R
4-14-77	45.9									10.8	
5-19-77	65.8	20.8		20.4							
5-26-77	56.9	22.6		17.6							
7-27-77	72.1	33.3	53.8	22.4	68.9	9.2	87.2	22.8	68.4	19.9	72.4
8-8-77	66.9			31.6	52.8	30.2	54.9	5.3	92.1	29.6	55.8
8-30-77	56.0	16.6	70.4			18.7	66.6	6.7	88.0	35.0	37.5
9-21-77	66.5	20.4	69.3	8.7	86.9						
10-24-77	58.8	16.3	72.3	24.8	57.8	19.2	67.3	26.8	54.4	39.4	33.0

APPENDIX II. CONTINUOUS CULTIVATION OF ALGAE IN THE LABORATORY

In this appendix laboratory chemostat studies are reported which were designed to test the theory of species control through selective biomass recycle which was developed in the previous Final Report and used in some of the outdoor culture experiments.

Continuous cultures with biomass recycling (cell feedback) have been described in theory (1,2,3,4) and experimentally (3,5,6). In these studies the limiting nutrient was dissolved in the feed. Using cell feedback at fixed dilution rate decreases the rate of washout of biomass. The cell density increases until the level of limiting substrate is lowered. Operation at higher dilution rates is possible because cell detention time is greater than hydraulic detention time. Since dissolved substrates are brought in faster at increased dilution rates and cell densities are generally higher, processes like biomass production and substrate destruction can occur at increased rates when biomass is recycled. This is especially useful when the influent concentrations of dissolved nutrients are fixed, as in the activated sludge process. However, in such aerobic processes, rates of oxygen transfer limit the extent to which recycling can be gainfully practiced.

In this report the analysis of a biomass recycled chemostat is extended to situations where the limiting nutrient is not dissolved in the influent and to the effects of biomass recycle on the outcome of competition among organisms. The limitations imposed by mass transfer of gaseous nutrients are discussed. Experimental results from light-limited algal cultures are presented to demonstrate the effects of recycling when the limiting nutrient

is neither carried in nor out hydraulically. Experiments with mixed cultures of algae show that biomass recycling can reverse the outcome of competition as well as allow the steady-state coexistance of two organisms on one limiting nutrient.

BIOMASS RECYCLING IN CONTINUOUS CULTURE OF A SINGLE ORGANISM

Figure 1 shows the flow diagram and biomass balance equation for a continuous culture with cell feedback. The system consists of a completely mixed reactor and a concentrator which allows recycling. Sketches of steady-state cell density versus dilution rate (of the system) with and without biomass recycling are shown in Figure 2 for different substrates which may limit the growth of the organism. For example, nitrogen, light, or CO_2 might potentially limit algal growth. A limiting nutrient is defined as any nutrient which controls the organism's specific growth rate. Empirically a limiting nutrient limits the biomass density at a given specific growth rate. Increasing the chemostat concentration of any limiting nutrient increases the biomass density unless there is instability caused by substrate inhibition at higher concentration. Different substrates may limit at different growth rates (7,8) as shown in Figure 2. There may also be multiple, simultaneous, nutrient limitation (nutrient interaction). The sketches shown in Figure 2 were drawn assuming no nutrient interaction, but the results described below are not changed qualitatively if several substrates are limiting simultaneously. Light and CO_2 simultaneously limit a photorespiring algal culture in the sense that increasing either the incident light intensity or the CO_2 partial pressure or both would increase culture density. Light and nitrogen may interact when both are in short supply since nitrogen limitation affects pigmentation and thus light absorption.

Most notably, biomass recycle affects the operation of a continuous culture by permitting independent variation of cell and hydraulic detention times through manipulation of the amount of cell mass recycled. At steady state the specific growth rate is no longer equal to the rate of dilution. Rather it is lower since recycle as well as growth increases the cell density in the reactor. Thus, at a fixed dilution rate, recycling a fraction of the cells leaving a chemostat always leads to increased culture density. The magnitude of this increase depends on the type of nutrient which is limiting growth.

The rates of inflow and outflow of nutrients which are dissolved in the feed are proportional to the rate of dilution. Gaseous nutrients, like oxygen and carbon dioxide, may be sparged directly into the culture vessel and thus the rate of entry may not depend on the rate of dilution. However, the rate at which dissolved gases flow out is proportional to the dilution rate. Light is entirely independent of dilution rate in its entry and exit from a continuous culture.

Consider two chemostats, one recycled (r) and one not recycled (nr), operated such that the organism's specific growth rate (μ) is the same in both. The cell mass balance equations give, at steady state, $\mu_{nr} = D_{nr}$ and $\mu_r = AD_r$ where $0 < A < 1$ (see Figure 1). The substrate balance equations (an energy balance equation for light) allow calculation of the biomass density if the level of limiting nutrient entering and leaving the culture is known and given the yield factor (which is the inverse of the cell quota for nutrients or an efficiency factor for energy sources). The ratio, $X_r/X_{nr}]_{\mu_r=\mu_{nr}}$, where X denotes biomass density, differs depending on the type of limiting nutrient. Since the growth rates are assumed equal, the limiting nutrient level is the same in both chemostats. Thus, even if yield factors are dependent on growth rate and nutrient level, they can also be assumed equal. When the substrate

is dissolved in the feed, $X_r/X_{nr}]_{\mu_r=\mu_{nr}} = 1/A>1$ (Figure 2A). The biomass density, and hence productivity, of the recycled chemostat is proportionately greater than that of the non-recycled chemostat by the fractional increase in system inflow rate. The maximum productivity of a recycled chemostat is greater because substrate can be brought in faster without washing out cells any faster. The net inflow of substrate, $D(S_{in}-S)$, is greater (D is greater, S_{in} and S are the same), with the difference going into cell mass. When the limiting nutrient is added to the chemostats at a constant rate, but washed out at the rate of dilution, then the cell density of the recycled chemostat may be lower than that of the non-recycled one. For instance, when a gaseous nutrient is limiting $X_r/X_{nr}]_{\mu_r=\mu_{nr}} = [(\mu/A)(C_{in}-C) + k_L\, a\, (C_g^*-C)]/[\mu(C_{in}-C) + k_L\, a(C_g^* -C)]$ (Figure 2B). Usually $C_{in} \simeq 0$ (or at least $<C$) and recycling decreases cell density somewhat ($D << k_L\, a$) at constant μ because it increases the rate at which substrate is washed out without increasing its inflow rate as much. The maximum productivity attainable is lower in the recycled chemostat. Recycling only increases maximum productivity when $C_{in} > C$. Thus, it magnifies the difference between nutrient concentration in the inflow and outflow changing productivity accordingly.

In light-limited cultures density is unchanged by recycling at constant specific growth rate. The amount of unabsorbed light energy which leaves the culture is the same in either case because the distribution of light contruls μ. The rate at which light enters the culture is independent of dilution rate, so that the same amount of light energy is converted into biomass as long as μ is constant. Density, pigmentation, and average specific growth rate are manifestations of the physiological state of the population. Density and pigmentation determine the distribution of light so that once a prescribed steady-state growth rate is attained, these assume values which are independent of dilution rate. Maximum productivity is determined by the incident light intensity and is unaffected by recycling.

From graphs like those in Figure 2A-C, one can infer what recycling does to biomass densities and substrate levels when the system dilution rate is held constant. For the case shown in Figure 2A, when recycling is initiated (keeping D constant), the cell density increases until the organisms have lowered the growth rate-controlling substrate level such that $\mu_r = A\mu_{nr} = AD$. Since the substrate level has been changed intracellularly (variable yield) and often extracellularly, X_r is not equal to X_{nr}/A. It is greater than that unless the apparent yield factor decreases substantially at the lower growth rate due to processes like endogenous metabolism, maintenance, and cell death. In these instances, the cell density may be less than X_{nr}/A at the lower growth rate.

When a sparged nutrient is limiting, the increase in biomass density with recycling at constant D is less than when light is limiting which is less than when a dissolved nutrient is limiting. This can be seen from the negative, zero, and positive slopes respectively of the arrows connecting points of equal μ on the non-recycled and recycled X versus D curves (Figure 2A-C).

As mentioned above, for a given set of conditions (feed composition, pH, incident light intensity and quality, partial pressures and sparging rates of gases, temperature, etc.) different nutrients may be limiting at different dilution rates. When different types of nutrients, say light, CO_2, and dissolved nitrogen are all in short supply, recycling may change which nutrient is limiting (Figure 2D). At each dilution rate it makes light more limiting relative to a nutrient dissolved in the inflow, and CO_2 more limiting relative to either one ($C_{in} < C$).

Throughout this discussion it has been assumed that $X_1 > X$, that is, biomass is concentrated and fed back to the reactor in a recycle stream which is more concentrated than the reactor. This, along with the condition that the biomass recycled must be concentrated from the reactor effluent, leads

to the requirement that $0 < A < 1$. The hydraulic dilution of inflow to the reactor with effluent from the reactor is $\alpha/(1+\alpha)$. The ratio of feed biomass density to reactor biomass density is $\alpha X_1/(1+\alpha)X$. $X_1 > X$ implies $X_{feed}/X > \alpha/(1+\alpha)$. That is, more biomass is fed back relative to the harvested effluent liquid. If $X_1 < X$, biomass is fed back in a recycle stream which is less concentrated than the reactor and $\alpha/(\alpha+1) > X_{feed}/X$. More of the harvested effluent if fed back relative to biomass. This is called effluent recycle. The definition implies that $1 < A < (1+\alpha)D$ and thus at steady state $\mu = AD > D$. Since D was defined as the dilution rate of the entire system, $(1+\alpha)D$ is the dilution rate through the reactor whether biomass recycling or effluent recycling is practiced. The former makes cell detention time longer than hydraulic detention time while the latter does the opposite. Arguments similar to those above, with $A > 1$, show that effluent recycle always decreases cell density at fixed dilution rate. It decreases maximum productivity if the limiting nutrient is dissolved in the feed, leaves it unchanged if light is limiting, and increases it if a sparged nutrient is limiting (and $C_{in} < C$). Since all of the changes are in opposite direction for biomass versus effluent recycling, the latter makes light less limiting relative to a dissolved nutrient and a sparged nutrient less limiting relative to either. Both types of recycling are cases of separation of cell versus hydraulic detention times.

In the absence of substrate inhibition ($d\mu/dS > 0$ for all finite S), biomass recycling at constant D can lead to more stable operation of a chemostat if the amount of biomass recycled is kept constant. Density fluctuations are damped by opposing changes in the ratio X_1/X (X_1 is a constant) as well as by changes in the rate of substrate consumption. If the recycle fraction, X_1/X, is kept constant, then density fluctuations may be increased at first.

The damping effect due to substrate consumption depends on the rate of change of growth rate and yield (if variable) with respect to substrate level, and on the biomass density. Since different types of limiting substrate affect biomass density differently with recycling, the return time from small perturbations is affected differently. These same considerations apply to effluent recycle, especially if a significant amount of biomass is recycled with the effluent. With inhibition at higher levels of external substrate, there are many possible outcomes (9). Biomass recycling can increase the range of dilution rates over which the chemostat is stable by lowering substrate levels. That is, it may bring the operating point to a region where $d\mu/dS > 0$. Since effluent recycle decreases the range of possible dilution rates (because $D > \mu$), it decreases the range of stable operation.

COMPETITION AMONG ORGANISMS FOR RESOURCES

Some processes such as the mass culturing of algae and sewage treatment involve open systems with mixed populations of organisms. Competititve exclusion requires that when organisms compete for a single limiting nutrient in ideal continuous cultures one organism will become predominant. In a chemostat at a fixed dilution rate one organism can absorb the limiting nutrient at a faster rate, lowering its availability to the less competitive organism. That is, if specific growth rate is plotted against extracellular substrate concentrations for each of two competing organisms, the one which leaves the least residual substrate at a given μ (=D) will exclude the other. These plots are often assumed to be rectangular hyperboles, but this is not generally the case. The growth rate is determined by levels of internal nutrient pools (10,11,12,13,14) and only indirectly by external substrate concentrations. The rate of nutrient uptake, and often the mechanisms involved, are determined by external and internal nutrient levels (13,15,16 17,18). However, even if the relationship between growth rate and internal

nutrients is hyperbolic and even if this is also true of the relationship between the rate of uptake and external nutrient concentration, the overall function for μ in terms of external nutrient is not a rectangular hyperbola unless the maximum uptake velocity and the K_M for uptake are true constants for all values of growth rate. Both have been found to vary with specific growth rate (13, 15, 16, 17). The maximum uptake rate for a particular mechanism is sensitive to the degree of nutrient deficiency. Dual uptake mechanisms, with different V_{max} and K_M may also exist. Thus, organisms have many ways of adapting to the degree of nutrient limitation, especially to severe conditions (19). When chemostats were operated over a large range of dilution rates, complicated relationships relating residual external substrate concentration and cell quotas, to growth rates emerged (20), including what has been called fast and slow adapted growth (13, 20). The same organisms apparently exhibited two maximum growth rates depending on what range of dilution rates was studied.

Since, at a particular growth rate, it is the rate of nutrient uptake that determines the outcome of competition, there can be many strategies for the subsequent use of the nutrient after absorption. The nutrient can be used to make biomass, pigments or uptake enzymes, extracellular products for scavenging nutrients, or excretion products which inhibit the growth of other organisms.

The outcome of competition between organisms can be affected by recycle. Selective biomass recycle can allow dominance of the normally less competitive organism or the stable coexistence of two organisms, in definite proportions, on one limiting nutrient. Interactions between organisms can be studied in these coexistence states.

Figure 3 presents a flow diagram and equations for a continuous culture with two organisms, a concentrator, and a recycle loop. The single limiting

nutrient may enter with the feed or independently of the hydraulic inflow. If the concentrator is not selective ($X_1^a/X^a = X_1^b/X^b$, i.e., does not distinguish between the organisms), the outcome of competition may change with biomass recycling according to the changing relative effectiveness of nutrient absorption by each organism. One organism may be more effective at low concentration of the limiting nutrient and the other more effective at higher concentrations. This is often discussed in terms of dilution rate control over species competition. (21,22,23,24,25).

An organism with a lower specific growth rate at all levels of limiting nutrient can still exclude the normally more competitive organism if it is selectively recycled. This requires a selective concentrator (i.e. $X_1^a/X^a > X_1^b/X^b$). Two examples of selective concentrators are sedimentation chambers which select by settling characteristics and screens which are size selective. Since these are often the most economical methods to concentrate microbial biomass, it can be advantageous for those types of organisms which are best concentrated by them to become dominant. Selective biomass recycling of one organism (say organism a in Figure 3) allows it to grow at a specific growth rate which is lower than the rate of dilution ($\mu = A^aD < D$). Thus, in a chemostat, organism a increases in density and depresses the concentration of limiting nutrient, thereby competitively excluding the non-recycled organism. The amount of recycle required is determined by the relationship between specific growth rates such that $\mu^a/A^a = D > \mu^b/A^b$. In this way an organism which would be predicted to dominate in a non-recycled chemostat could be eliminated or coexist in a selectively recycled chemostat.

To assure stable operation of a mixed culture it is necessary to keep the recycle parameter (X_1^a or X_{feed}^a) constant (4,6), rather than the recycle fraction discussed by Herbert (1). Otherwise an increase in the chemostat density of the recycled organism is augmented by an increase in the

amount recycled (in order to keep X_1^a/X^a constant). By controlling the recycle concentration, an increase in X^a lowers the ratio, X_1^a/X^a, which decreases dX^a/dt and dampens the fluctuation. Steady state coexistence is thus stabilized by recycle. Without recycle the coexistence states are only semi-stable (one eigenvalue zero, the other negative) at best (26). By varying the amount of recycle from zero to a critical amount (above which the recycled organisms takes over), all proportions of the two organisms are attainable.

EXPERIMENTAL MATERIALS AND METHODS

Organisms and Culture Conditions

Spirulina geitleri was chosen as the recycled organism because its filaments are easily concentrated by straining through a nylon screen with 26 μ openings. Typically, the filaments were about 6 μ in diameter and 100 to 400 μ long. Mean filament length was greater at lower growth rates. The filaments were like sine waves but with a small helicity. Wavelength was about 70-80 μ. A *Chlorella* species isolated from a sewage oxidation pond in Woodland, California, was used as the competing organism since it grew well at high pH and was easily separated from the *S. geitleri* by straining. The diameter of the *Chlorella* varied between 3-8 μ depending on the stage of the life cycle. Most cells were 3-5 μ.

Allen and Arnon's (27) blue-green algal medium was used with K_2HPO_4, NaCl, CaCl, $MgSO_4$, and trace metals at one-quarter strength, Fe-EDTA at 1/8 strength and with $NaHCO_3$ (1.25-3.0 gl^{-1}), pCO_2 (.003-.006 atm.), and $NaNO_3$ (.85-2.5 gl^{-1}) adjusted to insure light limitation. The pH was maintained at 8.60 $\pm$ .10 by adjusting the CO_2 content of the gas phase. Air/CO_2 and Ar/CO_2 were mixed using Matheson gas proportioners, humidified and bubbled into the cultures with fritted glass spargers at a rate of approximately 30 liter/hr. There was no foaming. Since the stock cultures of both the *Chlorella* and the *S. geitleri* were not axenic and recycling would be prohibitively difficult

to carry out axenically, the experiments were run non-sterilely. However, the experimental room was kept clean by periodic chemical disinfection and constant germicidal UV irradiation. Bacterial contamination never amounted to more than a fraction of a percent of the total biomass. Microscopic examinations were carried out verifying that algal contamination or zooplankton were never a problem. Concentrated samples were viewed to check for zooplankton. The room temperature was regulated to maintain culture temperatures at 26.0 $\pm$ 1.0°C.

The culture vessels were cylinders with outside diameter equal to 12.5 cm. Typically the vessels held 3600 ml when filled to a height of 32 cm. Wall growth was prevented by removing a culture and cleaning the vessel every three to five days. Level was controlled by L-shaped overflow tubes which withdrew liquid from below the air-liquid interface. This was necessary to prevent unwanted retention (internal recycling) of algae (28) especially filamentous algae, presumably due to surface tension. With a normal overflow tube the concentration of S. geitleri in the effluent was 20 to 50% of the chemostat concentration with denser cultures exhibiting the greatest difference. Even the Chlorella was 5-10% retained.

Cultures were illuminated from one side only by two 8-foot, ultra-high output "Vita-lite" fluorescent lamps placed 25 cm from the culture vessels. The spectrum of these bulbs closely matches the solar spectrum in the photosynthetically active region. Maximum irradiance was 15 W/m^2. The average irradiance was 10 W/m^2 over the illuminated half of the vessel. Irradiance was measured using a Lambda Licor LI-185 light meter calibrated in the visible to the solar spectrum. Cultures were operated under light-limiting conditions. This was tested by increasing the feed concentration of all nutrients as well as the CO_2 partial pressure of the sparged gas, keeping dilution rate, pH and temperature constant. This never resulted in increased cell density or changes in pigmentation. Increasing the incident light intensity always led

to higher cell densities.

Chemostats were operated using Buchler Polystaltic and Multistaltic peristaltic pumps which generally controlled flow rate to within less than a 6% variation over a period of weeks. Turbidostats were not so precise, with density exhibiting a total variation of about 20% around the mean value. Turbidity was measured outside of the growth vessel using a timer-controlled flush and fill sampling method (29). Fouling of the sampling tube still remained the greatest problem.

Recycling

Effluents were accumulated in stirred, darkened vessels sparged with Ar (or N_2)/CO_2. Each day the effluents were measured to determine flow rate and, if required, strained through a nylon screen with 26 μ openings, (Tetko Co., N.J.). Straining efficiency was determined by dry weights and Klett measurements and expressed as concentration of strained algae after resuspension in an equivalent volume divided by the concentration before straining. Since the Chlorella was retained with virtually zero efficiency, separation of the two algae was easily accomplished by straining. The concentrated S. geitleri was resuspended to a specified concentration in the feed vessel which was also stirred, darkened, and sparged with Ar(or N_2)/CO_2. Periodically, samples were withdrawn from the feed vessel just after this procedure and again the next day before wasting the excess. Thus, any changes in dry weight and chlorophyll content during 24 hours of dark anaerobiosis were monitored. Cell densities were determined as Klett units, using a Klett-Summerson Photoelectric Colorimeter with a No. 66 red filter and as ash-free dry weights (dried at 103°C, ashed at 550°C). It was established with the S. geitleri that ash-free dry weight equals (Klett density X 1.27 + 7.5) mg/l (Figure 5C). Recycle concentrations were measured in Klett units, but were frequently confirmed by dry weight. The recycle fraction was defined as the dry weight of the algal suspension in

the feed bottle divided by the dry weight of the culture in the chemostat. However, the controlled parameter (i.e. the parameter which was kept constant) was the concentration in the feed bottle, not the recycle fraction.

Pigment Estimation

Chlorophyll a and b were extracted by boiling for 45 seconds in 90% Methanol /10% water (V/V) at a pH greater than 8. For the green alga, a dichromatic method(30) was used to calculate chlorophyll a and b while Mackinney's (31) extinction coefficient was used for chlorophyll a for the blue-green alga. Phycobiliproteins were extracted from filtered samples by sonicating the filters in distilled water buffered at pH 6.5 with 10 mM potassium phosphate. A Heat Systems W200 R Sonicator was used on power setting 7 at 50% pulsing, for 8 minutes. The sonicates were centrifuged at 30,000 xg for 1 hour at 6°C. Rough estimates of the phycobiliprotein content were calculated from the absorbance at 620 nm (corrected at 750 nm) using $E^{1\%}_{1cm} = 70$. For some samples, a more accurate estimation was done by subtracting out the interference at 652, 620 and 562 nm due to chlorophyll a contamination, and then using the simultaneous equations of Bennet and Bogarod (32) except with the above $E^{1\%}_{1cm}$ for phycocyanin at 620 nm and an $E^{1\%}_{1\ cm} = 12\ 7$ for phycoerythrin at 562 nm. After centrifugation at 100,000 xg for 60 minutes, spectra of duplicate samples extracted in 10 mM phosphate buffer versus this buffer with 150 mM NaCl (as used by the above authors) were within 10%. Using these methods, the phycoerythrin content was never estimated to be more than 1% of the ash-free dry weight. Most likely phycoerythrin was not synthesized by the S. geitleri and these low values were due to systematic errors in the estimation procedure.

Counts

Algal counts were done manually using a hemocytometer. Generally only the Chlorella was counted because the large number of cells per ml improved

the counting statistics. Standard deviations were about 7-8% of the mean when over 3,000 cells were counted and 10-13% when only 1,000 cells were counted. Spirulina counts were much less reliable with standard deviations typically from 20-35% of the mean,or worse if the Spirulina content was very low.

Steady States

Steady states were defined by stable values of dilution rate, culture density (as Klett and ash-free dry weight), and chlorophyll a monitored for at least three detention times or three days, whichever was longer. Error bars on graphs indicate the maximum and minimum values obtained. For the measured quantities, the total error (the maximum value minus the minimum value divided by the mean) was generally less than 10%. Reproducibility of steady states was within about 10%.

EXPERIMENTS WITH BIOMASS RECYCLED, LIGHT-LIMITED, UNIALGAL CONTINUOUS CULTURES

Light-limited continuous cultures of the blue-green alga S. geitleri were operated with various amounts of the biomass recycled. It was empirically determined (at all but the fastest dilution rate without recycle) that only light was limiting growth. The data collected is shown in Table 1. The states were steady in all respects except one. In some states, the Spirulina would have been displaced by the Chlorella if a sufficiently large inoculation of tne latter had been made or, presumably, if one waited long enough. There was usually some Chlorella contamination since Chlorella and Spirulina chemostats were side by side, but less than .5-1% by volume. The steady states with cell density less than 100 mg/l could not be maintained free of Chlorella without recycling for much more than the three detention periods or three days required to establish the unialgal steady state parameters. As indicated in the methods, recycling was accomplished by suspending concentrated biomass in the feed vessel. Therefore, $A = 1+\alpha-\alpha X_1/X = 1-X_{feed}/X$. X_f/X is defined as the recycle fraction.

Biomass density is plotted against growth rate ($\mu = AD$) in Figure 4A for all steady states. The data is consistent with the assumption that physiological state (density, pigmentation, specific growth rate) and light distribution determine each other. From the graph one can approximately assign $\mu_{max} = .052 \pm .002\ hr^{-1}$ or that the minimum average generation time was 13 hours. When the limiting substrate is the energy resource, the substrate mass balance equation can also be written as an energy balance with yield coefficients representing conversion efficiencies. When light is limiting there is no mass balance equation, but one can equate the total absorbed light energy times on overall conversion efficiency to the energy of the biomass produced plus the maintenance energy (33). Figure 4B is a plot of the specific growth rate versus the quotient of the absorbed power and the energy content of the biomass density, assuming a heat of combustion of 5.5 kcal/gm. The intercept on the μ-axis is the negative of the specific maintenance coefficient. For the steady state unialgal Spirulina cultures $m = .005\ hr^{-1}$. The curve is diphasic. The slope of the line through any point and the point (0,m) is the overall conversion efficiency (efficiency of utilization of absorbed light energy, for biomass production and maintenance). This efficiency is constant and equal to .194 up to a specific growth rate of about $.0225\ hr^{-1}$. Then it falls off. The states with $\mu > .030\ hr^{-1}$ are not graphed because not all of the incident light was absorbed by these less dense cultures. It was too difficult to measure unabsorbed light even approximately. Absorption was 100% for all states shown in Figure 4B except those states clustered around $.0250 < \mu < .0300$ for which it was greater than 95%.

The point on the abscissa, with $\mu \simeq 0$, is usually inferred from stationary state batch cultures. In this experiment this point was obtained from a chemostat with $X_{feed}/X > .995$. Since the culture was dense straining efficiency was high ; between 97 and 99% of the algal biomass was retained

on the 26 μ screen. The extra 1-3% needed to reach 100% recycling (and $\mu = 0$) was obtained from the effluent of a culture with $\mu = .0088\ hr^{-1}$. The stationary culture obtained in this way was in steady state, continuously diluted with fresh media. This avoids complications due to the unsteady conditions of batch growth. The point on the abscissa is important to fix because m is really only defined as the specific maintenance coefficient of a non-growing culture. It is an assumption that m is constant at all growth rates. Since cell composition may change with growth rate there is really no reason, a priori to assume that maintenance energy does not.

Productivity, μX, is plotted against μ in Figure 4C . The productivity optimum is broad. Maximum productivity is the same for non-recycled and recycled cultures because the incident light intensity was fixed. Recycling cannot increase it as it can when the limiting nutrient is dissolved in the feed. (1). The net photosynthetic efficiency, defined as the energy content of the biomass produced at a given μ divided by the incident light energy, is of course less than c, the overall conversion efficiency of absorbed light. The net efficiency can be obtained from Table I by multiplying the productivity values by .037. Net photosynthetic efficiency at maximum productivity was about 0.16. All of the incident light was in the visible.

In general, several reasons can be given for the decline in c with increasing specific growth rates. Photorespiration can drastically reduce efficiency at higher μ if CO_2 is limiting because the more dilute cultures are subject to larger zones of high light. Photorespiration was not a factor in this experiment since doubling the CO_2 partial pressure while keeping pH constant never had any effect. Efficiency is also decreased in more dilute cultures if the zones of light intensity higher than saturating are increased. In these zones photons are absorbed but the energy is dissipated as heat because the conversion enzymes are saturated. Although it is often found that at higher steady state growth rates photosynthesis saturates at higher light

with composition changes such as decreased pigmentation. Saturation curves were not obtained for this experiment but changes in composition were minor (see below The low incident irradiance ($15 Wm^{-2}$ max., $10 Wm^{-2}$ avg.) may not have been higher than saturating, and so zones in which photosynthesis was saturated were probably not too significant. Light absorption was not measured for the most dilute cultures, but the overall efficiency was about .15 at $\mu \approx .028\ hr^{-1}$. This reduction from the maximum of .194 at lower μ may have been due in part to the effects of light saturation. Thermodynamically, efficiency should always decrease with increasing growth rate because of the increase in energy dissipation whenever a process rate is increased. Energy dissipated per unit of biomass produced must be greater at higher μ whether the growth reactions are occurring at a faster rate or whether the number of reactions per unit of biomass (a change in cell composition) is increased.

Figure 4D shows that, at a fixed dilution rate, recycling may increase or decrease productivity, under light-limitation, depending upon whether it brings the density closer to or further from the optimal density. When the limiting nutrient is dissolved in the feed, recycling always increases productivity at a given D.

At the onset it was not known whether there would be a lag time when cells stored in darkened, anaerobic feed bottles were reintroduced into the illuminated, aerated culture vessels. Since, for the same μ, the recycled cultures attained about the same density as non-recycled cultures (Figure 4A any lag time effects were less than the experimental reproducibility of about 10%. A turbidostatically controlled culture attained twice the dilution rate when recycled 50% (I-3 and I-8 in Table I). This is what would be expected if there was no lag. The turbidostat keeps cell density constant, which keeps specific growth rate constant by controlling the light distribution. With 50%

recycling, $\mu_r = D_r/2$. Since a turbidostat keeps μ_r equal to μ_{nr}, D_r must become twice D_{nr} at steady state. This is true to the extent that storage of the biomass to be recycled does not change either pigmentation (which would change light levels) or introduce a lag time (decrease the growth rate). Although the turbidostat runs indicated that there was no lag, errors were large with turbidostatic operation. Table II compares analysis of algae before and after a period of 24 hours of dark anaerobiosis. The differences were small. Hence, it can be concluded that there was only a small (undetected) lag time, if any.

Figure 5 shows pigment concentrations as a function of ash-free dry weight (ash averaged 8% of the total dry weight) for steady states. Chlorophyll a increased linearly with dry weight, averaging about 2% of the ash-free dry weight (Figure 5A).

Since the phycobiliprotein content was only measured approximately, the linear relationship with dry weight (Figure 5B) may be only approximately correct. Allophycocyanin plus phycocyanin averaged 12.4% of the ash-free dry weight. Algal cultures have generally been observed to exhibit self-shading adaptation (34,35). As the density increases (with fixed incident irradiance) the percentage of pigment also increases. This definitely was not the case in this experiment with Chlorophyll a content. Allophycocyanin appeared to be a constant % of the dry weight also, but its estimation was subject to possible error. There is some indication (see Table I) that the % of phycocyanin did increase with dry weight. In any case, adaptation to shading did not seem to be significant. The density was varied over several orders of magnitude. However, the incident light intensity was low. In general the decrease in pigmentation is more pronounced in the range of light intensity above the saturation value (36). The correlation between Klett density and ash-free dry weight is shown in Figure 5C. The high degree of correlation(r^2=.998) indicates that on can quickly and accurately determine biomass concentration using this colorimeter

COMPETITION BETWEEN TWO ALGAE IN LIGHT-LIMITED CONTINUOUS CULTURES

Figure 6 shows the three types of steady states that can be obtained by recycling different amounts of Spirulina: a unialga Chlorella culture ($\mu^a/A^a < \mu^b/A^b$), a mixed culture, with 90% Spirulina and 10% Chlorella ($\mu^a/A^a = \mu^b/A^b$), and a unialgal Spirulina culture ($\mu^a/A^a > \mu^b/A^b$). These steady states resulted from setting X^a_{feed} = 0, 57 and 130 mg/l respectively. Single species chemostats were operated at the same dilution rate and then intermixed. The increase of the more competitive alga from a small inoculum is shown in Figure 7. In Figure 7A the recycling fraction was very large until a sizeable population of Spirulina was established. Since the Chlorella culture had been recently isolated, continuous cultures of this alga were operated throughout the experimental period to monitor any changes in culture characteristics. Table III shows the results from these chemostats and turbidostats. A small increase in average cell size (dry weight/cell) was observed as well as decreases in pigmentation and dry weight per Klett unit. None of these changes occurred quickly enough to affect the analysis of competition experiments. These trends continued in Chlorella chemostats that were operated after this experiment was terminated.

The biomass concentrations of the organisms in the coexistence steady states assume definite values determined by the input level of nutrients, the dilution rate, and the amount of recycle. In principle they can be calculated for any dilution rate given the substrate level in the chemostat, the relationships between the specific growth rates and substrate level, and the amount of recycle of each organism. This information gives D, μ^a, X^a_{feed}, μ^b, X^b_{feed} from which X^a and X^b can be calculated. If one organism is not recycled, then its growth rate-substrate relationship need not be known. Its biomass density is determined from the yield factors of both organisms (at the particular growth rates) and the steady state substrate balance equation

Thus far it has been assumed that the organisms only interact indirectly through competition for the same substrate. In the absence of additional interaction, the light levels in the mixed culture experiment shown in Figure 6B, in which only the Spirulina was recycled,should have been the same as in the unialgal Chlorella culture (operated at the same dilution rate) since for the Chlorella μ was equal to D in both cases. Measurement of light levels within a light-limited culture is difficult for several reasons. Optical density of the culture is not a useful measure of extinction since it measures only that fraction of the scattered light intercepted by the detector. The undetected scattered light is still available for absorption by cells. The optical density at 750 nm of the unialgal, recycled Spirulina culture in Figure 6C was less than that of the unialgal Chlorella culture in Figure 6A (.73 vs. 1.3) indicating that less light was scattered away from the detector. In situ light intensities were measured approximately by submerging the light probe from the quantum meter in a test tube at corresponding points in the cultures. These readings confirmed that the available light was significantly lower in the recycled Spirulina culture. This was expected since the Chlorella was not competitive at this dilution rate with 130 mg/l of the Spirulina recycled. The optical densities together with the underwater light measurements indicate that the Spirulina scattered much less light relative to the amount it absorbed than did the Chlorella. However, a further complication is that light is not truly a single limiting substrate, i.e. the algae compete with pigments of differing absorption spectra. The underwater readings were done on both the absolute energy scale (which is calibrated to the visible solar spectrum) and the photon counting scale. The average energy per photon was the same, within 0%, whether the readings were taken in air in front of the cultures (facing the solar spectrum lamps) or submerged in the culture of blue-green or green algae, indicating that the different energy-frequency distributions had approximately

similar means.

Underwater light measurements of Spirulina and Chlorella cultures with the same Klett density were within 10% of each other. Given the approximate nature of the measurements, Klett is used below as a measure of the light levels in a culture since it correlated well with in situ light measurements. Klett measurements from Chlorella and Spirulina cultures were also strictly additive (that is, 500 ml of a Chlorella culture of Klett = 150 plus 500 ml of a Spirulina culture of Klett = 100 rendered a mixture of Klett = 125).

The Klett densities of steady state coexistence states were less than the corresponding Chlorella cultures run at the same dilution rates. Culture III-3 was mixed into culture I-10 to get the mixed culture of Figure 6B . The Klett right after mixing was 127. It rose 10% shortly thereafter but fell back to about 125 and remained near this value at steady state. The Klett of culture III-3 was about 158. Thus on the basis of Klett density, the Chlorella needed more light in mixed culture than in unialgal culture, to grow at the same rate. The Chlorella biomass of the steady mixed culture can be estimated by using the weight per cell of culture III-3. Of the approximately 172 mg/l mixed culture density at steady state, about 17 mg/l were Chlorella and thus 155 mg/l were Spirulina. Using X^a_{feed} = 57mg/l, D = .0347 hr^{-1}, and X^a = 155 mg/l, the growth rate of the Spirulina was about .0220 hr^{-1} at a total Klett density equal to 125 in mixed culture and .0232 hr^{-1} at Klett 128 in unialgal culture. The recycle fraction was .37 in mixed culture. So the Chlorella grew significantly more slowly in mixed culture than when grown alone, while the Spirulina grew about the same.

In another mixed culture experiment with only 30 mg/l of Spirulina recycled, the Chlorella again failed to drive the Klett density as high as it did when growing alone. The Spirulina grew at approximately the growth rate predicted on the basis of the Klett density of the mixed culture. The recycle fraction was .31. The proportions were 70% Spirulina/30% Chlorella.

Since the nature of this inhibition was not determined we can only speculate as to the cause. It is possible that the Spirulina excreted a growth-restricting chemical into the medium. Experiments using filtrates from Spirulina cultures as growth media for other algae are planned. Also, the different absorption spectra of the two algae lead to varying levels of competition for photons in several bands. The light quality in mixed culture is different from each of the unialgal cultures. This altered spectrum could affect the ratio of reducing power to ATP generated by each algae, which would affect the specific growth rate.

DISCUSSION AND CONCLUSION

The analysis of the chemostat with recycle has been extended and generalized, emphasizing that the basic feature of recycling is the separation of cell, nutrient and hydraulic detention times. Cell detention time can be made longer or shorter than hydraulic detention time by feeding back effluent which has been made more or less dense with biomass than the reactor. Biomass and effluent recycle have opposite effects on the availability of nutrients to the organisms. Availability is definied by the relationship between the rate of entry and exit of nutrient relative to the cell detention time. Differences in availability arise because inflows and outflows of cells and the various kinds of nutrients are related differently to the hydraulic flow. Nutrients which are dissolved in the feed become more available when biomass is recycled at a given dilution rate because the nutrient throughput rate is faster than the rate of cell washout. These nutrients become less available when an effluent depleted of cells is recycled. A gaseous nutrient shows the same trend but to a lesser degree because it is diffused in at a fixed rate but washes out hydraulically. Thus its availability relative to a dissolved nutrient decreases with biomass recycling (and increases with effluent recycling). More generally, nutrient throughput can be made independent of hydraulic detention time if the nutrient can be dosed into the reactor in a concentrated form. Light is totally independent of

hydraulic flow and thus changes in its availability are always intermediate between those for gaseous and dissolved nutrients (Figure 2).

The experiments (Figure 4) with light-limited cultures of S. geitleri demonstrate that in general cell density(and pigmentation)determines productivity because the distribution of light controls specific growth rate. Recycling (biomass or effluent) increased or decreased productivity at a fixed dilution rate depending on whether density was brought closer to or further from the optimal density. Biomass recycling always increased density. Effluent recycling would decrease it since more light is required to support the higher growth rate. The productivity maximum was found to be very broad under the conditions of the experiment. The high maximum photosynthetic efficiency for visible light of 16% for the Spirulina and 20% for the Chlorella was achieved in part because the incident light intensity was near the saturation level and photorespiration was absent. Large losses in maximum photosynthetic efficiency occur when light levels are higher, due to the significant amount of absorption which does not lead to chemical energy conversion.

Cell recycle was possible without continuous concentrating because Spirulina cells were degraded very little during 24-48 hours of storage in darkened, anaerobic effluent and feed vessels. Changes in pigmentation and dry weight were negligible. The absence of any detectable lag time upon reintroduction into the growth vessel indicates that any changes in physiological state were quickly reversible

Special overflow tubes which sampled beneath the air-liquid interface were necessary to prevent internal recycling of the algae. Since this recycling can be significant, caution is required in interpreting the results of continuous culture experiments in which normal effluent ports are used. Neglect of the recycling leads to overestimation of specific growth rate and any quantity derived from it.

Competition between organisms is affected by changes in their detention times. These changes are magnified if the biomass of one of the types is recycled, or if one is selectively excluded during effluent recycling. Effluent recycle can be appropriately labeled "anti-recycle" when considering the excluded organism. Figure 6A and C show how selectively lengthening the detention of one organism, using mechanical methods, reversed the outcome of competition. Biomass or effluent recycle can stabilize coexistence of organisms under circumstances which would normally lead to exclusion (Figure 6B). Interactions between organisms, like the inhibition of Chlorella growth by Spirulina, can be studied if a simple method of recycling can be devised to produce coexistence states.

The separation of hydraulic and cell detention times could have applications in many areas of microbial technology. The mass cultivation of algae is an example where this is an important consideration. Economical harvesting of the algal biomass has been a major problem due to the small size of many algae. In this report , selective biomass recycling of filamentous and colonial sewage-grown algae was studied . These larger forms are cheaply concentrated by microstraining. It was found that most filamentous algae would not grow well in the sewage under the conditions tested and that colony formation was inhibited at dilution rates fast enought to yield high productivity. At moderate dilution rates recycling was unnecessary since colonial algae were predominant without it. However, effluent recycling is effective in achieving high productivity as well as efficient substrate utilization as long as a means of harvesting is available. This is important in integrating sewage treatment with the recovery of nutrients in the form of algal biomass. Effluent recycling is also necessary in single-cell protein production (using Spirulina, for example) where high productivity can only be achieved by using high concentrations of dissolved nutrients making light the limiting factor. In such a process the

nutrients removed by the algae would be added back to the recycled effluent with a small amount of make-up water. The extent of the recycling may be limited by the accumulation of inhibitory products of metabolism and/or lysis. Other means of selection for the desired organisms are required since effluent recycle selects against the harvestable organism.

Cell recycling is practiced in the activated sludge process to increase the rate of sewage treatment per unit of reactor volume (38). High biomass densities are achieved at fast dilution rates using recycle. Sludge recycling may have an effect on species dynamics in activated sludge since it selects for those organisms, or associations of organisms, which settle well. In a laboratory study (5) recycling "greatly enhanced the flocculating and settling characteristics of the cells." The authors suggested that predation, mortality and nutrient depletion in the sedimentation chamber might have been responsible for this. Although differences in conditions in the aeration and sedimentation units, as well as floc formation due to collisions between particles in the mixed reactor (enhanced by the recycled sludge particles acting as nuclei) are significant factors in sludge settleability; recycle is also a factor in selecting for organisms which settle well.

NOMENCLATURE

A	ratio of specific growth rate to dilution rate of system
C	concentration of gaseous substrate dissolved in reactor, $mg\ell^{-1}$
C_{in}	concentration of gaseous substrate dissolved in the system inflow, $mg\ell^{-1}$
C_g^*	hypothetical concentration of gaseous substrate which would be in equilibrium with gas in bulk gas phase, $mg\ell^{-1}$
c	overall efficiency of conversion of light energy into utilizable energy
D	flow rate into the system divided by reactor volume, hr^{-1}
E	net light energy absorbed by a culture, joules
F	hydraulic flow rate into the system, hr^{-1}
k_La	volumetric mass transfer coefficient, hr^{-1}
m	maintenance coefficient, hr^{-1}
Q	cell nutrient quota, mg of intracellular nutrient/mg dry wt. of biomass
S	concentration of extracellular substrate in reactor, $mg\ell^{-1}$
S_{in}	concentration of substrate dissolved in the system inflow, $mg\ell^{-1}$
X	concentration of biomass in reactor, mg dry wt. ℓ^{-1}
X_1	concentration of biomass in recycle stream, mg dry wt. ℓ^{-1}
X_f	concentration of biomass in feed line to reactor, mg dry wt. ℓ^{-1}
V	volume of reactor
Y	yield coefficient, mg mg^{-1}
α	ratio of recycle flow to inflow to system
μ	specific growth rate, hr^{-1}
μ_{max}	maximum specific growth rate, hr^{-1}

TABLE TITLES AND EXPLANATIONS

TABLE I. STEADY STATES FROM UNIALGAL *SPIRULINA* CULTURES

T = 26 ± 1°C; pH = 8.55-8.70;

An asterisk denotes turbidostatic operation rather than chemostatic operation. Allophycocyanin (mg ℓ^{-1}/%VSS) was measured to be 17.8/4.8, 5.8/4.7 & 34.5/4.7 for cultures I-5, I-7 and I-19 respectively. Phycocyanin (mg ℓ^{-1}/%VSS) was 36.1/9.8, 7.8/6.2 and 73.6/9.9 for the same cultures. The phycobiliprotein estimation for culture I-19 was made before steady state was reached, at a density of 730 mg ℓ^{-1}. Values in parentheses indicate the total percent variation (the maximum value minus the minimum value divided by the mean value x 100).

TABLE II. EFFECT OF DARK ANAEROBIOSIS ON ASH-FREE DRY WEIGHT AND CHLOROPHYLL *a*

Numbers are from results of analyses performed on strained and washed resuspensions of effluents which were collected over the preceding 24 hours and from samples obtained after another 24 hours of dark anaerobiosis in the feed vessel.

TABLE III. STEADY STATES FROM UNIALGAL *CHLORELLA* CULTURES

T = 26.0 ± 1°C; pH = 8.55 - 8.70.

Asterisks indicate turbidostatic rather than chemostatic operation. Values in parentheses indicate total percent variation.

APPENDIX II. TABLE I. STEADY STATES FROM UNIALGAL SPIRULINA CULTURES

Culture	Recycle Fraction, X_f/X	Dilution Rate, hr^{-1}	Specific Growth Rate, hr^{-1}	Ash-Free Dry Wt. mg/l	Klett units	Productivity, μX, mg/l	Chlorophyll a mg/l	Chlorophyll a %VSS	Allophycocyanin + Phycocyanin mg/l	Allophycocyanin + Phycocyanin %VSS
I-1	0	.0507(2)	.0507(2)	15	10(10)	.8	--	--	--	--
I-2	0	.0352(5)	.0352(5)	86(6)	59(3)	3.1(1)	1.86(4)	2.15(8)	8.5(10)	9.8
I-3*	0	.0292(17)	.0292(17)	125(4)	85(6)	3.68(3)	2.23(1)	1.78(3)	--	--
I-4	0	.0155(6)	.0155(6)	226(8)	177(8)	3.51(9)	4.59(7)	2.00(7)	--	--
I-5	0	.0086(5)	.0086(5)	334(9)	266(8)	2.9(13)	6.49(5)	1.95(7)	51.5	14.3
I-6	0	.0088(10)	.0088(10)	382(12)	295(10)	3.4(14)	7.21(10)	1.89(6)	53.1	13.9
I-7	.23(10)	.0358(4)	.0280(7)	125(8)	85(10)	3.45(7)	2.43(14)	1.94(13)	14.5(10)	11.0
I-8*	.50(12)	.0575(30)	.0288(30)	137(12)	92(13)	4.0(35)	2.61(13)	1.90(-)	--	--
I-9	.52(7)	.0522(4)	.0251(14)	129(13)	96(5)	3.35(23)	2.47(12)	1.92(9)	16.0(4)	12.4
I-10	.32(8)	.0350(6)	.0238(6)	178(11)	128(7)	4.20(12)	3.30(4)	1.86(13)	21.8(15)	12.2
I-11	.34(7)	.0352(6)	.0232(5)	175(8)	125(5)	4.06(10)	3.24(11)	1.85(5)	21.3(10)	12.2
I-12	.50(2)	.0366(3)	.0184(4)	235(5)	188(4)	4.34(9)	4.70(10)	1.99(6)	30.0(15)	12.7
I-13	.49(2)	.0366(5)	.0185(5)	233(10)	192(5)	4.31(6)	4.73(13)	1.93(13)	29.3(14)	12.6
I-14	.50(3)	.0346(7)	.0172(6)	242(4)	187(3)	4.15(9)	4.94(4)	2.04(7)	33.2(15)	13.7
I-15	.52(9)	.0357(10)	.0171(12)	236(5)	181(7)	4.04(18)	5.02(9)	2.12(10)	30.0(13)	12.7
I-16	.54(1)	.0336(1)	.0155(2)	225(1)	173(1)	3.50(3)	5.41(2)	2.40(3)	--	--
I-17	.50(10)	.0161(6)	.0082(14)	357(5)	286(10)	2.92(22)	8.11(6)	2.27(8)	--	--
I-18	.72(7)	.0354(3)	.0099(18)	356(10)	288(10)	3.60(21)	7.33(9)	2.06(12)	--	--
I-19	.99(1)	.0348(10)	.0001	930(8)	714(8)	<.1	18.1(4)	1.92(11)	112.2	15.1

APPENDIX II. TABLE II. EFFECT OF DARK ANAEROBIOSIS ON ASH-FREE DRY WEIGHT AND CHOLOROPHYLL *a*

	I-7	I-7	I-9	I-9	I-18	I-12
VSS Before/VSS After	1.01	0.97	0.93	1.07	1.05	0.97
Chlorophyll *a* Before/ Chlorophyll *a* After	1.06	1.00	0.99	1.04	1.02	0.99

APPENDIX II. TABLE III. STEADY STATES FROM UNIALGAL CHLORELLA CULTURES

Culture, Date	Dilution Rate hr^{-1}	Ash-Free Dry Wt. mg/l	Productivity mg/l	Chlorophyll a mg/l	%VSS	Chlorophyll b mg/l	% Chlor. a	Klett	Counts
III-1, Jan.-Feb.	.0352(2)	165(10)	5.82(10)	7.4(6)	4.5	2.9(10)	39	165(0)	26165(2)
III-2 Feb.	.0346(4)	162(5)	5.62(5)	7.8(20)	4.8	2.9(11)	37	162(3)	24192(10)
III-3 Mar.	.0349(7)	162(3)	5.59(10)	7.1(4)	4.4	3.0(10)	43	158(7)	22548(6)
III-4 Mar.	.0345(7)	173(2)	5.97(5)	7.3(5)	4.3	3.3(14)	45	153(4)	23214(8)
III-5* Jun.	.0350(12)	162(12)	5.67	6.3(7)	3.9	2.5(12)	40	139(10)	--
III-6* May-Jun.	.0431(16)	101(17)	4.28(18)	3.7(13)	3.7	1.5(18)	39	85(15)	12122(11)
III-7 Apr.	.0534(4)	88(15)	4.72(16)	3.3(13)	3.8	1.4(16)	41	77(8)	11976(15)

FIGURE 1. Flow diagram of a continuous culture with cell feedback and three types of potentially limiting nutrients. Biomass balance equations and steady state conditions are shown for the non-recycled and recycled cases. V-volume of the reactor; F-volumetric flow rate of the system; $D \equiv F/V$ dilution rate of the system; X, X_1 - biomass concentrations in the reactor and recycle stream, respectively; S_{in}, S-inflow and reactor concentrations of a nutrient which only enters the reactor hydraulically; C_{in}, C- inflow and reactor concentrations of a nutrient which is sparged into the reactor as well as entering hydraulically; μ - net specific growth rate, defined as the rate of production of biomass (per unit volume) due to growth, divided by the biomass (per unit volume); α - ratio of the recycle flow rate to the inflow rate; $A \equiv 1 + \alpha - \alpha X_1/X$. If $X_1 > X$, then biomass is recycled and $0 < A < 1$. A dot over a symbol indicates its first derivative with respect to time. nr refers to a non-recycled chemostat. r refers to a recycled chemostat.

FIGURE 2. Sketches of tne steady state reactor biomass density versus dilution rate, with and without biomass recycling. A = .5. Arrows connect points of equal μ.

A. The limiting nutrient is dissolved in the influent.

B. The limiting nutrient is mainly sparged into the reactor, i.e., $C_{in} << C$. $K_L a$ is the mass transfer coefficient. C_g is the concentration of dissolved gas that would be in equilibrium with the sparged gas if no organisms were present.

C. Light is limiting.

D. X vs. D if the input nutrient levels are as in A-C. The limiting nutrient at each D is that nutrient which supports the lowest density. It is assumed that there are no interactions of nutrient limitation. ——— , density limited by the dissolved nutrient; ------- , density limited by light;..... , density limited by the sparged nutrient.

FIGURE 3. Completely mixed continuous culture with two competing organisms (a,b), a concentrator, and a recycle loop. The biomass balance equations around the reactor are shown for both species, as well as three classes of steady states. The inequalities are the minimum requirements for stability of the steady states. See Figure 1 for meaning of symbols.

If $X_1^i > X^i$, then $0 < A^i < 1$ and species i biomass is recycled.

FIGURE 4. Steady states of light-limited Spirulina cultures.

Symbols indicate approximate recycle fractions: ○ - 0.00; □ - .25; △ - .33; X - .50; ◇ - .75; + - 1.00. Error bars indicate maximum deviation from the mean.

A. Cell density vs. specific growth rate (AD).

B. Specific growth rate vs. power absorbed per unit biomass (expressed in energy units).

C. Productivity (μX) vs. specific growth rate.

D. Productivity vs. recycle fraction.

FIGURE 5. Steady states of light-limited Spirulina cultures.

See Figure 4 for meaning of symbols.

A. Chlorophyll a content at steady state. Chlorophyll a, mg/l = (.197 x ash-free dry weight + .11) mg/l, r^2 = .990.

B. Allophycocyanin + phycocyanin content; mg/l = (.124 x ash-free dry weight + 1.13) mg/l, r^2 = .985.

C. Ash-free dry weight = (1.27 x Klett density + 7.5) mg/l, r^2 = .998.

FIGURE 6. Three types of steady states attained in light-limited chemostats with Spirulina geitleri (organism a) and Chlorella sp. (organism b). All chemostats were run at the same dilution rate. In all cases $X^b_{feed} = 0$. All effluent and feed bottles were sparged with N_2/CO_2 (99.5%/0.5%, V/V) to prevent algal respiration and decomposition, and darkened to prevent growth. With this technique, no growth outside of the culture vessel occurred, nor was any lag time due to storage of algae under non-growth conditions observed.

A. Chlorella predominates when $X^a_{feed} = 0$. $D = .0348\ hr^{-1}$. 22,550 cells/ml is the average of days 0-3, prior to mixing in Spirulina (arrow on day 3) which yielded a culture with 45% Chlorella by weight. The steady state attained was 100% Chlorella. At least 3,000 cells were counted in each assay. (Culture I-14 was mixed into Culture III-3 yielding Culture III-4 at steady state).

B. Steady state coexistence when $X^a_{feed} = 57$ mg/l. $D = .0347\ hr^{-1}$. Days 0-3 show Chlorella counts in the unialgal culture of Spirulina before the unicellular alga was mixed in on day 3 (arrow by day 3) resulting in a culture with 70% Spirulina by weight. The steady state composition was 90% Spirulina by weight. $X^a_{feed}/X^a = 0.35$ at steady state. Days 19-21 show Chlorella counts after the entire contents of the chemostat was strained (arrow on day 18). At least 3,000 Chlorella cells were counted for each assay on days 4-19. (Culture

C. *Spirulina* predominates when X^a_{feed} = 130 mg/l. D = .0344 hr^{-1}.

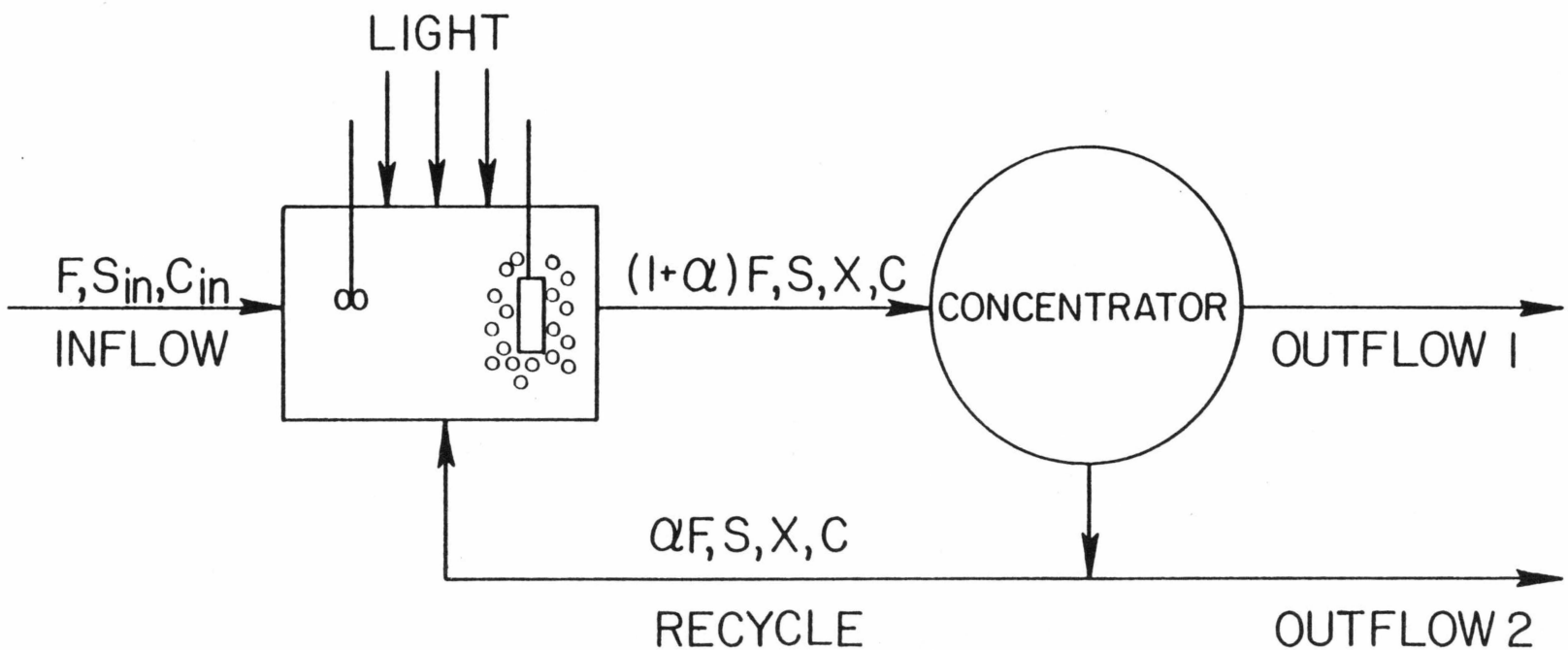
Days 0-3 show harvestability of the unialgal *Spirulina* cultures (about 93% harvestable). On day 3 *Chlorella* was mixed in, yielding a culture with 67% *Spirulina* by weight. The open square on day 10 indicates the results of *Chlorella* counts which showed less than 1% *Chlorella* by weight. (Culture III-3 was mixed into Culture I-14 yielding Culture I-15 at steady state).

FIGURE 7A. Increase of the filamentous algae from a small inoculation (7% *Spirulina* by weight on day 0) into a chemostat (D = .0353 hr-1) with *Chlorella*.

Recycling was variable (as shown). —●— —●— : *Chlorella* counts (approximately 1,000 cells counted). —▲——▲— : *Spirulina* counts (about 100 filaments counted).

B. Predominance of *Chlorella* in a turbidostat (K1 = 85 [12%]) without recycling. The arrow on day 2 indicates mixing *Chlorella* into the unialgal turbidostatic culture of *S. geitleri*, resulting in a culture with 9% *Chlorella* by weight (10% by Klett). Days 0-1 show *Chlorella* counts prior to their being mixed in. The steady state attained is virtually 100% *Chlorella* (*Chlorella* counts: —●— —●— ; *S. geitleri* counts: —▲——▲—). About 1,000 *Chlorella* cells were counted for each assay. The number of filaments counted ranged downward from 200, depending on the amount of *Spirulina* present in the culture.

APPENDIX II. FIGURE 1. FLOW DIAGRAM OF A CONTINUOUS CULTURE WITH CELL FEEDBACK AND THREE TYPES OF POTENTIALLY LIMITING NUTRIENTS.

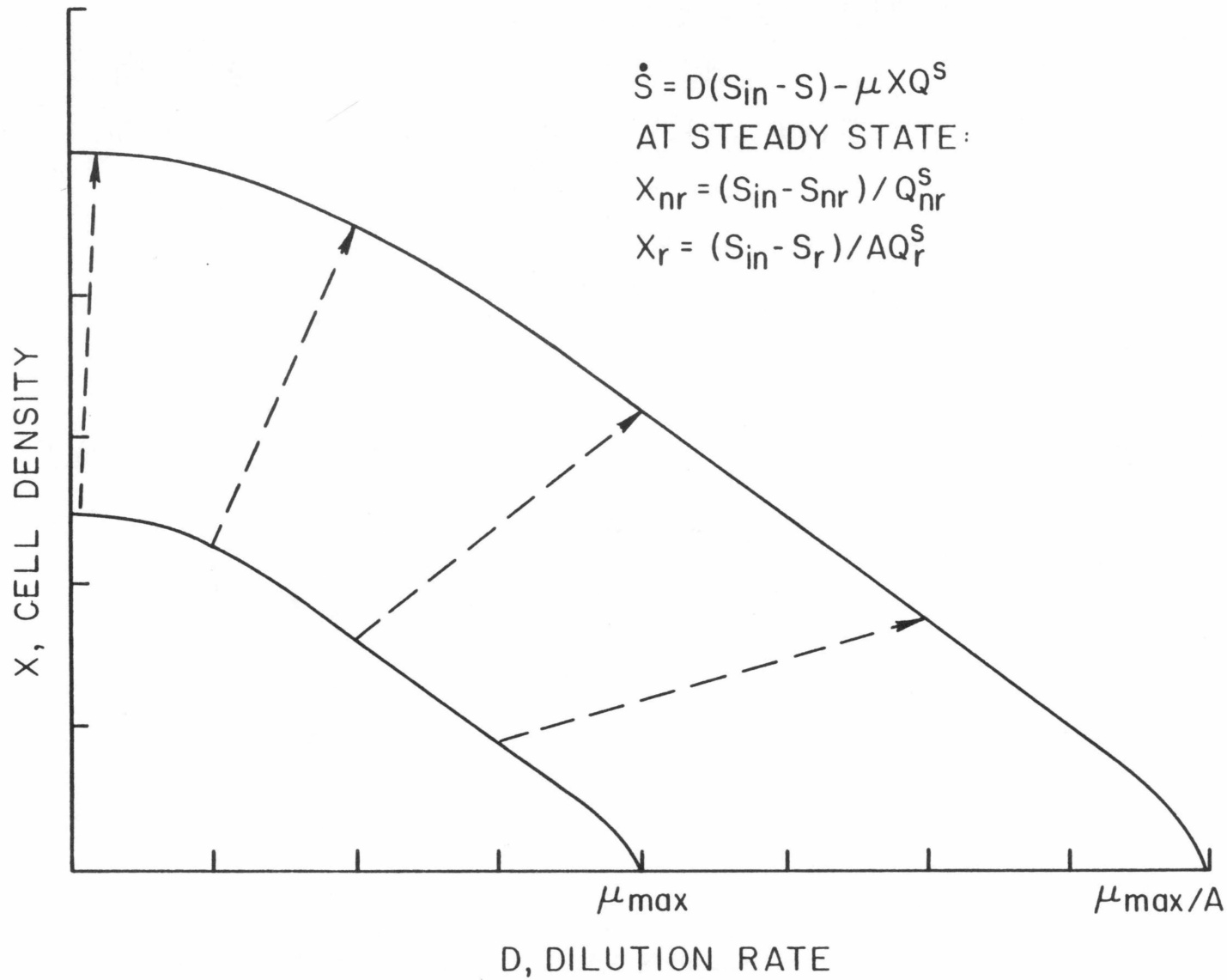


NO RECYCLE: $\dot{X} = \mu X - DX$, AT STEADY STATE $\mu = D$

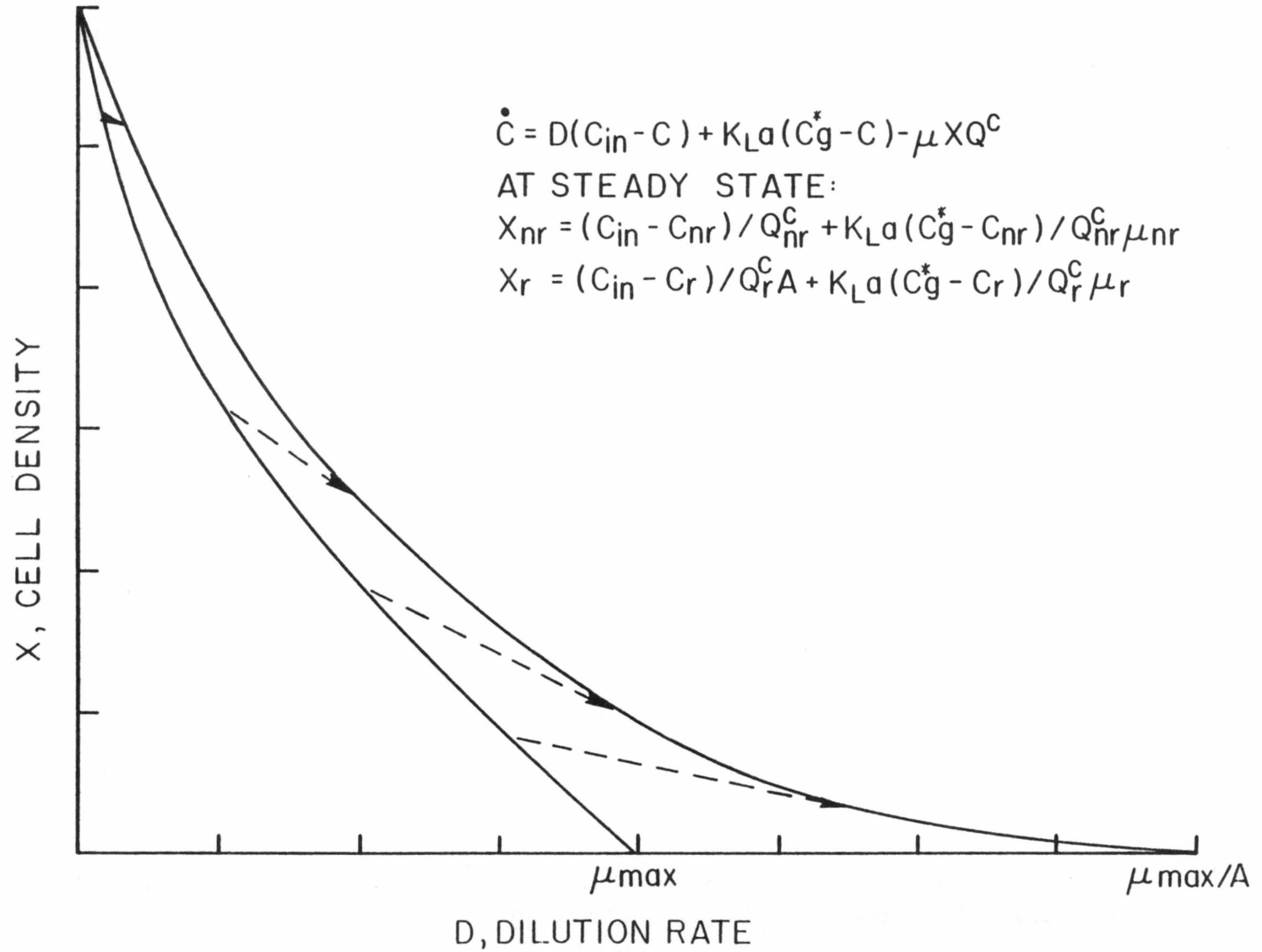
RECYCLE: $\dot{X} = \mu X - (1+\alpha-\alpha X_1/X)XD$

$= \mu X - AXD$, AT STEADY STATE $\mu = AD$

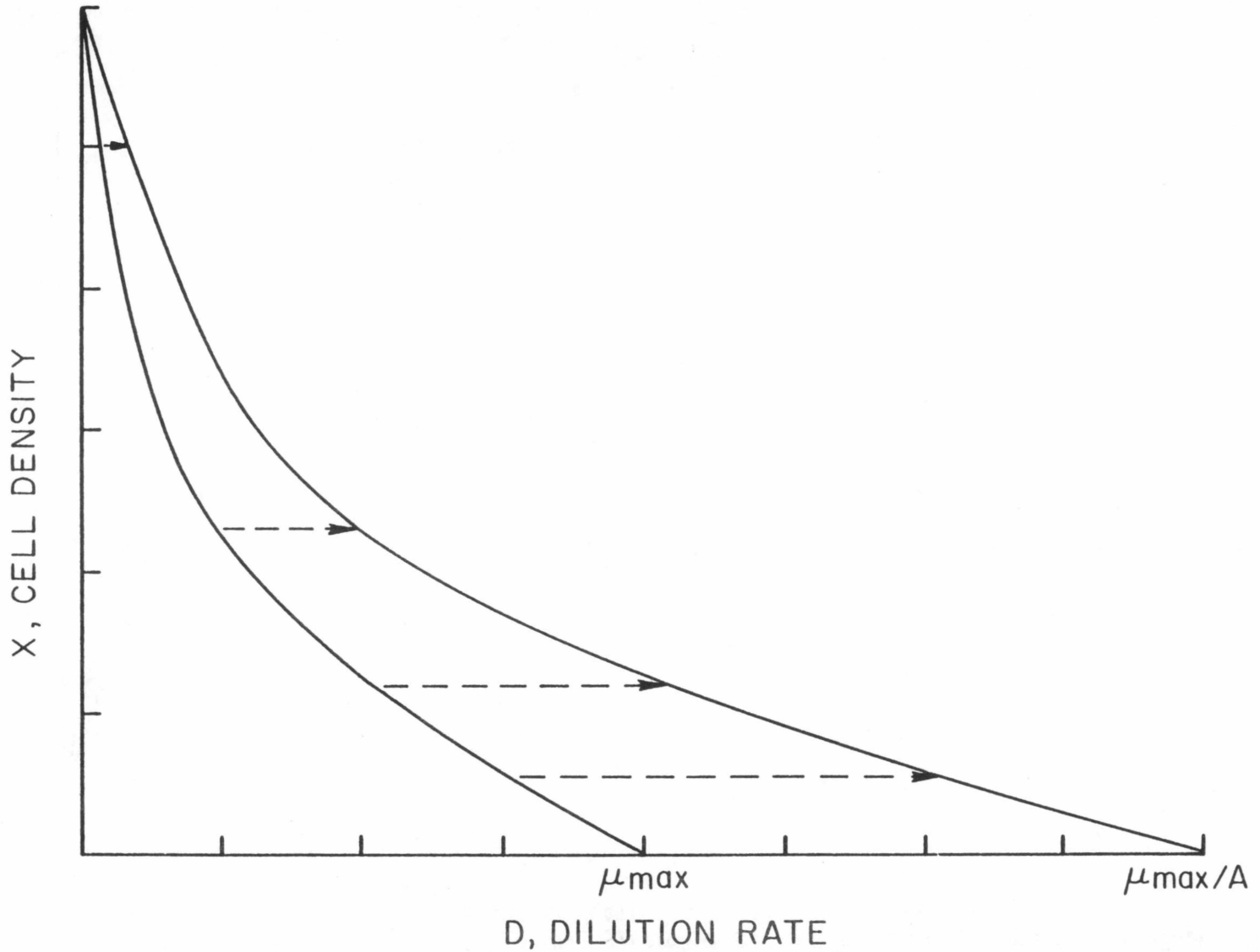
APPENDIX II. FIGURE 2A. SKETCHES OF THE STEADY STATE REACTOR BIOMASS DENSITY VERSUS DILUTION RATE WITH AND WITHOUT BIOMASS RECYCLING



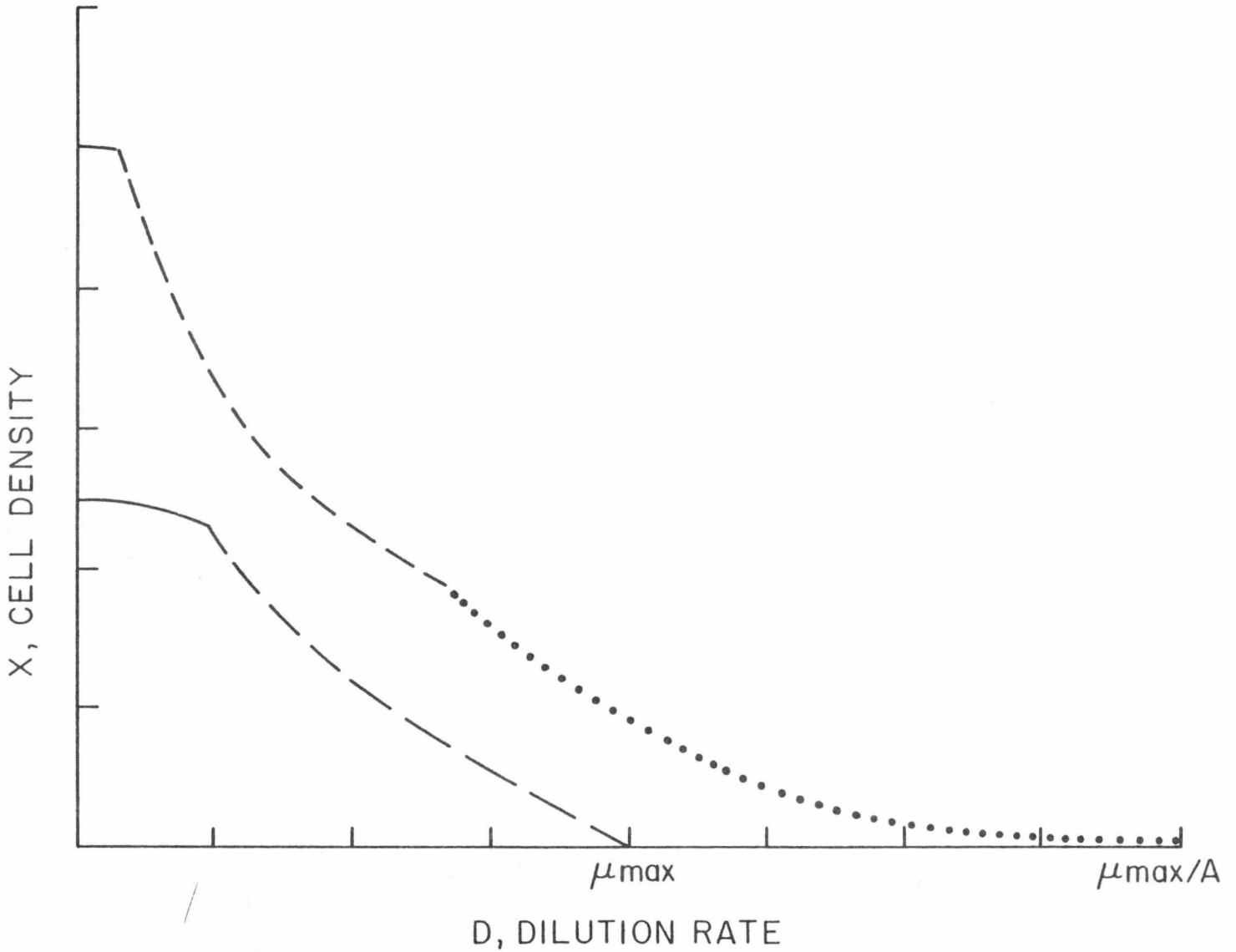
APPENDIX II. FIGURE 2B. SKETCHES OF THE STEADY STATE REACTOR BIOMASS DENSITY VERSUS DILUTION RATE WITH AND WITHOUT BIOMASS RECYCLING



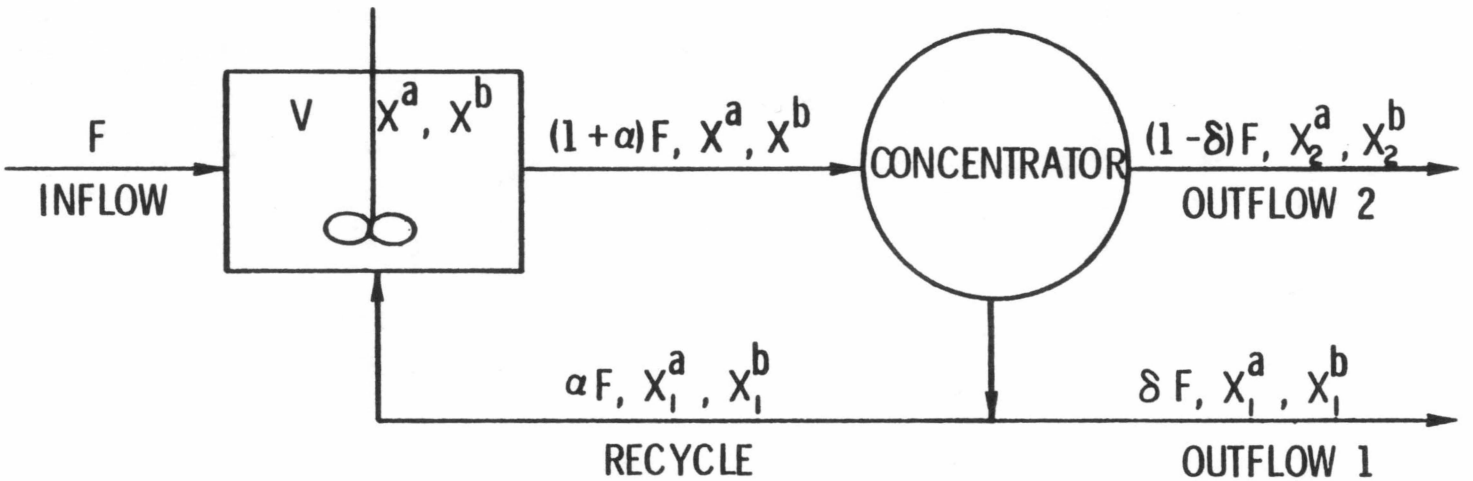
APPENDIX II. FIGURE 2C. SKETCHES OF THE STEADY STATE REACTOR BIOMASS DENSITY VERSUS DILUTION RATE WITH AND WITHOUT BIOMASS RECYCLING



APPENDIX II. FIGURE 2D. SKETCHES OF THE STEADY STATE REACTOR BIOMASS DENSITY VERSUS DILUTION RATE, WITH AND WITHOUT BIOMASS RECYCLING



APPENDIX II. FIGURE 3. COMPLETELY MIXED CONTINUOUS CULTURE WITH TWO COMPETING ORGANISMS (A,B) A CONCENTRATOR, AND A RECYCLE LOOP



$$\dot{X}^a = X^a\mu^a - X^a(1+\alpha-\alpha X_1^a/X^a)D = X^a(\mu^a - A^aD)$$
$$\dot{X}^b = X^b\mu^b - X^b(1+\alpha-\alpha X_1^b/X^b)D = X^b(\mu^b - A^bD)$$

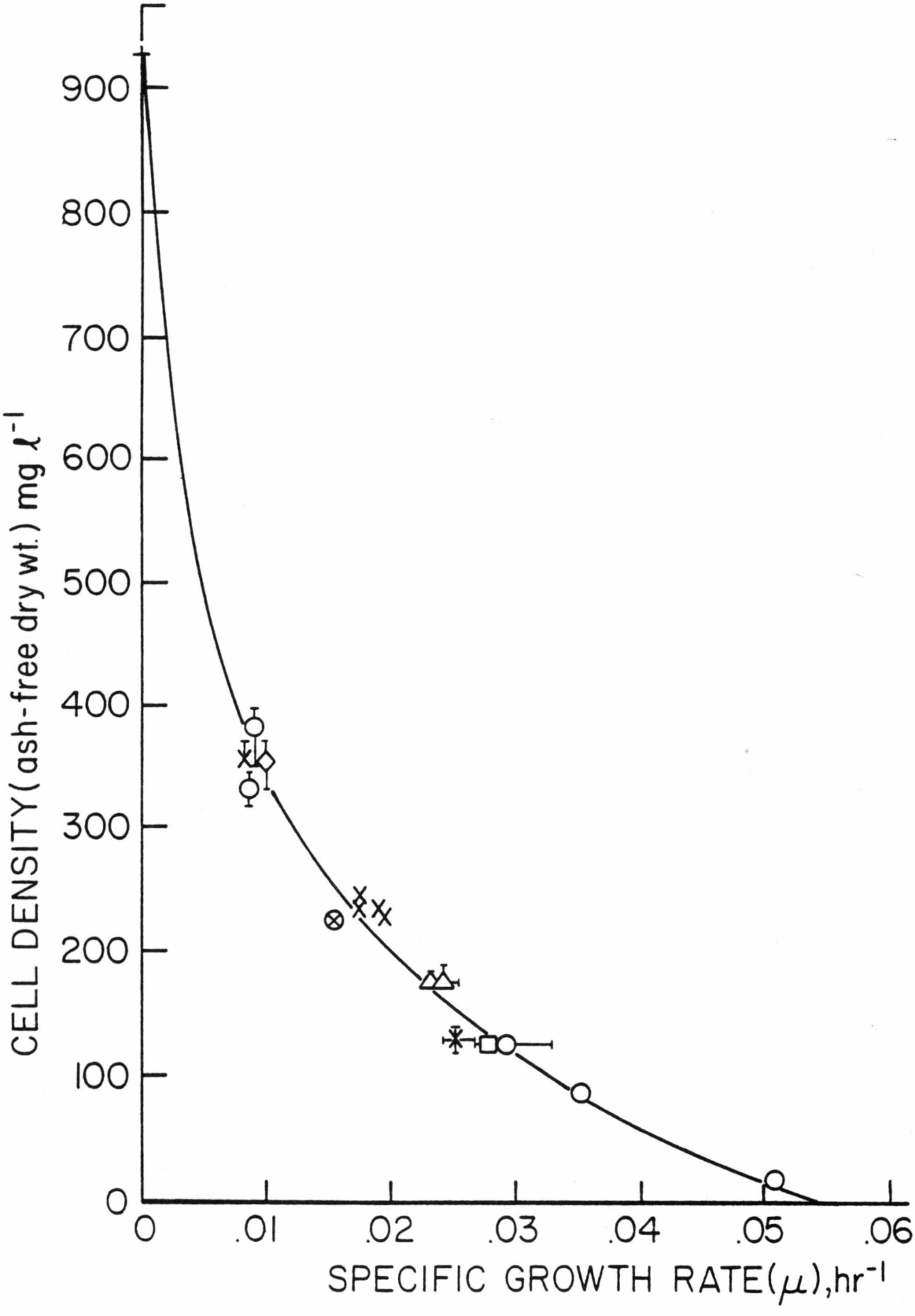
NON-ZERO STEADY STATES:

i) $X^a = \bar{X}^a;\ X^b = 0;\ \mu^a/A^a = D > \mu^b/A^b$

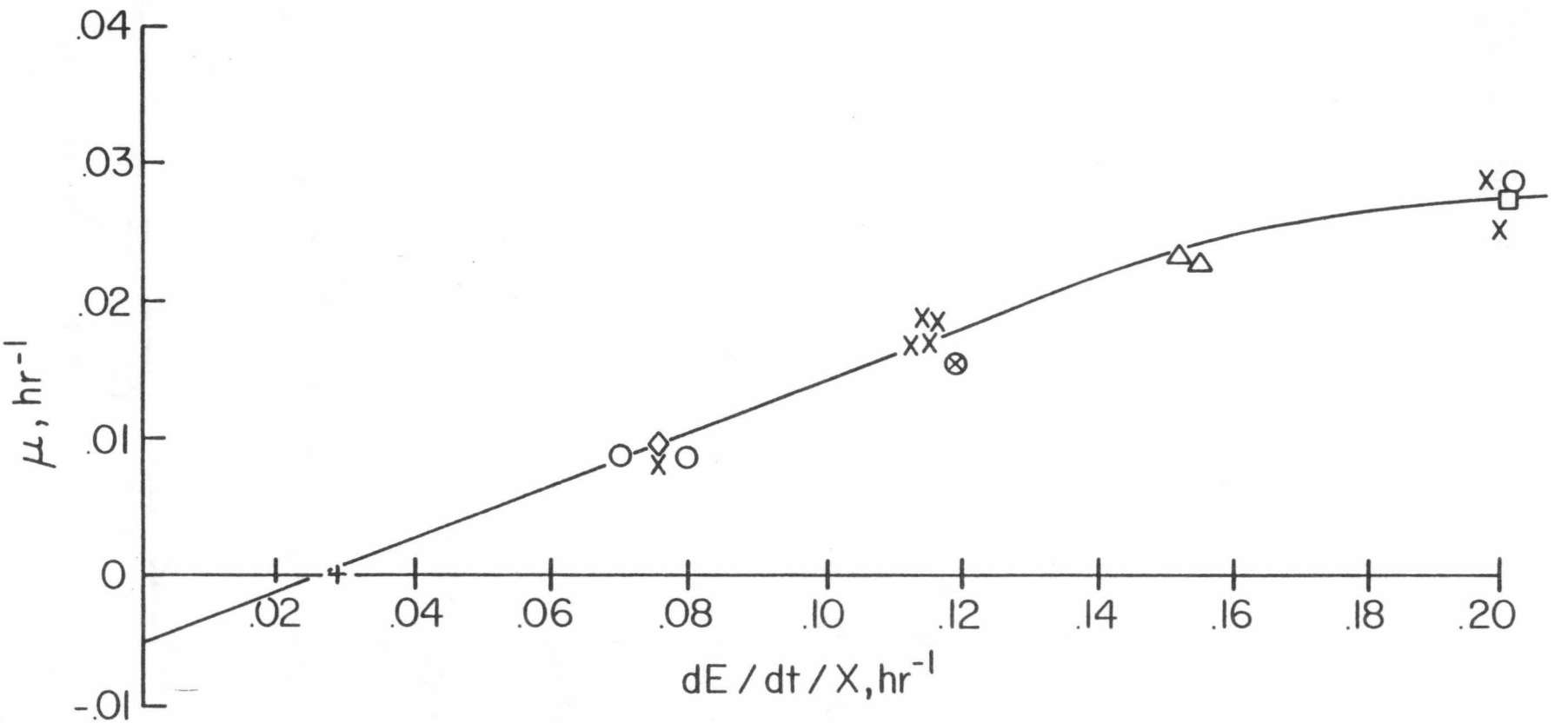
ii) $X^b = \bar{X}^b;\ X^a = 0;\ \mu^b/A^b = D > \mu^a/A^a$

iii) $X^a = \bar{X}^a;\ X^b = \bar{X}^b;\ \mu^a/A^a = \mu^b/A^b = D$

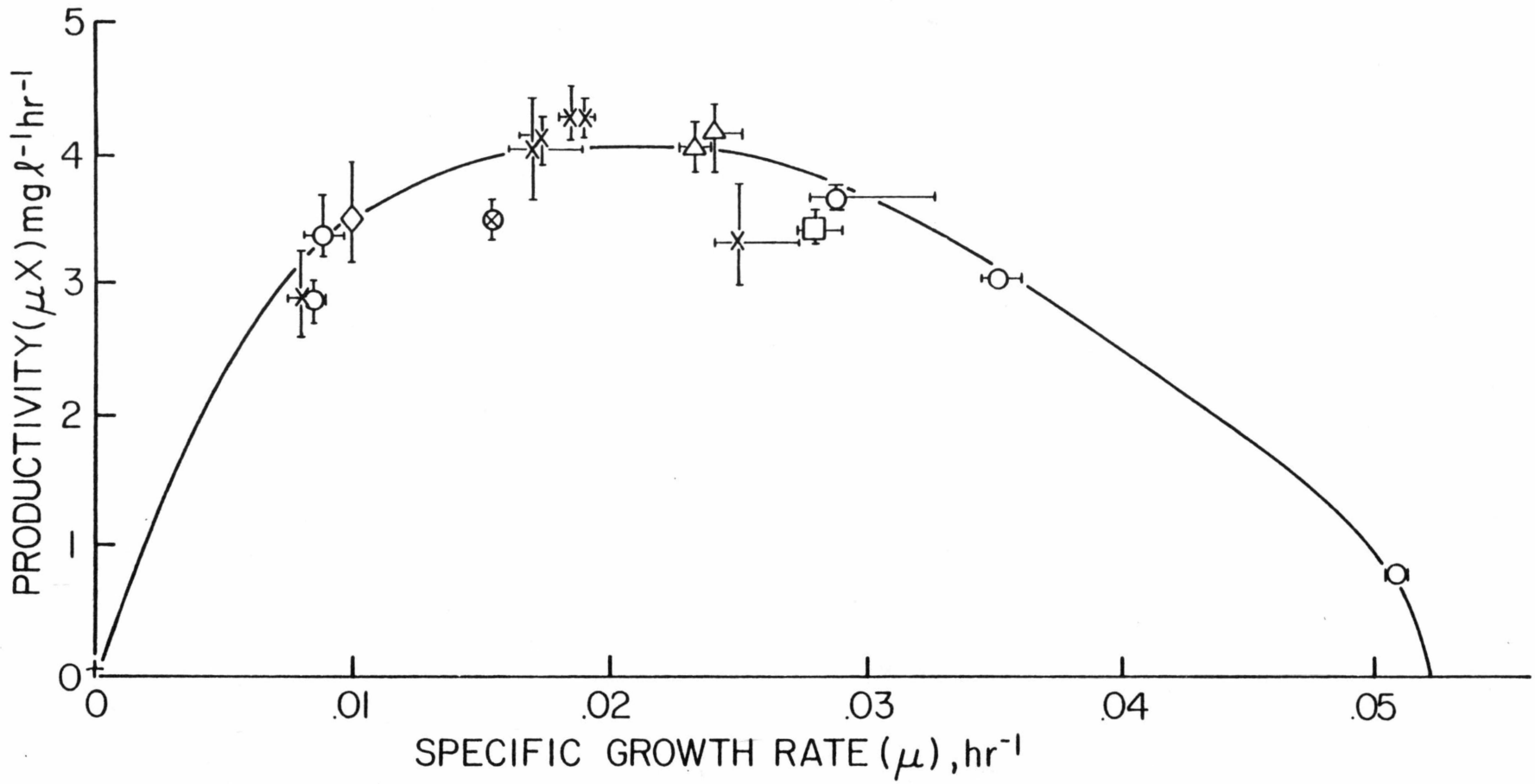
APPENDIX II. FIGURE 4A. STEADY STATES OF LIGHT-LIMITED SPIRULINA CULTURES



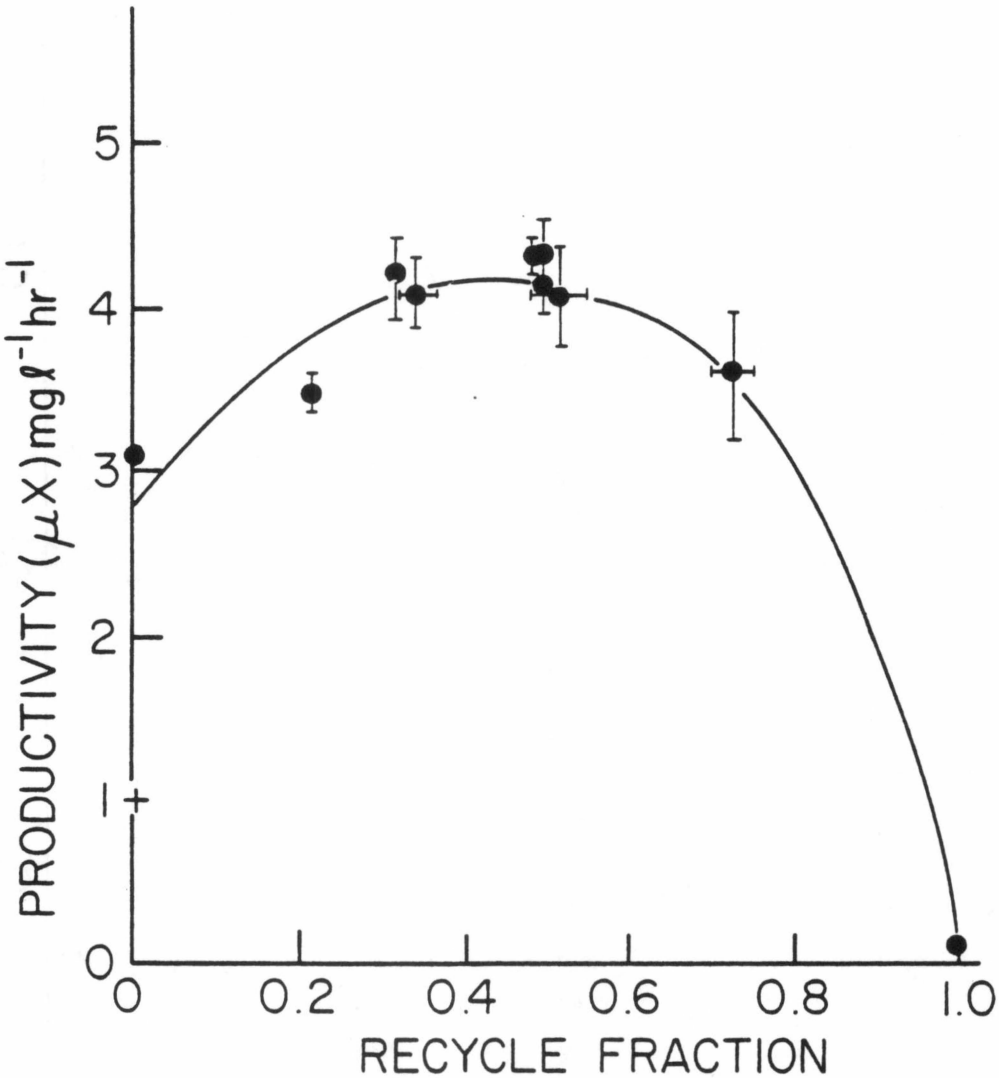
APPENDIX II. FIGURE 4B. STEADY STATES OF LIGHT-LIMITED SPIRULINA CULTURES



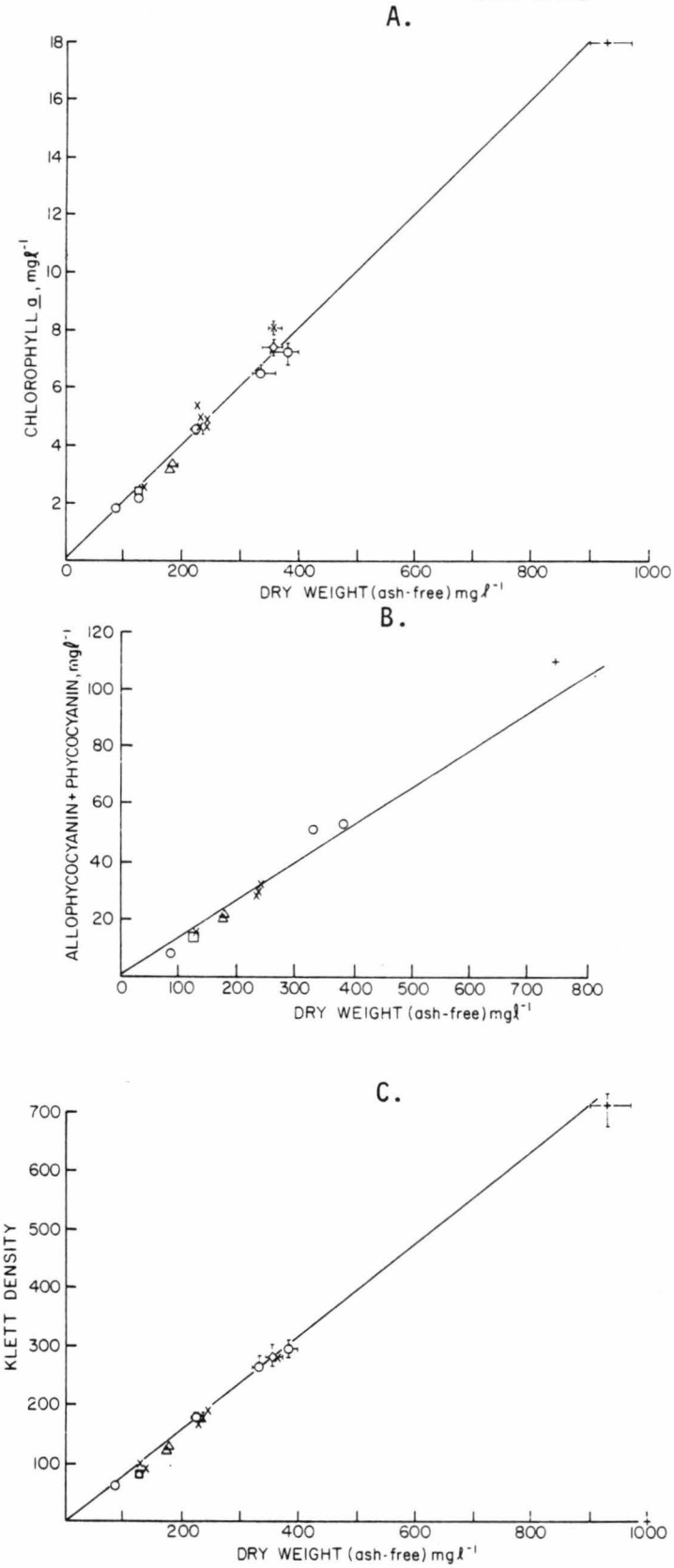
APPENDIX II. FIGURE 4C. STEADY STATES OF LIGHT-LIMITED SPIRULINA CULTURES



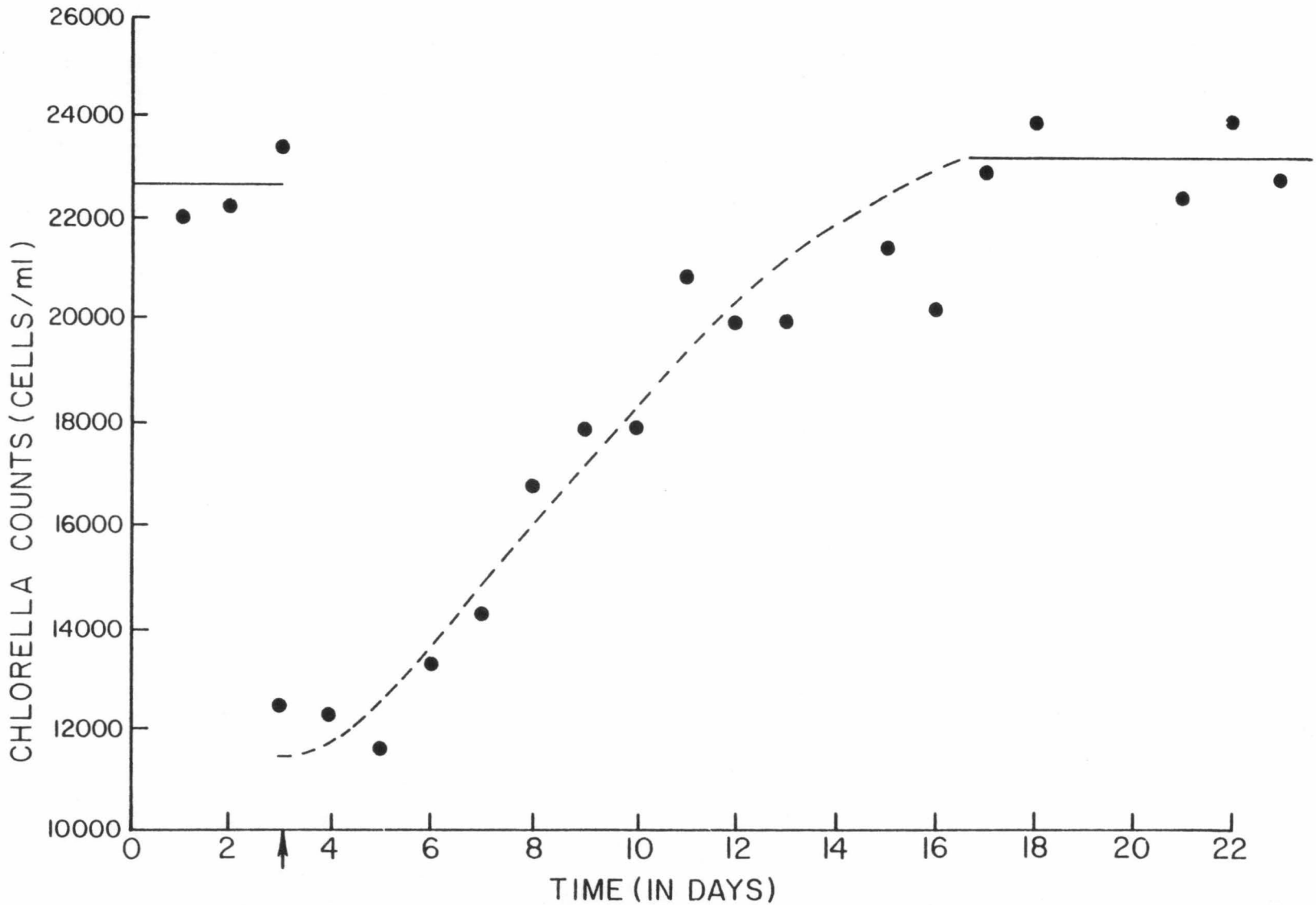
APPENDIX II. FIGURE 4D. STEADY STATES OF LIGHT-LIMITED *SPIRULINA* CULTURES



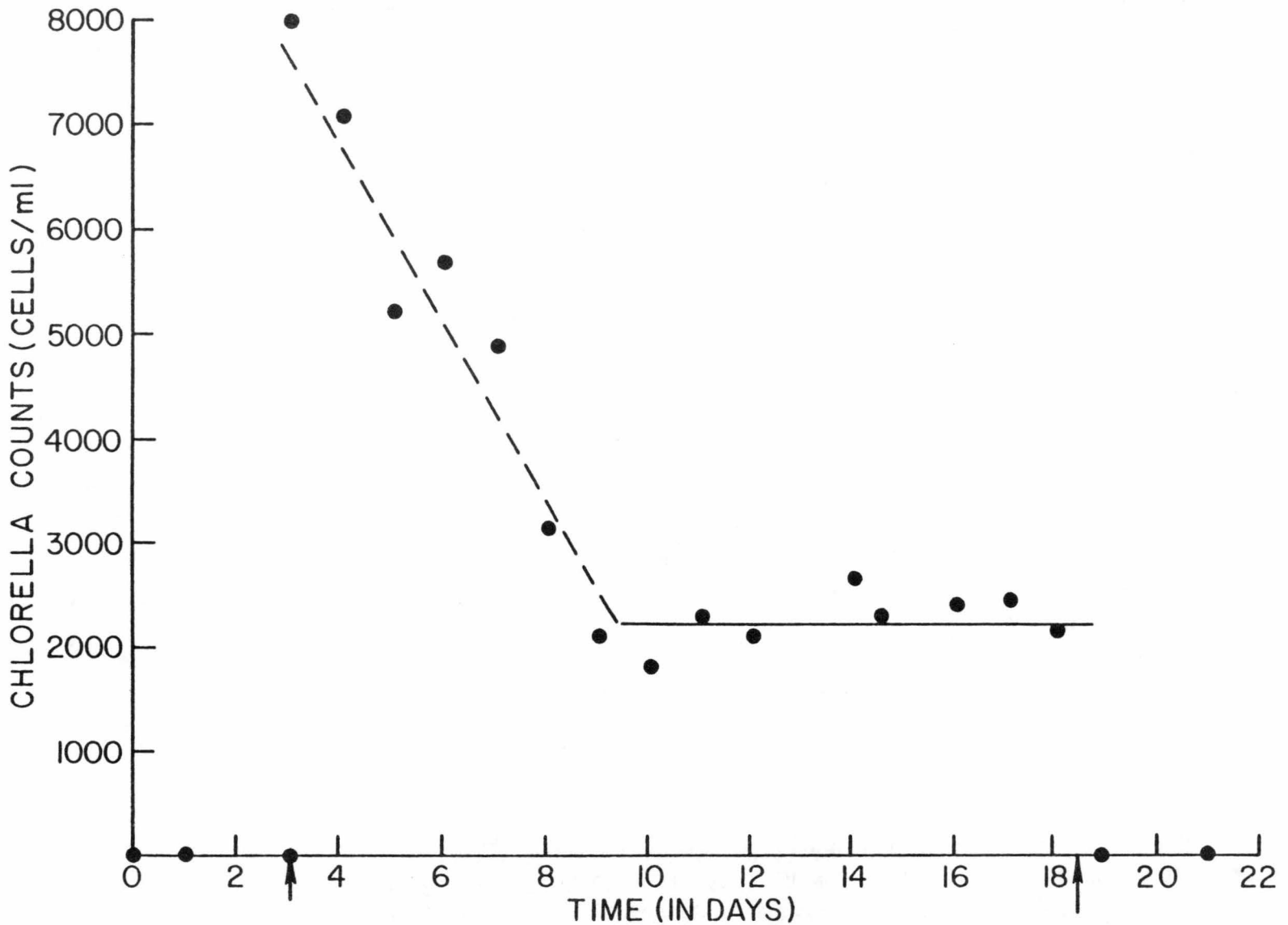
APPENDIX II. FIGURE 5. STEADY STATES OF LIGHT-LIMITED *SPIRULINA* CULTURES



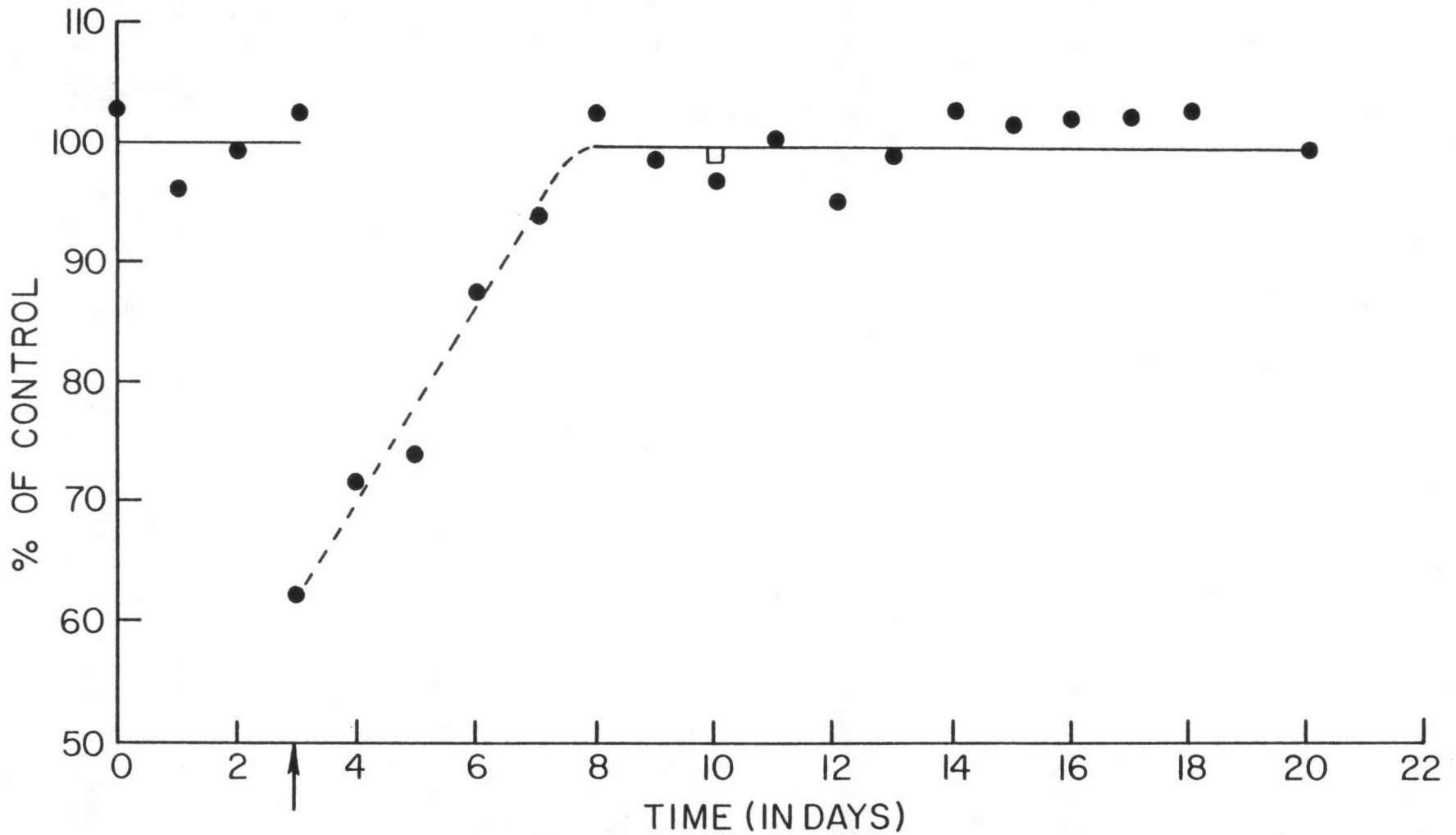
APPENDIX II. FIGURE 6A. THREE TYPES OF STEADY STATES ATTAINED IN LIGHT-LIMITED CHEMOSTATS WITH SPIRULINA GEITLERI (ORGANISM A) AND CHLORELLA SP. (ORGANISM B).



APPENDIX II. FIGURE 6B. THREE TYPES OF STEADY STATES ATTAINED IN LIGHT-LIMITED CHEMOSTATS WITH SPIRULINA GEITLERI (ORGANISM A) AND CHLORELLA (ORGANISM B)

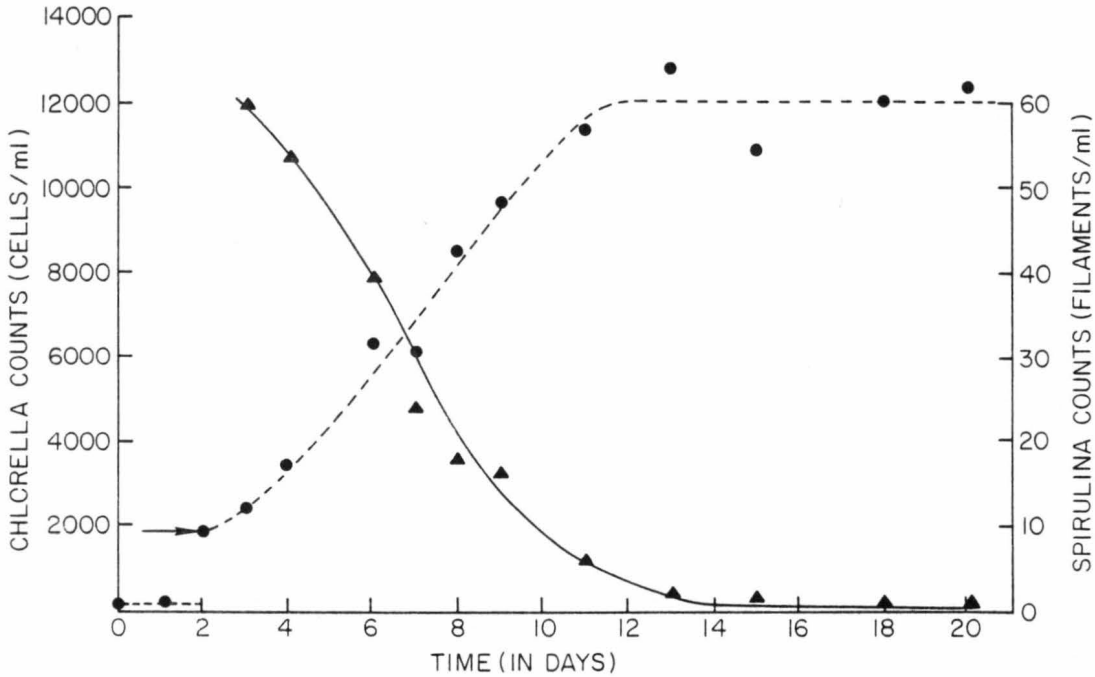


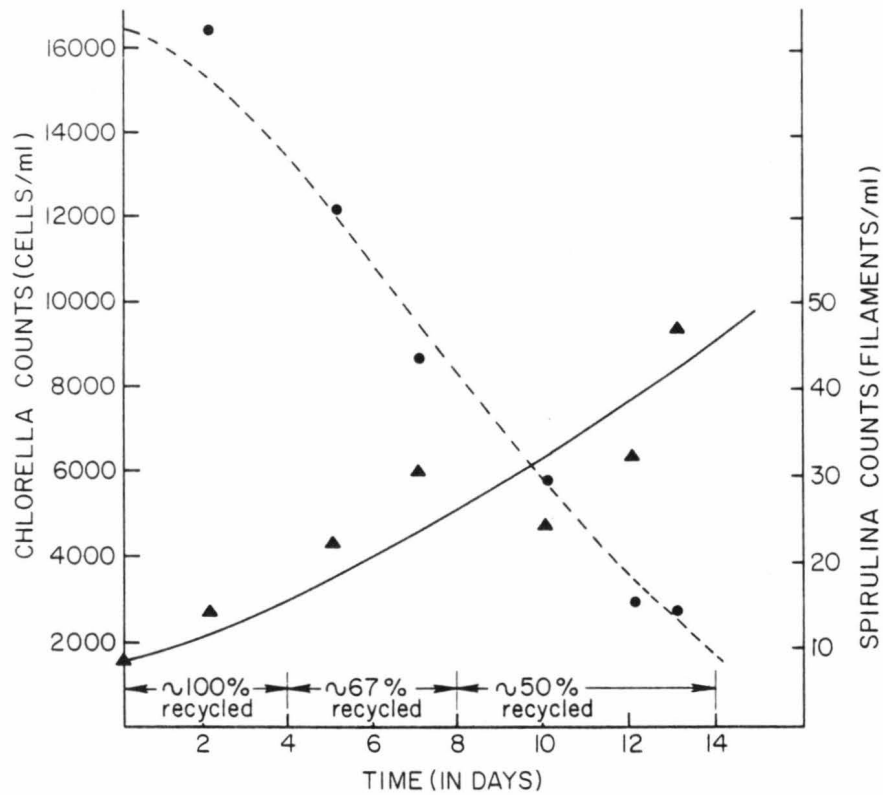
APPENDIX II. FIGURE 6C. THREE TYPES OF STEADY STATES ATTAINED IN LIGHT-LIMITED CHEMOSTATS WITH SPIRULINA GEITLERI (ORGANISM A) AND CHLORELLA (ORGANISM B)



APPENDIX II. FIGURE 7A. INCREASE OF THE FILAMENTOUS ALGAE FROM A SMALL INOCULUM

7b. PREDOMINANCE OF CHLORELLA IN A TURBIDOSTAT WITHOUT RECYCLING





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