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SPECIES CONTROL IN LARGE-SCALE ALGAL BIOMASS PRODUCTION

Final Report

By
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Work Performed Under Contract No. W-7405-ENG-48

Lawrence Berkeley Laboratory
Sanitary Engineering Research Laboratory
University of California
Berkeley, California

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FOREWARD

This report covers the work related to algal biomass production carried out during 1976 by the Algae Research Project at the Sanitary Engineering Research Laboratory of the University of California, Berkeley. The work was initiated in January 1976 with a contract entitled "Species Control in Large-Scale Algal Biomass Production" funded by the Fuels from Biomass Program of the Division of Solar Energy, Energy Research and Development Administration (Contract No. W-74-05-ENG-48). This project, awarded to the Lawrence Berkeley Laboratory, had for its objective: to "explore diligently all aspects of algal species control in algal biomass production ponds in order to promote the growth of the most efficient, most harvestable, and most fermentable algae possible."

The ERDA project "Species Control in large-Scale Algal Biomass Production" was continued October 1976 with a new contract awarded to the Universit of California, Berkeley under a different title: "An Integrated System for Solar Energy Conversion of Solar Energy Using Waste-Grown Filamentous Blue-Green Algal Biomass," (Contract No. E(04-3)-34). The work described was also partially supported by a contract entitled "Fertilizer Production with Nitrogen-Fixing Blue-Green Algae," funded July 1976 by the Research Applied to National Needs Section of the National Science Foundation (Grant No. AER 76-10809).

The authors with to thank the many people that helped in the execution of the work and preparation of the report. Marida Norgaard helped in the laboratory studies of Spirulina batch cultures; Mark Henriquez worked on the turbidostatic controls; Friedrick Janko and Philip Caskey were in charge of chemical analysis. Drs. David Jenkins and Alex Horne, respectively Director and Acting Director of SERL, are gratefully acknowledged for making available the facilities and some equipment for this research.

The authors wish to express their particular appreciation to Dr. Roscoe Ward, Chief, Fuels from Biomass, Solar Energy Division, Energy Research and Development Administration, whose interest in this project and understanding of its requirements made this research possible.

ABSTRACT

The present crisis in natural gas supplies is expected to worsen within a decade; new technologies capable of providing alternative methane sources must be rapidly developed. A multiplicity of approaches to synthetic natural gas production through bioconversion exists. This project deals with the development of biomass production technology for planktonic microalgae.

A review of this subject details the use of such algae in waste treatment and the problem of harvesting the many types of microscopic algae occurring in ponds. The solution of this problem is deemed to be development of algal species control techniques which allow cultivation of desirable algal types harvestable through the most economic methods available. Two methods of algal species control are presented in detail--the theory of size selective biomass recycle and the selection and cultivation of nitrogen-fixing blue-green algae.

In the outdoor experiments, circular 3-m^2 ponds were used and were fed daily settled sewage, were mixed with paddle wheels, and were maintained at a variable detention time and fixed depth (10 inches). The ponds were inoculated with the filamentous blue-green alga, Oscillatoria (obtained from local oxidation ponds). These algae were harvested with microstrainers--slowly rotating screens equipped with a backwash and capable of economically harvesting sewage-grown filamentous or colonial microalgae. The harvested algae were partially returned to the ponds. This technique was demonstrated to help in culture maintenance and predominance. Due to poor growth, the Oscillatoria culture could not be maintained for long against an invading Scenedesmus sp. The naturally appearing alga--Micractinium, a colonial, spiny green alga which harvested well with microstrainers--was much more successful in maintaining itself in the experimental ponds. However, even this species could not survive adverse conditions despite extensive recycling. Selective biomass recycle is not a sufficient tool in itself for sustained species control.

The outdoor experimental ponds were effective in removing chemical oxygen demand (COD) and ammonia-nitrogen (NH_3-N) from influent sewage, achieving removals of 50% and 80%, respectively. A lesser removal of orthophosphate, 25%, was obtained. Effluents from the ponds, after microstraining, generally contained less than 30 mg/ℓ total suspended solids (TSS), thus meeting 1977 EPA discharge standards. Total solar energy conversion efficiencies were up to 2% on a weekly basis. Carbonated effluents from such ponds should be ideal for cultivation of nitrogen-fixing blue-green algae.

Laboratory experiments concentrated on demonstrating the selective recycle theory. A mixture of Spirulina (a filamentous blue-green alga) and a small unicellular green alga could be maintained with either organism predominating, depending on recycling. Maintenance of a fixed total algal density should make this technique compatible with high productivities. The growth requirements of two Spirulina species were studied in batch cultures in the laboratory where they were found to be similar to other blue-green algae. Tolerance of high salts and alkalinities accounts for the predominance of Spirulina under

these conditions. Small outdoor sewage ponds inoculated with Spirulina confirmed these findings and demonstrated the effect of long detention times on culture maintenance.

An economic analysis of algal biomass production indicates the feasibility of algal biomass production and conversion to methane and fertilizer as by-products of sewage or liquid waste treatment assuming the availability of species control technology. Algal harvesting by microstraining is an economically favorable method for small-scale waste treatment systems (10 MGD) and could become sufficiently inexpensive for large-scale systems (100 MGD) to allow algal biomass production with minimal waste treatment credits. Significant contributions to local gas requirements could be made by such systems. Large-scale algal biomass production for agricultural fertilizer and methane production are economically plausible. Nutrient integrated systems for large-scale centralized natural gas production will require substantial research and development efforts. Development of such large-scale systems is a future extension of the research presented herein.

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I. INTRODUCTION

THE PROBLEM

The winter of 1976-1977 marks an historic turning point for the U.S. The long predicted natural gas shortage became critical during the most severe winter this nation has had in a hundred years. After having become dependent on natural gas for a major portion of energy supplies, suddenly there was not enough to go around, at least not at the controlled prices. Natural gas price controls were instituted in the 1930's as a price floor, and converted in the 1950's into a price ceiling. Low natural gas prices encouraged increased consumption (which more than doubled and tripled in the U.S. from 1950 to 1970) while exploration and production was discouraged. Domestic production of natural gas reached a maximum and declined as liquid fuel production had earlier. In 1976 over half the petroleum consumed in the U.S. was imported and domestic natural gas production declined about 10%. In January of 1977 came the first major demonstration of the eventual effects of these trends.

At present the outlook for natural gas is uncertain. Conservation, both voluntary and price dictated, can postpone the next crisis a few years. Increased exploratory and production efforts, imports of natural gas, and shifts in industrial usage could further stretch supplies until about the 1990's. After that some believe that domestic production could again increase through discovery and development of large new fossil sources. However, the existence of such reserves or the technologies for their exploitation remain to be proven. Thus, our country needs to rapidly develop by the mid to late 1980's, alternative fuel production technologies capable of making a significant contribution to natural gas supplies. These technologies should be able to produce a major portion of the U.S. natural gas needs by the end of the century.

BIOCONVERSION

Bioconversion, the purposeful production of fuels from biomass, has only recently been introduced into the consiousness of energy planners and policy makers. The recent realization of the limitations of nuclear energy, coal conversion, or centralized solar electric systems, has increased

awareness of alternative energy sources, of which bioconvesion is perhaps the most important. "Biomass" can be converted into liquid fuels, electricity, or substitutes for natural gas. These alternatives are probably its greatest potential.

Organic wastes have long been considered a potential fuel source and are already utilized in some industrial or agricultural processes (e.g. bagasse). The appeal of energy from wastes has been limited by the rather small overall contribution they can make to the overall U.S. energy supplies. However, the search for a single, ultimate solution to the energy crisis is now a luxury that can no longer be afforded. Partial solutions and even minor energy sources must be fully developed and utilized. Two specific recent examples are the coal-solid wastes joint firing (1) and the use of dairy or feedlot wastes for on-site natural gas production through anaerobic digestion. (2) Many more such technologies must be developed. The large variety of possibilities together with the large number of proponents for diverse approaches makes choosing between them difficult. Sorting out the conflicting claims and establishing the most practical and economical methods of waste recovery for energy conversion will require substantial research and development support. This could result in the contribution of perhaps two percentage points of national fuel consumption by the late 1980's from agricultural and municipal wastes.

More difficult to determine and predict is the potential of the purposeful cultivation of plants for conversion to fuels. Again a bewildering variety of schemes and numberless plants are proposed as perpetually renewable energy sources. Only by making favorable assumptions about, for example, tree farming or sugar cane production (e.g. the development of technology capable of increasing productivity while decreasing capital, energy and labor inputs) is it possible to develop favorable cost analyses for their utilization as energy sources (3). The large requirements for land, waters, fertilizer and sunshine limit such schemes. Ambitious plans for ocean farms (not subject to such considerations) have come up against many biological and engineering problems. (4) Freshwater aquatic plants are presently more a nuisance and environmental problem than a potential energy source.

A broad range of alternative projects must be supported to allow selective elimination of those ideas least likely to succeed and promotion to the pilot and demonstration stage of those projects judged favorably. Such research

projects would require but a fraction of the research support needed by, for example, coal gassification or breeder reactor development. However, the aggregate expenditures for all bioconversion research will have to be substantial if a significant contribution to national energy sources is expected before the end of the century.

This report details one approach to bioconversion: the cultivation of microscopic algae and conversion of the harvested biomass to methane and fertilizer. Microalgae biomass could be produced as a by-product of liquid waste treatment or for its own sake. This report concentrates on the first possibility. Consideration of microalgal biomass for bioconversion requires a review of present microalgal cultivation, harvesting, and conversion technology.

MICROALGAE CULTIVATION

Microalgae are microscopic plants. A wide variety of types exists and many thousands of species have been described. (See Table 1 for some that are discussed in this report). Microalgae have been the subject of increasing study by limnologists and microbiologists over the past one-hundred years. (5, 6). The importance of these plants as the basis of all aquatic food-chains has been the main impetus for this research; their use in elucidating fundamental biological processes such as photosynthesis has been their attraction to biochemists.(7)

Since the 1940's interest has developed in the cultivation of micro-algae for feed and protein production. (8) The high protein content of microalgae (normally above 50%) and their presumed high productivities (based on extrapolations from laboratory experiments) gave the impetus to this research. By the 1950's, a small-scale industrial production of Chlorella was established in the Far East (Japan and Taiwan). The market for the Chlorella is in specialty foods; total production to date does not exceed 500mt/year. The technical difficulties of culture contamination (by undesirable algal species), quality control, harvesting and processing have not been resolved. Present practice is to grow the algae heterotrophically (on acetic acid) under carefully controlled conditions. Costs of production are high and the product sells for above $10/kg dry weight.retail.

TABLE 1

ALGAE MOST ABUNDANT AND WIDESPREAD IN OXIDATION PONDS (23)

RANK	GENUS
1	Chlorella
2	Ankistrodesmus
3	Scenedesmus
4	Euglena
5	Chlamydomonas
6	Oscillatoria
7	Micractinium
8	Golenkinia

More recently, attention has focused on cultivation of Spirulina, a filamentous blue-green algae. These algae have been a traditional food source for people around certain alkaline African lakes where they grow naturally. Spirulina was also a source of protein for the Aztecs before the conquest by the Spaniards. It can easily be harvested by fine screens and is sundried before consumption. Considerable research is being devoted to Spirulina, and a 25-acre pilot production pond has been established by the Sosa Texcoco Company near Mexico City (9). The site is part of a large evaporation pond for bicarbonate mining; the resulting alkaline and saline conditions are ideal for Spirulina. Production is given as about 1 ton/day, cost factors are kept confidential but appear to be well below the selling price of about $5/kg. Expansion of Spirulina production is anticipated and these algae could become economically important wherever naturally favorable growing conditions (e.g. alkaline waters) exist. The high pigment content of microalgae is presently the economically valuable component. If prices are reduced, a large market as feed additives is possible.

OXIDATION PONDS

The use of microalgae in sewage treatment is an ancient and inadvertent practice predating the medieval moat. Many lakes and other fresh water bodies in the U.S. are presently overpopulated with microalgae growing on nutrients from sewage treatment plant effluents. Oxidation ponds are shallow impoundments in which sewage is introduced for stabilization. The first such pond was constructed at San Antonio, Texas near the turn of the Century. Oxidation ponds became quite popular in the Dakotas and in WWII they were widely used by the military. This gave the impetus to develop a scientific basis for the mode of action and optimal engineering design of oxidation ponds. The algal populations of oxidation ponds were demonstrated to be the major causative agents in the process of waste stabilization: the algae photosynthetically generate oxygen required for bacterial oxidation of the organic wastes (10). The development of this concept of photosynthetic oxygenation, together with equations relating pond size to local insolation and waste flows, resulted in widespread adoption of oxidation ponds, particularly by smaller municipalities, agricultural processors and industries. (11) Several thousand oxidation ponds are presently used in the U.S., from Alaska southward. Most are small, only a few acres in size, however, many systems

are several hundred acres in size. Presently oxidation ponds are the only widespread and large scale utilization of algal mass cultures. By substituting solar energy for the electrical energy used in conventional waste treatment systems they also represent a large scale (albeit inefficient) method of solar energy conversion.

Oxidation ponds presently are not designed for either maximum algae biomass production or algal species control. The requirements of waste treatment can be met by long detention times (weeks to months) and deep (3-8ft) ponds; this maximizes pathogen destruction and minimizes algal concentrations in pond effluents (due to settling and respiratory losses of the algae biomass produced.) Ponds receiving raw sewage (primary ponds) generally exhibit an anaerobic sludge zone where fermentations result in considerable waste breakdown and concomittant methane evolution. Odors, a major concern in waste treatment systems, are prevented by maintenance of an aerobic surface layer established by the photosynthetic activity of the algae. Ponds incorporating anaerobic and aerobic zones are termed "facultative". Mixing in these ponds is usually accomplished by wind action and some effluent recirculation.

Maximization of algae production requires shallow depths, mechanical mixing, and short dentention times to achieve optimal algae concentrations. Such ponds, termed "high rate", have been used in experimental algal production facilities as well as in a few oxidation pond systems. High rate ponds are up to 20 inches (50cm) deep, divided into channels up to 75 ft. wide and mixed by pumps or paddlewheels at a relatively low velocity (about 5 cm/sec) sufficient to keep the microalgae from settling. The reason that high rate ponds exhibit higher production of algae biomass is the greater relative light penetration afforded by the shallower depth, prevention of thermal stratification by slow mixing, and the diminished loss of algae due to settling. High rate ponds are considerably cheapter to construct than facultative ponds; however, their mixing requirements and higher operational costs diminish the capital savings. The general economics of algae production ponds is discussed in Section VI, it suffices to say that algae biomass production is not limited by capital or operational costs of such systems (except for land costs near urban areas).

MICROALGAE HARVESTING

Recognition of the role of microalgae in oxidation ponds has led to the realization that microalgae biomass could become a valuable protein resource or a substrate for methane fermentations (12). However, it proved difficult to develop effective and economical methods for the harvesting of sewage-grown microalgae. Centrifugation was too capital and energy intensive and efficiency of harvesting was also dependent on the specific gravity of the algae. Chemical flocculation with lime or alum requires large amounts of chemicals (about equal to the biomass recovered for alum and twice the biomass for lime) and involves careful operations and expensive flocculation and sedimentation (or flotation) chambers. Also, the chemical coagulants must be extracted from the harvested algae before they are used for feeds or fermentations. Straining, filtration, or sedimentation of algae gave poor results, except for sporadic instances. Since these are the potentially cheapest methods their failure is of interest. Oxidation pond algae are of a great variety of types, the most common are listed in Table 1. These algae are each represented by several different species, and many other genera are also reported in oxidation ponds. They may occur as almost pure monocultures, or as a mixture of two to scores of different species. Size and shape as well as less visible characteristics such as density and surface charge distribution are of great importance in microalgae harvesting techniques. For example, cell density and shape affect the performance of centrifugation, surface charges determine flocculation and size is important in filtration. The present lack of control over oxidation pond algae does not allow selection of any one optimal harvesting technique.

Recently US EPA 1977 Discharge Standards for waste water treatment plant effluent have increased interest in algae removal from oxidation pond effluents. Although the fate of these algae and their role in eutrophication is doubtful (13), they are deemed a source of suspended solids (and potential BOD load) so that their removal was dictated by EPA regulations. This has led to construction of several large scale multi-million dollar algae removal plants, all of the chemical flocculation type. However, their high capital costs (and rapidly increasing operational costs) together with the realization of the very high costs of achieving PL 92-500 goals with present technology, have resulted in an

EPA announcement that oxidation ponds effluents of < 1 MGD would be exempt from the 1977 standards applied to conventional sewage treatment plants. This is a welcome development, but should not discourage interest or funding for development of alternative, more economical microalgae harvesting technology to some extent the research and development required for algae biomass production from oxidation ponds must be justified in terms of their energy and fertilizer production potential.

The economics and effectiveness of presently available or proposed algae harvesting technologies are detailed in Chapter VI. All presently available harvesting technology could be made more economical and/or more effective if the algae in the oxidation ponds were under at least a moderate form of control. For example, centrifugation is presently prohibitively expensive (in terms of capital investment and energy utilization); cultivation of microalgae of higher density could decrease engineering requirements sufficiently to allow construction of very large throughput fiberglass bowls of reasonable costs. Control over algal species with optimal charge distribution would similarly ease flocculation requirements in terms of both chemicals used and flocculation stability. The growth in ponds of filamentous or colonial algae would allow cheap filtration or straining methods, particularly microstraining for which much experience exists in removal of water reservoir algae. The inherent physical difficulty of concentrating very large volumes of a dilute suspension of microscopic algae, whose dimensions are often below 5 microns (0.0002 inches), does not allow optimism about any cheap and simple technique which allows recovery of the algae biomass without requiring some degree or type of species control. In Chapter VI are reviewed some of the novel or proposed algae harvesting technologies. Their interest lies mainly in their novelty (which allows undue optimism about unknown costs), none can overcome the thermodynamic barriers aginst algae concentration created by the high entropy content of oxidation ponds due to their dispersed and variable algal population. Thus no proposed algae harvesting technology not based on at least a modicum of algal species control can be both economical and effective. Species control could even allow a reversal of algae dispersion prior to application of a physical concentration method. Many microalgae

are known for properties such as movement toward (or sometimes away from) light, clumping and autoflocculation, flotation and sedimentation, etc. Utilization of anyone of these abilities could achieve a sufficient concentration factor (5 to 10 fold) in an inexpensive primary harvesting pond or chamber so that a relatively more expensive secondary harvesting system could be employed. This is of importance when microalgae systems are developed whose sole function is to provide biomass for methane fermentations and/or fertilizer. The relatively low value of these commodities would require algal biomass production at about 1-3 ¢ /lb (ash-free dry weight). Even relatively cheap harvesting systems like microstraining approach the product costs. Considering land preparation pond construction and operational costs, harvesting costs must be well below this, or about two to three mils per lb (Section VI). This could only be achieved by either very simple sedimentation systems followed by a second stage using either a similar principle or a mechanical system like microstraining. Such harvesting methods will obviously be developed when algae population dynamics are both predictable and fairly well controllable. Since all necessary "hardware" is already at a sufficiently advanced stage of engineering development, the research must focus on the development of the "software"--the specific control processes using allowable (e.g. low cost) pond operations which achieve desired aims of algal species management and control. This is the underlying theme and purpose of this project. The choice of filamentous blue-green algae and microstraining promoted in the proposals is only one of the possibilities that need to be developed. Thus this project is already expanding past that one, limited, combination of algae type and harvesting technique to include (by experimental necessity) colonial green algae and sedimentation.

The present approaches to algal species control are detailed in the next section, this introduction concludes with considerations of how much microalgae biomass can be produced and how it may be converted to methane and/or fertilizer.

MICROALGAE BIOMASS YIELDS

Algae biomass production should be expressed as ton/acre/yr (or MT/hectare/year) of volatile algal solids. Presently, little data exists

which is directly useable in this form: microalgae productivity is generally determined in small scale, short duration experiments and extrapolated to give tons/acre/year. The values reported range from 10 to 50 gm/ m^2/day (14) (equivalent to 15 to 75 tons/acre/year). The higher values refer to sewage grown algae in the tropics, an origin doubly suspicious. However, the inclusion of large amounts of suspended organic matter in the harvested "algae" as well the potential for photoheterotrophic growth might indeed make such values realistic in areas of high insolations and raw sewage strengths. A recent report from Israel (15) indicates a 20% photoheterotrophic and a further 35% non-algal component in harvested (chemical flocculation) experimental high rate oxidation pond effluents. However, such productivity, if sustainable, would apply only to limited location and production volumes. For large-scale algal biomass production proposals, a lower, more conservative value must be chosen. The best approach is to choose a generally attainable and sustainable yield in terms of photosynthetic conversion efficiencies. Data from this laboratory and others shows that high rate microalgae production ponds can sustain and surpass a 4% average conversion efficiency of photosynthetically active sunlight In Table 2 are listed the expected maximal rates of biomass that can be produced as a function of latitude and conversion efficiency. By correcting these values for local cloud cover the actual expected yields for any given location may be calculated. Use of a 4% photosynthetically available conversio efficiency remains to be demonstrated in practice on a sufficiently large scale and might not be achieved initially on a sustained basis. Nevertheless the value is conservative on basis of presently available data and should be feasible in most cases with sufficient operational practice.

There is another aspect of algal biomass yields: the total amount that can be produced on a given input of nutrients (other than light, which always should be limiting). The cheapest source of nutrients are the waste streams of municipalities, agriculture or industries. Utilization by the algae of the nutrients contained therein renders the dual benefit of waste treatment and biomass production. Table 3 gives "typical" sewage compositions in the U.S. and their algal growth potential assuming 40%C,

TABLE 2

POTENTIAL MAXIMUM ALGAL PRODUCTION AT VARIOUS LATITUDES AND SUNLIGHT ENERGY CONVERSION EFFICIENCIES (in g m^{-2} day^{-1})

LATITUDE DEGREES	FOR ASSUMED VISIBLE SUNLIGHT ENERGY CONVERSION EFFICIENCY									
	1	2	3	4	5	6	7	8	9	10
0	4.68	9.36	14.15	18.7	23.4	28.2	32.6	37.5	42.2	46.8
10	4.57	9.17	13.7	18.3	22.9	27.4	32.0	36.7	41.3	45.7
20	4.35	8.71	13.1	17.4	21.8	26.1	30.7	34.8	39.1	43.5
30	3.99	7.97	11.9	15.9	19.9	23.9	27.9	31.8	35.9	39.9
40	3.36	6.71	10.1	13.4	16.8	20.2	23.5	26.8	30.1	33.6
50	2.82	5.64	8.46	11.3	14.1	16.9	19.7	27.5	25.4	2.8
60	1.64	3.28	4.93	6.57	8.22	9.87	11.5	13.1	14.8	16.4

TABLE 3

TYPICAL U.S. DOMESTIC SEWAGE COMPOSITIONS (in mg/ℓ) (22)

	RAW	SETTLED
Total Solids	1275	1035
Suspended Solids	250	105
Biochemical Oxygen Demand	300	195
Chemical Oxygen Demand	950	400
Total Nitrogen (as N)	65	53
Total Phosphorus (as P)	15	13
Total Available Carbon	-	100
Total Alkalinity (as Ca CO_3)	130	130
Algae Growth Potential (assuming a content of 40%C, 8%N, and 0.5%P in algae volatile solids)		
Carbon	-	250
Nitrogen	-	660
Phosphorus	-	2,600

8%N and 0.5%P. Sewage flows and compositions are very variable and so are algae. N may vary from 3 to 15% of dry weight and P from 0.2 to 2%. In practice, the production of algal biomass containing 7 to 13% N and 0.5 to 1.5% P is probably feasible. It is clear that P is present in excess in municipal sewage and complete utilization during algal biomass production would require additional C and N. These may be provided from flue gases (or other CO_2 sources) and biological nitrogen fixation (using filamentous blue green algae). Water could also become a limiting nutrient.

Phosphate detergents are the single most important source of phosphates in the U.S. municipal wastes; wherever they are banned and substituted for by N-based detergents algal biomass production on sewage would thereby be limited. Even with P-containing detergents algal biomass produced from sewage for methane and fertilizer would be limited below local usage. Additional sources of phosphorous would be required to allow algae biomass production beyond that possible from local liquid waste flows. Of course, other nutrients might also become limiting: Mg, Mn, salts, trace metals, all of which are found in excess in sewage but might eventually be utilized to the point of becoming limiting. However, quantitatively and economically P is the most important; (except for saline algae like Spirulina or Dunaliella). Algal biomass production above that feasible from wastes requires "nutrient integration" or fertilization of the ponds with phosphates. Nutrient integration is the process by which the effluents for the methane digester are recycled to the aerobic high rate ponds where their nutrients are reutilized. The validity of this idea was proven experimentally about 20 years ago (16). Another idea would be to add mineral phosphate fertilizer to the algae ponds, which would selectively encourage the nitrogen fixing blue-green algae. This would only be possible where strong local markets for nitrogenous fertilizer exist. The phosphate fertilizer is recovered in the fermentation residues along with the nitrogenous fertilizer produced in the ponds. Since these pond systems would be located near the site of consumption (less than 5 miles) transportation of the fertilizer sludge would not be a limiting factor (see Chapter VI). This concept of algae cultivation for simultaneous methane and fertilizer production offers several attractive possibilities: it would be a fairly controllable system (in terms of harvestable species) mostly independent of particular local

waste streams and adaptable to the size required by local agricultural usage (the methane and carbon dioxide being more transportable than the fertilizer). This process, like tertiary waste treatment, is dependent on the cultivation of nitrogen-fixing algae. Nitrogen fixation is a reductant and ATP requiring process which detracts from the carbon fixation required for methane production. Although there is no direct experience, (and no theoretically sound values for the energetics of nitrogen fixation), it is doubtful that nitrogen fixation would lower biomass yields more than about 10%. Nitrogen-fixing algae biomass production might become the preferred process in many localities for agricultural fertilizer production.

PROJECT OBJECTIVE

The objective of this project was to develop microalgal cultivation technology which would allow the establishment in sewage oxidation ponds of microalgal species large enough to be harvested by microstrainers. Such algae could be filamentous or colonial blue-green or green species. Thus, strictly speaking, this project had for its objective "size control;" any species of appropriate dimensions to be harvestable would be suitable for this purpose.

The primary method by which the objective was to be reached was by the cultivation of filamentous blue-green, or other suitable, algae in small experimental sewage oxidation ponds. Determination of the factors responsible for maintenance of such algae, and pond operations required, was the primary goal of that phase of the research. For this purpose, development of the theoretical background to algal species control was required (Chapter II). The pond system developed and methods used in pond operations are detailed in Chapter III and the results of pond operations in Chapter IV. Laboratory studies on species control and microalgal cultivation were to be undertaken under this project and these are detailed in Chapter V. (species control experiments) and Chapter VI (*Spirulina* cultivation).

The objectives of sewage oxidation pond monitoring were abandoned as it could not be carried out because of the greatly curtained funding for this project and it was deemed unlikely that facultative oxidation ponds could provide information applicable to this project. The final chapter presents an economic analysis of algal harvesting and various

scale algal biomass and bioconversion systems.

Although the objective of this project was the production of microalgal biomass for the purpose of methane and fertilizer production, no effort has yet been made to improve the available bioconversion technology for microalgal biomass. However, the rudimentary level of knowledge in this area will not allow complacency for long. The information available on sewage-grown microalgal fermentations is contained in a few publications (16,17,18) and several reviews (19,20). The data may be summarized to state that about 50-70% of the caloric content of the algal volatile solids (about 10,000 BTU's/lb) may be converted into methane. Thus, a conservative value is that 5,000 BTU's of methane may be derived from each pound of algal biomass (ash-free), correspondir to 10 MBTU's/ton of algae. The residue from the algal biomass should find use in fertilizer applications (21). The potential of algal biomass production will not be defined until more, larger-scale and longer duration algal biomass fermentation studies have been carried out. These are required to establish operating parameters for large-scale engineering designs. These designs must be capable of great economics in digesting algal biomass.

II. ALGAL SPECIES CONTROL THEORY

INTRODUCTION

As detailed in Chapter I, cultivation of specific types or species of microalgae is advantageous, even required, in any mass cultivation processes. Specifically, cultivation of microalgae which are harvestable by inexpensive methods is a precondition to economically viable biomass production systems. The basic premise of this project is that algal species control is possible and that the techniques required for its achievement will not add significantly to the costs of the biomass produced. Microbial species control has traditionally been used in mankind's food processing technology; alcoholic fermentations and cheese production are the best known examples. Nevertheless, the premise that low cost microbial species control is possible in large-scale "open" systems such as oxidation ponds is not universally accepted. Indeed, industrial scale fermentations are usually carried out under strictly controlled conditions, often using axenic cultures under sterile conditions. In other large-scale microbial cultures applied in waste treatment processes (e.g. activated sludge, anaerobic fermentation), few efforts are made for controlling the microbial organisms to the species or geotype level.

The desirability of growing filamentous or colonial microalgae which are easier to separate from oxidation ponds has been repeatedly suggested. In particular, the potential use of microstrainers for low-cost harvesting of such algae is generally appreciated (22, 26). However, the technology by which species control might be achieved has yet to be developed.

Ecological literature contains much useful information on algal blooms and physiology. The passivity of the ecological science, however, gives few guide posts on how species control may be achieved in managed artifical systems. The highly eutrophic conditions in oxidation ponds are not approached by even heavily polluted natural systems. Therefore, oxidation pond biology must be considered a distinct, presently underdeveloped branch of phytoplankton ecology. Up to now, no in depth limnological study has been made of an oxidation pond. Several reports exist of the biology and phycology

of oxidation ponds but these are descriptive and contain insufficient data by which to correlate population dynamics with operational characteristics.

Selection and succession of microalgal species has been studied in the growth of diatoms on secondary effluent-seawater mixtures. The results indicated that temperature was a determining factor in the predominating species and that other factors, particularly detention time, also had important effects in determining species composition (24). Temperature is considered an uncontrollable factor in outdoor mass cultivation of algae. Of course, it might be possible in some situations to achieve a certain degree of control (e.g. by using cooling water from a power plant, or artificially upwelled ocean water, etc.) but such applications would be limited.

Detention time is a more controllable and flexible control parameter for ponds. Although detention time is usually thought of in terms of liquid residence time; nutrient and organismal detention times are equally important and also manipulatable to a considerable extent. For algal production ponds, the concept of detention time should be reformulated in terms of incident light energy since that is the normally limiting factor. Since light and area are related, detention times per unit area are a better engineering concept. Thus, depth and hydraulic detention times are related variables in algal biomass systems.

Since algal species composition in a pond is affected by the hydraulic detention time, nutrients, culture density and other phyto and zooplankton species, it should be possible to exert some species control by manipulating the relative detention times of these various components.

How to selectively effect the relative detention times of selected nutrients or organisms is the key problem. Obviously, only limited selectivity or variations will be possible in large-scale systems. The second problem is the determination of the effects which these operations will have on algal species in the ponds. In this section, one approach, of many possible, is discussed--the use of selectively increasing the detention time of large (harvestable) algal species. Another species selection process developed theoretically involves selective nutrient limitation. N deficiency would result in filamentous nitrogen-fixing blue-green algae.

To be able to control algal species in ponds (whether for harvesting, agriculture, or feed production), the specific factors affecting algal population dynamics must be understood. A few generalizations may be readily made. When high-strength wastes (e.g. food processing wastes) are serially diluted and incubated in the light, different biotypes will appear in the various dilutions; the most concentrated wastes will contain only anaerobic fermentative bacteria, the photosynthetic bacteria appear in the first dilution followed by a graduation of flaggelated euglenoids, blue-green algae, green algae, and finally diatoms and nitrogen-fixing blue-green algae. Such successions may also be observed in outdoor ponds--from heavily loaded anaerobic ponds to the final "polishing" or "tertiary" ponds. In general, it is only one or two algal species that make up over 95% of the algal biomass in sewage ponds that are not allowed to fully stabilize (through rapid detention and heavy loading). However, in ponds operated at lighter loadings and longer detention times, a large variety of algal species is usually present.

In operating oxidation ponds the five most commonly encountered algae are small colonial or unicellular species (Table 1). The filamentous blue-green alga Oscillatoria, sixth on this list, is usually found at higher temperatures (it being the predominating algae reported from Indian oxidation ponds) and in ponds receiving low nitrogenous wastes (such as cannery wastes). The many environmental, biological, operational, and even historical factors which affect algal population dynamics in ponds interact in unknown ways as to make understanding algal population dynamics very difficult. In outdoor systems, it is not even possible to achieve a steady-state due to constantly changing conditions. Perhaps the greatest problem is the lack of enough detailed ecological or limnological studies of operating oxidation ponds. For example, it is not known to what extent seeding of ponds with specific algae affects the establishment or subsequent history of algae in the ponds. The low species diversities and rapid succession of the predominating algal species in ponds indicates an unstable ecology which can be affected by many environmental conditions. Obviously, strong species selection techniques are required.

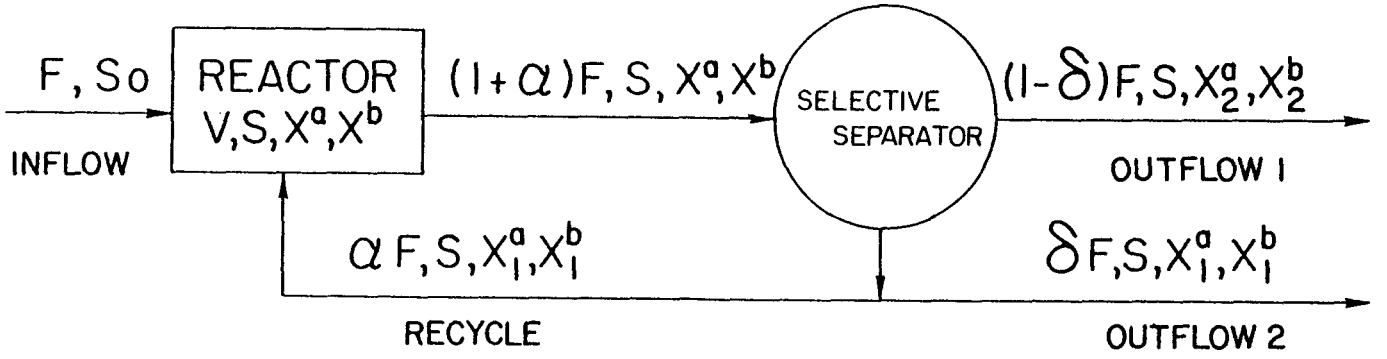
THEORY OF SPECIES CONTROL THROUGH SIZE SELECTIVE RECYCLING

In this section we propose a method for controlling species in continuous microbial cultures where some selective separation of competing species is possible. We prove that recycling (reinoculation) of the desired species will result in its dominance even if its growth rate is lower than that of the unwanted species.

Maintenance of desirable species is a major problem in industrial microbial fermentations and waste treatment processes. As discussed above, harvesting of algae grown in outdoor sewage oxidation ponds is hampered by the normal predominance of small algal species which cannot be economically removed (25, 26). Removal of the algae from pond effluents could simultaneously result in water reclamation and production of algal biomass (27) suitable for methane fermentation (28). The problem would be solved if filamentous or large colonial algae which can be cheaply strained from ponds could be made to dominate algal populations.

For many purposes, algal sewage ponds, like other microbial reactors, can be approximately described by continuous culture theory. As usually formulated (29), this theory relates cell concentration, substrate concentration, and dilution rate in completely mixed continuous cultures containing a single species. The theory has been developed to include biomass recycle (30), a technique used in waste treatment processes such as activated sludge and in anaerobic fermentations. For a long time, ecologists have studied species competition in natural bodies of water and consequently continuous culture theory has been adapted to multispecies systems (31-37). By combining the equations which describe biomass recycle and species competition in continuous cultures, we are able to prove a theory of species control through biomass recycle.

In Figure 1 is shown the flow diagram and mass balance equations for a completely mixed continuous culture with one limiting nutrient, two microbial species that compete for that nutrient, and selective recycling. For example, in algal ponds, the two species can be the unicellular alga Chlorella and the filamentous blue-green alga Oscillatoria. Any separation method, whether dependent on property differences other than size (e.g.



REACTOR MASS BALANCES:

$$\dot{S} = D(So - S) - X^a \mu^a / y^a - X^b \mu^b / y^b$$

$$\dot{X}^a = X^a \mu^a - X^a (1 + \alpha - \alpha\beta^a) D = X^a \mu^a - X^a A^a D$$

$$\dot{X}^b = X^b \mu^b - X^b (1 + \alpha - \alpha\beta^b) D = X^b \mu^b - X^b A^b D$$

FIGURE 1. COMPLETELY MIXED CONTINUOUS CULTURE WITH ONE LIMITING NUTRIENT, TWO COMPETING SPECIES, AND SELECTIVE BIOMASS RECYCLE

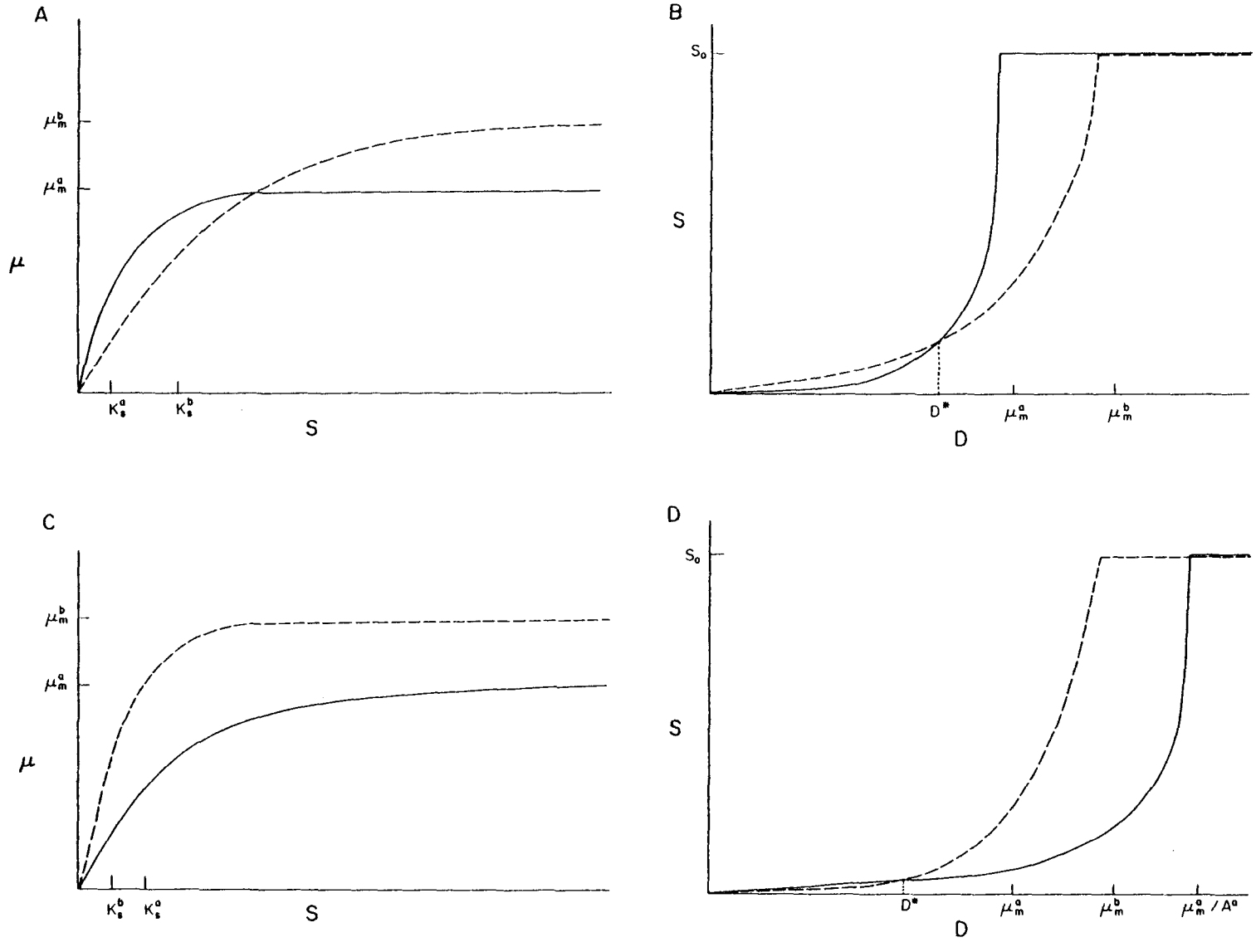


FIGURE 2. SELECTING SPECIES IN A CHEMOSTAT

CAPTION TO FIGURE 1:

Completely mixed, continuous culture with one limiting nutrient (S), two competing species (a, b), and biomass recycle using a selective separator which favors the slower growing species "a". V = volume of reactor; F = volumetric inflow rate; D = dilution rate $\equiv$ F/V; S_o = influent concentration of limiting substrate; S = concentration of limiting substrate in reactor; X^i, X_1^i, X_2^i = concentration of species i in reactor, recycle stream and second outflow, and first outflow respectively; μ^i = specific growth rate of species i = $\mu_m^i S/K_s^i + S$; α = ratio of recycle flow to inflow; δ = ratio of second outflow("product" stream) to inflow; $\beta^i \equiv X_1^i/X^i$; $\alpha \beta^i/(1 + \alpha)$ - ratio of cell mass recycled to cell mass coming out of reactor ($0 < \alpha \beta^i/(1 + \alpha) < 1$) $A^i \equiv 1 + \alpha - \alpha \beta^i > 0$; Y^i = yield constant for species i. Since the separator favors species a, $\beta^a > \beta^b$ and $\beta^a > 1$. A dot over a symbol indicates its derivative with respect to time.

CAPTION TO FIGURE 2:

Selecting species in a chemostat. ———— species "a"; ———— species "b". A.Monod growth curves where $\mu_m^b/K_s^a < \mu_m^b/K_s^b$, $\mu_m^a < \mu_m^b$. B. Steady-state concentrations of limiting nutrient versus dilution rate for each species along in a chemostat without recycling. C. Monod growth curves where $\mu_m^b/K_s^b < \mu_m^a/K_s^a$, $\mu_m^b > \mu_m^a$. Steady-state concentrations of limiting nutrient versus dilution rate for each species alone in a chemostat with recycling. $A^a = 0.6$, $A^b = 1$.

density, movement, charge, etc.) would serve equally well. Selective recycling would be accomplished by a microstrainer, i.e. a rotating fine mesh screen. The term species is used in its strict taxonomic sense only for defined systems. In open systems such as ponds the term refers to general population types characterized by the selectivity of the separator. In single species reactors recycling allows dilution rates which are greater than the organism's specific growth rate and increased cell densities, thus leading to increased rates of substrate utilization and higher productivity. The extent to which these benefits are attained is lower if the limiting nutrient is not present in the influent, e.g. oxygen in aerobic processes and light in algal ponds or other bioconversion systems. The complications imposed by sunlight being a limiting nutrient that is time dependent but dilution rate independent does not alter the general conclusions of this report. A continuous system under such a limitation would be operated with density regulation (turbidostatically) or nutrient regulation (nutristatically) rather than with dilution rate regulation (chemostatically).

When two or more species are present in a completely mixed continuous culture, the ecological principles of competitive exclusion applies. As stated by Levins (38), at equilibrium "the number of species cannot exceed the number of distinct nutrients." (This should be restated in terms of limiting nutrients.) The principle is based on the mathematical analyses of Lotka (39) and Volterra (40). Its applicability to continuous cultures was theoretically proven by Stewart and Levin (36) and experimentally demonstrated by Tieman (37). In these studies, species selection is accomplished in the usual manner, i.e., by using the dilution rate to control nutrient concentrations. In Figure 2A-B, it is illustrated how this occurs. Species "a" has a higher affinity for the limiting nutrient and thus grows faster at low nutrient concentrations. Species "b" has the greater maximum specific growth rate. If each were alone in a chemostat without recycling, Monod growth kinetics lead to the S versus D curves shown. The equations developed here are applicable to any situation where growth rate is a piecewise, continuous monotonically increasing function of substrate. The Monod equation, indeed, does not necessarily apply to a number of phenomena encountered in continuous cultures or algal ponds. Considering both species in the chemostat, the graph indicates that for values of D that are less than D*, then species "a" cannot compete. Dilution rate

control in chemostats with one limiting nutrient is effective only when the Monod functions for the two species intersect. Species control by recycling, on the other hand, works not only under these conditions but also when dilution rate control is ineffective. In Figure 2C is shown the Monod functions for two species, one of which has a lower specific growth rate at all substrate concentrations. Greater recycling of the slower growing species (species "a") can result in steady-state situations where its standing population again reduces the substrate concentration to a point where the other species is washed out. An obvious special case is when the recycling of species "a" allows the dilution rate to be increased so much that it is greater than the maximum specific growth rate of species "b". As can be seen from the graph, for a given amount of selective recycling, there may be dilution rates for which the recycling does not compensate enough for the slow growth rate.

We now prove that differential recycling can also accomplish species control. Consider the flow diagram and mass balance equations in Figure 1. There are two non-zero stationary states of the system, i.e., states which satisfy the condition that $\dot{S} = \dot{X}^a = \dot{X}^b = 0$:

$$\bar{X}^b = 0;\ \bar{X}^a = \frac{Y^a}{A^a}(S_o - \bar{S});\ \bar{S} = \frac{K_s^a A^a \bar{D}}{\mu_m^a - A^a \bar{D}};\ \mu^a = A^a \bar{D} \qquad (1)$$

$$\bar{X}^a = 0;\ \bar{X}^b = \frac{Y^b}{A^b}(S_o = \bar{S});\ \bar{S} = \frac{K_s^b A^b \bar{D}}{\mu_m^b - A^b \bar{D}}\ \mu^b = A^b \bar{D} \qquad (2)$$

The following restrictions must also be imposed:

$$\mu_m^i > A^i \bar{D};\ 0 < A^a < 1;\ 0 < A^b < 1 + \alpha\ ;\ S_o > \bar{S} \qquad (3)$$

(A bar over a symbol indicates its stationary state value). In each case, one of the species dominates completely. Intuitively, this should occur when the supplemented growth rate (true specific growth rate enhanced by recycle) of one species is greater than the supplemented growth rate of the other. The following summarized proof of stability closely follows the

method used by Koga and Humphrey (41) for the stability of a chemostat without recycling and containing only one species. One first transforms variables so that the stationary state values of S, X^a, and X^b form the origin of a new coordinate system. Thus, let $X^a = \bar{X}^a + x^a$, $\bar{X}^b + x^b$, and $S = \bar{S} + y$. After substituting these transformations into the mass balance equations, one linearizes them with respect to the new variables x^a, x^b, y and substitutes in the stationary state values $\bar{X}^a$, $\bar{X}^b$, $\bar{S}$ for the case under investigation. Case (1) is analyzed here, but case (2) gives results that are similar. This results in the following system of three linear first order differential equations where the variables represent deviations from the stationary state of case (1):

$$\dot{y} = (-\bar{D} - \frac{R}{Y^a})\, y - \frac{A^a\bar{D}}{Y^a}\, x^a - \frac{1}{Y^b}\, \frac{\mu_m^b \bar{S}}{K_s^b + \bar{S}}\, x^b$$

$$\dot{x}^a = Ry + 0 \cdot x^a + 0 \cdot x^b \quad \dot{x}^b = 0 \cdot y + 0 \cdot x^a + (\frac{\mu_m^b \bar{S}}{K_s^b + S} - A^b\bar{D})\, x^b \qquad (4)$$

$$R \equiv \bar{X}^a A^a\bar{D} (\frac{1}{\bar{S}} - \frac{1}{K_s^a + \bar{S}}) = \frac{Y^a (\mu_m^a - A^aD) [S_o (\mu_m^a - A^a\bar{D}) - A^aD K_s^a]}{A^a \mu_m^a K_s^a} > 0 \qquad (5)$$

If the solutions to these equations tend to zero with increasing time, then the system approaches the stationary state (the new origin), making this state locally stable. General solutions to the above system of equations are of the form:

$$x^a = \sum_{i=1}^{3} B_i e\lambda i^t; \quad x^b = \sum_{i=1}^{3} C_i e\lambda i^t; \quad y = \sum_{i=1}^{3} D_{ie}\, \lambda i^t \qquad (6)$$

The λ are the roots of the characteristic equation $\det (M - \lambda I) = 0$, where M is the matrix of coefficients of the system of equation (4) and I is the identity matrix. It is apparent from the form of the general solutions that the stationary state is stable if all of the λ_i have negative real parts. Solution of the characteristic equation yields three roots:

$$\lambda_1 = \frac{1}{2}\left(\frac{Y^a\bar{D} + R}{Y^a}\right) - \frac{1}{2}\sqrt{\left(\frac{Y^a\bar{D} + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a}}$$

$$\lambda_2 = \frac{1}{2}\left(\frac{Y^aD + R}{Y^a}\right) + \frac{1}{2}\sqrt{\left(\frac{Y^a\bar{D} + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a}}$$

$$\lambda 3 = \frac{m^b\bar{S}}{K_s^b + S} - A^b\bar{D}$$

Now, $\left(\frac{Y^a\bar{D} + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a} > \left(\frac{Y^aD + R}{Y^a}\right)^2 - \frac{4Y^aR\bar{D}}{(Y^a)^2} = \left(\frac{Y^a\bar{D} - R}{Y^a}\right)^2 > 0$

The first inequality is obtained because $0 < A^a < 1$. Thus, all of the roots are real only. Also, $\frac{Y^a\bar{D} + R}{Y^a}$ and $\frac{4A^aR\bar{D}}{Y^a}$ are both positive because A^a, R, $\bar{D}$, and Y^a are all positive. Thus, λ_1 is negative by inspection and $\left(\frac{Y^aD + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a} < \left(\frac{Y^aD + R}{Y^a}\right)^2$. The latter implies that

$$\sqrt{\left(\frac{Y^a\bar{D} + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a}} > \frac{Y^a\bar{D} + R}{Y^a}, \text{ or } -\frac{Y^a\bar{D} + R}{Y^a} + \sqrt{\left(\frac{Y^a\bar{D} + R}{Y^a}\right)^2 - \frac{4A^aR\bar{D}}{Y^a}} < 0$$

So, λ_2 is also negative. λ_3 is negative if $\bar{D}\ (= \frac{\mu^a}{A^a}) > \frac{m^b\bar{S}}{A^b(K_s^b + \bar{S})}\ (= \frac{\mu^b}{A^b})$

Thus, we have confirmed the intuitive notion of stability, i.e., $^a/A^a$ $^b/A^b$. Stability of the second stationary state requires $\frac{\mu^b}{A^b} > \frac{\mu^a}{A^a}$. However, since A^b may be greater than 1, λ_1 and λ_2 may be complex numbers having real parts that are negative.

As the separator was assumed to favor species "a", this second stationary state only occurs when the selectivity of the separator is not sufficient

to make up for the slower growth of species "a". This happens because A^a and A^b are assumed independent of dilution rate, but the ratio $\frac{\mu^a}{\mu^b}$ usually changes with dilution rate.

Since the stationary states are stable, the recycling can be intermittent rather than continuous. In this way unwanted species are washed out only when their cell densities have become great enough to be a problem.

This theory may be visualized by the following qualitative argument. If a pond is heavily inoculated with harvestable species and all the algae strained from the pond are reintroduced into the pond, then the inoculated algae, because they are not washed out of the pond, will have an advantage over the other, faster growing, non-harvestable algae. Eventually all of the algae in the pond will be of the desired, harvestable type and the amount recycled to the ponds can be reduced. To prevent the original algae from coming back, the fraction of harvested algae that must continuously be recycled is proportional to the ratio of the relative growth rates. In practice, this recycling fraction must be larger since harvesting is neither completely selective nor totally effective. Thus, the growth rate of the desirable harvestable algal species must not be too far below that of the unwanted single-cell algae. The decreased detention times or algal concentration required by cell recycling also set limits to the allowable recycling. It is important to note that size selective recycling is not a species-specific control method. Any harvestable alga which spontaneously appears in the ponds will be recycled and become dominant if it grows faster than the inoculated strain. Inoculations and recycling might be alternated so as to allow true algal species control. Another type of size selective recycle may be visualized by the following example. In a pond operated at a one-week detention time, the predominating alga Chlorella is accompanied by daphnia (water fleas) which graze on the Chlorella, and by a small percentage of Micractinium whose spines discourage their use as a food. The daphnia do not diminish the Chlorella population because the daphnia cannot grow fast enough to prevent their washout. If a relatively coarse screen is used to remove the daphnia from the pond effluents and recycle them into the pond, a population explosion of daphnia will quickly lead to a diminished Chlorella population and then followed by predominance of the undelectible

Micractinium. Although such biological control of algal species remains to be demonstrated, its application to algal ponds have already been observed in laboratory chemostat cultures: a rotifer invasion helped maintain Spirulina by selectively consuming a smaller unicellular green alga. Although the above proof applies in a strict sense only to a defined system, selection by recycling can be a major factor in many open systems. Experiments (detailed in Chapter V) with small-scale outdoor sewage ponds have demonstrated that harvestable algae can be maintained as the dominant population through recycling. Another system where recycling is already practiced is the activated sludge process for liquid waste treatment. In this process organic wastes are oxidized by microbial action in an aerated reactor and the microbial biomass produced is settled in a sedimentation tank (clarified). The rationale given for recycling is that a high rate of substrate utilization per unit volume is attained through lowered detention time and increased cell density. In addition, it has been frequently observed that the recycling imposes the settling characteristics of the biomass in the clarifiers (42). How recycling enhances settling in activated sludge remained an unsolved problem which now can be explained in terms of the theory of species selection proved above.

The theory developed here applies to any physical or chemical method which causes a particular species to dominate in the recycle stream. Thus, it is relevant to many microbial processes of both practical and ecological importance.

NITROGEN FIXATION

Besides organismal and hydraulic loads and detention time, nutrient supplies can also be controlled in efforts at algal species control. The principal nutrients are sunlight, carbon, nitrogen, and phosphorus. Although not yet considered, salts and minor nutrients might eventually become as important in species control as the major nutrients and pond operations. One problem is that few nutrient effects can be a priori predicted for theoretical reasons or based on experience. It is likely that higher pH, sodium, and ion concentrations might help shift conditions in favor of blue-green algae; however, much more data is required to determine their actual effects under specific conditions. Thus, only the major nutrients are presently considered for species control and only nitrogen can be assumed to have desirable and predictable effects.

Sunlight should always be the limiting nutrient, and photosynthetic efficiency should be maximized through proper pond operations (depth, hydraulic loads and detention time, algal concentration, etc.). Species control techniques which result in less than optimal productivities would represent a real cost of such technology. However, it is expected that small shifts in sunlight inputs and absorption could result in large effects on relative algal growth rates without significantly affecting productivity. Carbon should not be allowed to become a limiting nutrient; addition of carbon dioxide to ponds through a carbonation mechanism is a necessity of all pond systems. Although this has not yet been practiced in large-scale ponds, it appears technologically and economically feasible. A dual, related function is accomplished throuch carbonation--carbon supply and pH control. Both carbon dioxide and light input additions are independent of hydraulic flows and can be individually controlled. It is possible to also vary other nutrient flows separately from the hydraulic detention time.

Under conditions of nitrogen limitions in natural situations, the nitrogen-fixing procaryotes and their symbiotic associations have strongly selective advantages. It can be assumed that in algal biomass production ponds, this also would hold. Only nitrogen-fixing blue-green algae would be capable of growing under such conditions. Nitrogen-fixing blue-green algae, particularly planktonic forms, are all of the filamentous type and are particularly suitable for the harvesting approach taken in this project. Also of potential significance are the gas vacuoles of these algae which theoretically could allow a high preconcentration in a simple short detention time flotation pond. (It was observed during the operation of the Oscillatoria ponds described in the next section that the algal samples collected for laboratory analysis formed a thin floating scum within fifteen minutes of sample collection). Filamentous nitrogen-fixing blue-green algae can be classified as heterocystous (e.g. Anabaena, Nostoc, Gleotrichia, etc.) and non-heterocystous (Oscillatoria, Plectorema, Phormidium, etc.). The key distinction are the heterocysts, specialized cells known to contain the site of nitrogenase. It is the heterocysts which allow algae possessing them to simultaneously fix nitrogen and produce oxygen. Non-heterocystous blue-green algae must alternate nitrogen fixation and photosynthetic oxygen evolution. Although both algal types could be grown in ponds under nitrogen-fixing conditions, the heterocystous would be preferable since pond operations would be simpler.

Experience in cultivation of blue-green algae is lacking and actual productivities are difficult to estimate. The spontaneous growth of such algae in eutrophic lakes, rice paddies, and some oxidation ponds allows optimism about the success of this approach to algal cultivation and species control. Further data needs to be gathered before much more can be said on the subject. No specific effort was made to grow nitrogen-fixing blue-green algae outdoors during the first year of this project. Efforts were directed toward strain isolation and laboratory studies. Efforts at outdoor mass culture of blue-green algae are expected during the current year.

SPECIES CONTROL AND ALGAL PRODUCTIVITY

Productivity per unit area and time of photoautotrophic organisms is maximized only when the culturing system is operated with light as the limiting nutrient. Growth potential, maximum total algal biomass yield, is determined by the concentration of nutrients present in the liquid growth media. Many empirical and mechanistic growth models have been developed for light-limiting conditions giving a relationship between specific growth rate and local monochromatic light intensity (intensity a single algal cell experiences). This is then integrated over wavelength and depth to produce macroscopically applicable relationships between cell density or productivity and specific growth rate. These models generally fit the data only qualitatively. Respiration, photorespiration, photoinhibition, heterotrophy, non-algal turbidity, temperature changes, nutrient interaction, light reflection, species effects, and many other factors are either not treated or are poorly incorporated into the models. In general, qualitative estimates based on the known and speculated mechanisms of light and algae interactions do as well or better in predicting productivity. Since net productivity is proportional to net photosynthetic efficiency by definition, maximizing this efficiency is a major criterion for designing and operating algal production ponds (harvesting is the other criterion). Photorespiration and photoinhibition, of course, decrease efficiency. The former can be alleviated with carbon dioxide sparging and the latter can be lessened through high culture densities. There is a trade-off though, since dense cultures contain (by weight) more chlorophyll and light harvesting apparatus which requires maintenance energy. Very dense cultures, thus, require more energy for

maintenance of structure and function not directly associated with growth. Dilute cultures, on the other hand, experience a higher average light intensity, causing many algal cells to be in a region where the light is above the saturating level. It also stimulates photorespiration (if carbon dioxide is below .2%). Very dilute cultures lose efficiency due to light transmission and increased photoinhibition. Thus, an optimum density can be expected. Since there is a gradient of light intensity in any algal culture, all phases of the light curve are experienced. The non-productive phases, inhibitory levels and levels above saturation, should be minimized by shallow depths and optimal detention times. One of the least tractible problems involved is the changing incident light intensity. Thus, there is a different set of "optimized" pond parameters for each incident intensity.

The relationship between productivity and harvestability is most important in mass culturing of algae. This report is concerned with selective recycling as a means of achieving a highly harvestable pond population. As mentioned above in the section on recycling, in chemostats, productivity is always increased by recycling if the limiting nutrient is contained in the liquid feed. This is mainly due to the constant replenishment of nutrient even with changing detention time. The rate at which light enters a culture is independent of detention time. Thus, recycling may increase or decrease productivity. It will increase productivity if the increase in density (caused by recycling) brings the culture closer to the optimal density (i.e., the culture started out too dilute). Productivity decreases with recycling if the culture is already more dense than optimal. This decrease is not significant, as our experiments show, for the portion of the density vs. specific growth curve where the two variables are close to inversely proportional (here $P=\mu X$ = constant). Most mathematical models of productivity predict an unsymmetrical curve of P vs. μ, with a region at moderately high densities that is fairly flat. Turbidostatic operation of ponds avoids this problem altogether. Since a given algal density results in a unique culture specific growth rate under light limitation, holding the density constant will maintain a given productivity regardless of recycling. Productivity ($=\mu$ X, where μ = AD) can be maximized by holding the density at its optimal value. Recycling increases the flow through the system without decreasing the productivity. This approach is difficult only if a minimium detention time is necessary to make nutrients in sewage available or to breakdown chemical

inhibitors. Some strains of algae seem to be able to grow on sewage at very short detention times. Others (Spirulina, for example), appear to require a long detention time when grown on sewage. For nitrogen-fixing blue-green algae, a decrease in productivity of about 10 to maximally 20% is predicted due to the metabolic resources devoted to the reaction. If a reasonable energy value is assigned to the nitrogen produced, a real increase in productivity would result.

III. EXPERIMENTAL POND OPERATIONS

OBJECTIVES AND SUMMARY

Previous experimental operations of oxidation ponds have focused on the results of various operational parameters on pond performance. A series of experiments was carried out according to a predetermined plan which varied detention times, depth, mixing, and recirculation. Along with sewage analysis (BOD, suspended solids, nutrients) and some pond parameters (DO, pH, temperature, etc.), occasional experiments also contain phycological data (algal counts and identification, chlorophyll, etc.). These studies were quite sufficient for determining the relationships of the variables with environmental factors (insolation) and pond performance. The algal culture behaved similarly regardless of the algal species found in the pond so few attempts were made to correlate phytoplankton composition with pond performance. Indeed, it is doubtful whether such correlations could be made on the basis of available data.

For the purposes of this project, this approach was no longer sufficient. The primary aim was to achieve a certain pre-set goal (maintenance of harvestable algae) and pond performance. This had to be achieved by continuous feedback in order to counteract changing conditions. Since it is not yet clear how variables such as mixing or depth affect species composition, these were kept constant in the initial series of experiments performed and only detention time and recycling were used as experimental variables. The aim was to establish correlations between pond operations and algal species compositions which will determine the best approach to algal species control. The initial approach was to inoculate harvestable algae collected from oxidation ponds. When harvestable algae appeared spontaneously in the ponds, the approach was changed to maintenance of these cultures.

The initial series of experiments were unsuccessful in demonstrating the maintenance of Oscillatoria at the dilution rates employed, and in most (except for the first which was terminated within one week of start-up), the filamentous algae were eventually displaced by Micractinium. However,

high algal removal efficiencies (up to 95%) were attained by microstraining with the 26-μm opening fabric employed, thus demonstrating the "harvestability" of the Oscillatoria. Microstrainer performance correlated well with the size distribution of the algae present, and in addition, higher culture densities proved beneficial to separation efficiencies.

Two major problems in the maintenance of filamentous blue-green algae were revealed by the experiments: (1) filament breakup was probably due to toxic effects of the settled sewage, and (2) other large, colonial algae such as Scenedesmus and Micractinium were harvested and, consequently, recycled along with the Oscillatoria. Competition experiments showed that that alga, Oscillatoria, was maintained longer in a pond where harvested biomass was recycled but that in both ponds its population eventually dwindled to insignificant levels.

Because of the success of Micractinium, a second series of experiments in which these algae were alternately inoculated at high and low levels was run. In all but the last two experiments Micractinium either maintained or established itself as the predominant algal species, whether or not harvested biomass recycling was employed. This trend was even true for one pond where effluent biomass was recycled. The last two experiments differed in that harvested biomass recycling, for the initial 10 days, did enable Micractinium to increase from a low level to a high (predominant) level whereas in the control pond its population remained low. However, in the recycled pond, the Micractinium population sharply decreased at the end of the experiment.

Harvested biomass recycling did appear to beneficially affect observed algal separation efficiencies, although this effect was predominantly due to the higher culture densities maintained by the recycling. Abrupt increases in dilution rates decreased culture harvestabilities by causing filaments or colonies to break up.

POND SYSTEM DEVELOPMENT

Sewage Supply

Continuous availability of fresh sewage was essential to the microalgal cultivation experiments. Thus, the SERL sewage handling and distribution system is designed to dependably deliver raw, clarified or screened municipal

sewage as needed with no more than two hours detention prior to its entry into the ponds.

Source of the sewage is a trunk sewer serving a residential section of the City of Richmond, California. Either of two independent pumping stations--one having pumps supplying approximately 400 liters/min of continuous flow or the other functioning intermittently to supply an average of 100 liters/min--can be used to lift sewage from the sewer through a pressure conduit about 0.5 kilometers long to SERL (Sanitary Engineering Research Laboratory). At SERL, the sewage normally enters a (10,000 liter) sedimentation basin, allowing either raw (before the clarifier) or settled (after about one-hour detention) sewage to be drawn off and delivered to the ponds. Unfortunately, during the course of the experiments described herein, the clarifier was shut down for repairs, necessitating the use of a smaller (1000-liter) clarifier located adjacent to the ponds. After clarification, the sewage passed through a smaller holding basin from which the ponds were fed by gravity flow. At no time was the sewage allowed to stagnate in the holding basin; a steady flow was continuously circulated through the clarifier and holding basin.

The 10,000-liter clarifier is presently on-line, increasing the flexibility of the Richmond pond system by enabling settled sewage to be fed to the high-rate pond. In addition, a DSM screening unit is now in place to provide a coarse-screening stage for the raw sewage.

Pilot-Scale Ponds

The primary function of the pilot-scale ponds used in the cultivation experiments was to simulate conditions obtained in larger, field-scale systems. Three factors determine, to a large extent, the similtude: depth, surface to volume ratio, and mixing. By far, the most stringent of these criteria is that of mixing; depth is easily simulated and surface:volume ratios decrease to plateaus at about 3-m^2 (round ponds) and 6-m^2 (rectangular ponds); above these sizes the reductions in surface to volume ratios with increasing size become less significant as evidenced in Table 4 . In order to simulate in the small ponds the uniform mixing action attained through turbulent flow in the long channels of a field-scale high-rate pond, paddle wheels were used. Paddle wheels serve to uniformly accelerate the fluid flow across the width of a channel, a great advantage where channels are relatively short.

TABLE 4

SURFACE:VOLUME RATIOS FOR VARIOUS SIZED CIRCULAR AND RECTANGULAR VESSELS[a]

Horizontal Area m^2	Circular Vessels S:V Ratio m^{-1}	Rectangular Vessels[b] S:V Ratio m^{-1}
0.2	11.8	13.5
0.4	9.6	10.7
0.8	8.0	8.7
1.6	6.8	7.3
3.2	6.0	6.4
6.4	5.4	5.7
12.8	5.0	5.2
25.6	4.7	4.8
51.2	4.5	4.6

[a] Depth = 25 cm

[b] Length:width = 2

3-m^2 Circular Ponds

The results reported herein were mostly achieved in these ponds, shown by a photograph in Figure 3 . A schematic of the ponds, Figure 4 , shows in plan view the overall dimensions of the ponds and the placement of the paddle-wheels. Normal operational depth for the ponds is 25 cm which corresponds to a liquid volume of 950 liters. The nominal horizontal area of the ponds is 3.8 m^2; however, the actual exposed area (after subtracting for shading due to the 0.4 m^2 of paddle wheel area, in addition to shading by the center baffle, freeboard and the microstrainer units) is about 3 m^2.

The ponds were slow-mixed at 3 cm/sec (1 rpm paddle rotation) 23 hours daily with 1 hour of fast mixing (20 cm/sec, 6 rpm) at dusk. Mixing speed was automatically controlled by a 24-hr timer which activated a relay alternately engaging Minarik speed controllers, one set at the slow speed and one set at the fast speed.

The primary disadvantage found with the circular ponds is their small volume. At a typical dilution rate of 0.25/day, only 250 liters of pond effluent and sewage influent are exchanged each day. Because the pilot-scale microstrainers required a minimum flow of 1.5 liters/min, the resultant period of drawdown was short, usually 3 hours or less. It was decided that representative data could be obtained only if sewage was withheld from the ponds during harvesting, otherwise sewage solids would be mixed with the algal solids as determined by the ash-free dry weight analysis. The harvesting operation typically began about 8:30 AM and was completed by noon. In experiments involving algal biomass recycle, the requisite portion of the harvest was not returned to the pond until the harvest was complete. The volume of harvest was measured by collecting the microstrainer effluents into graduated plastic 110-liter vessels.

12-m^2 Rectangular Ponds

Current experiments with sewage-grown Scenedesmus cultures are proceeding in these ponds. The ponds are used in tandem with a smaller (2.4 m x 1.2 m) vessels used to receive the harvester effluent, thus allowing accurate control of total flow volumes. Maximum depth is 46 cm; the present experiments are being run at 30 cm. Culture depth is maintained continuously at

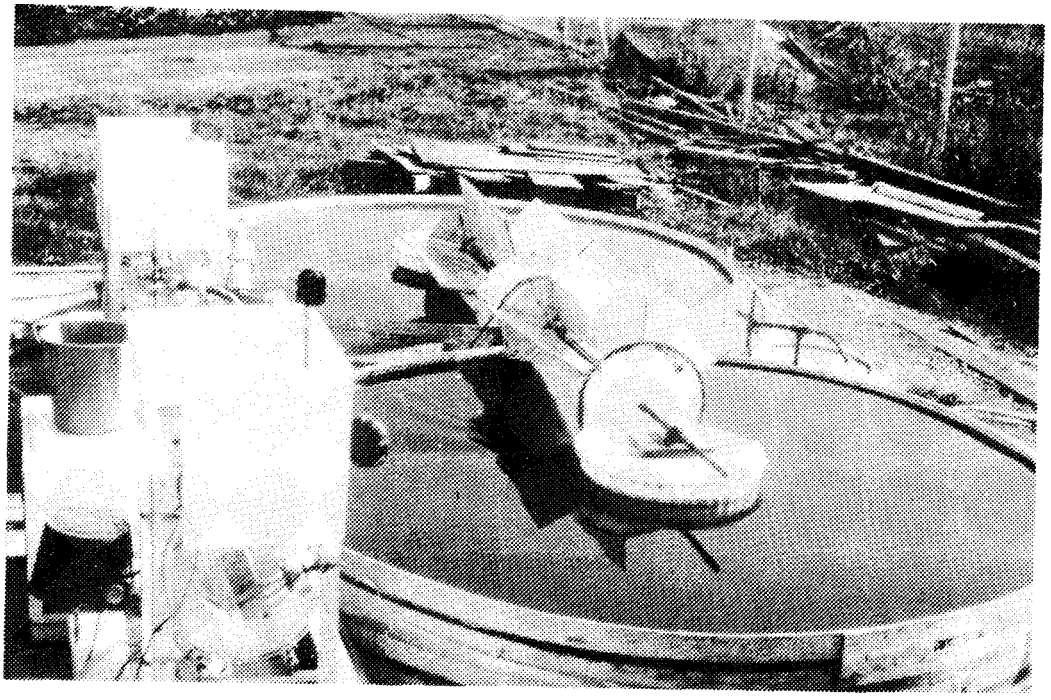


Figure 3. THREE-m^2 ALGAL-PRODUCTION PONDS AND HARVESTERS

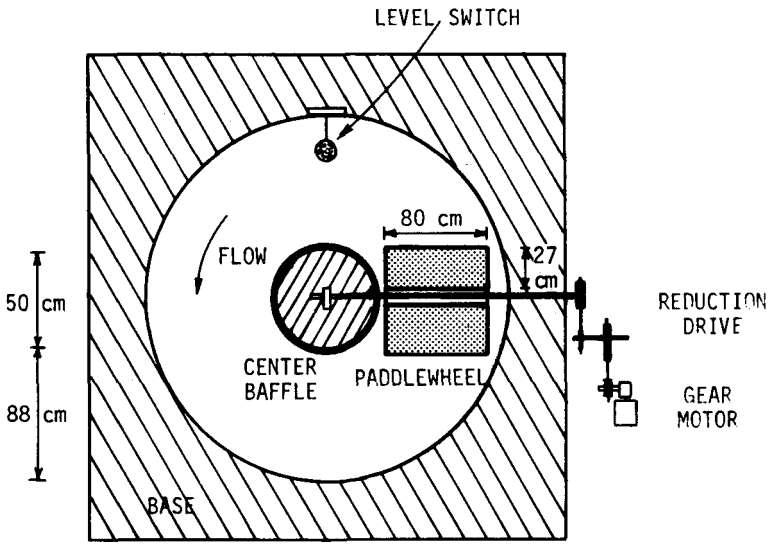


FIGURE 4. PLAN VIEW OF 3-m^2 ALGAL PRODUCTION POND

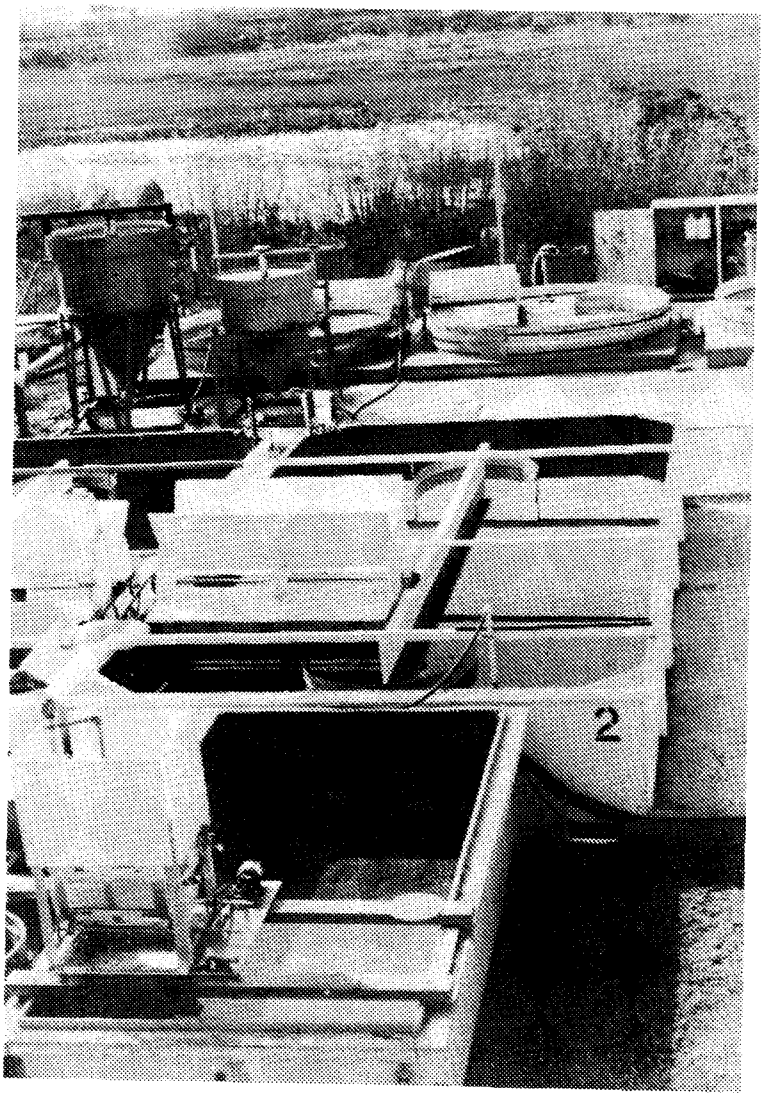


FIGURE 5. TWELVE-m^2 ALGAL-PRODUCTION POND. SHOWN IS MP-2 WITH ITS PADDLEWHEEL MIXER, CENTER BAFFLE AND FLOW DEFLECTORS AT EITHER END. ALSO VISIBLE ARE THE MICROSTRAINER AND EFFLUENT-RECEIVING BASIN (FOREGROUND) AND SEWAGE SEDIMENTATION AND STORAGE BASINS (LARGER AND SMALLER CONICAL VESSELS, RESPECTIVELY, IN BACKGROUND)

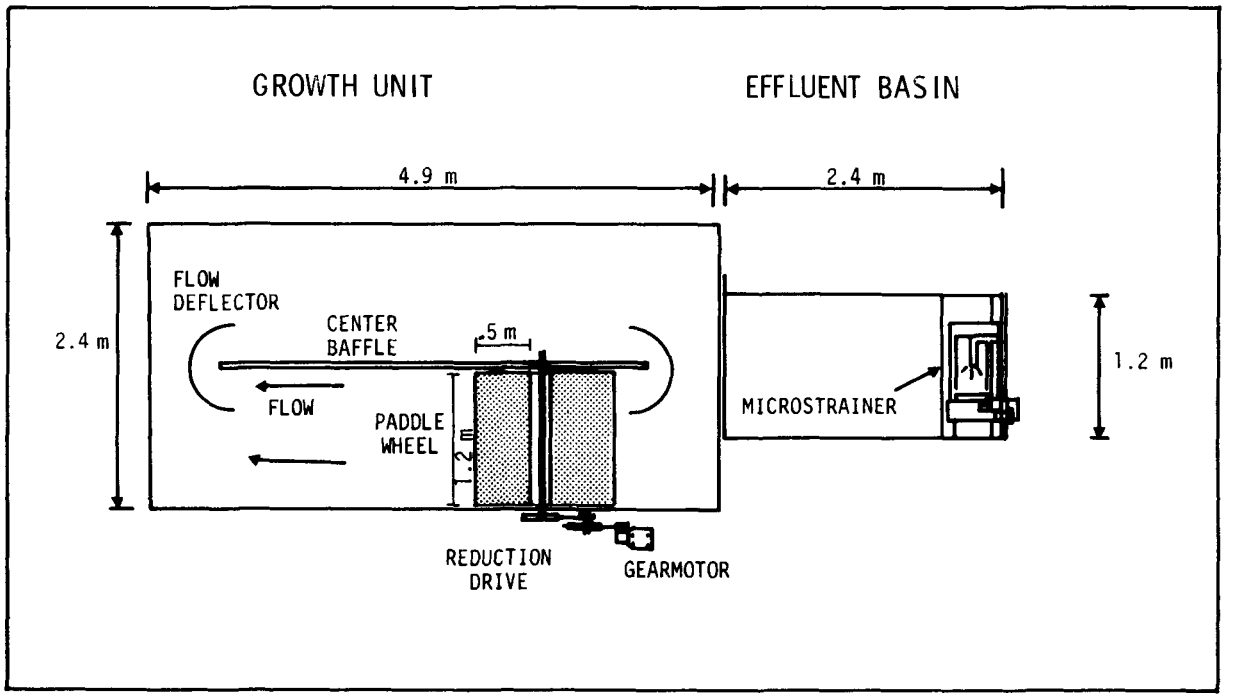


FIGURE 6. PLAN VIEW OF 12-m^2 ALGAL-PRODUCTION POND AND HARVESTING SYSTEM

the prescribed setting by level switches activating solenoid valves; thus, during harvesting, the inflow rate closely approximates the outflow pumping rate. The dilution period is 5 hours and as the days increase in length the length of this period will be increased. The ponds are continuously slow, mixed at 5 cm/sec (1.25 rpm paddle rotation) with no fast mixing. (Refer to Figures 5 and 6 for photographic and schematic views of the 12 m^2 ponds and effluent basins). Volume of the ponds at 30 cm is 3600 liters.

Field-Scale Ponds

Concern has been expressed regarding scale-up factors involved in translating results obtained with pilot-scale ponds into design factors for field-scale units, especially as regards areal production rates (e.g. grams algae m^2/day). Two field-scale ponds are available at SERL where such scale-up factors can be determined.

0.1-Hectare Deep Facultative Pond

Studies are now being initiated to study the nitrogen cycle, carbon balance and algal population dynamics of SERL's facultative pond. The unit closely approximates many typical installations in California, being about 2 meters deep at the center with sloping sides. The inflow (normally raw sewage) enters at the bottom center of the pond, with the effluent drawn off just below the surface.

0.25 Hectare High-Rate Pond

The high-rate pond is an asphalt-lined rectangular pond which can be operated between 15 cm and 60 cm in depth. Mixing is accomplished by three 5-HP low-head propeller pumps; these pumps can be operated singly, in pairs or all three simultaneously. A stream of pond culture is continuously strained through a DSM unit to remove large predator organisms (e.g. Daphnia, large rotifers); a portion of this stream is then microstrained with the effluent wasted to the sewer. Volume of the high-rate pond at 30 cm is approximately 10^6 liters. A plan view of the experimental algal growth pond system is shown in Figure 7 .

SEWAGE POND SYSTEM

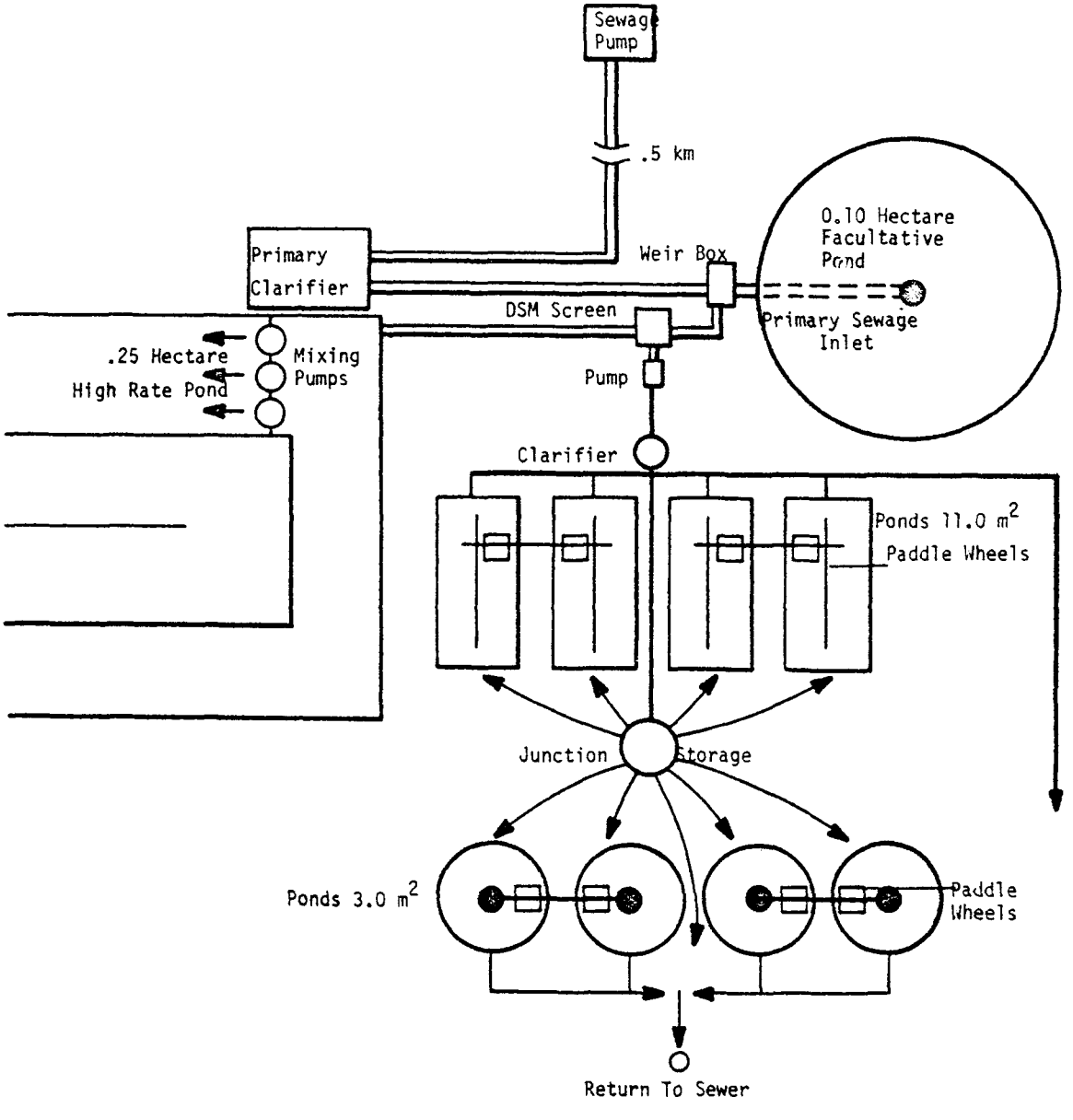


FIGURE 7. PLAN VIEW OF RICHMOND FIELD STATION EXPERIMENTAL ALGAL GROWTH POND SYSTEM

Microstrainers

Although microstrainers are a commonly applied water treatment process commercially available (e.g. Crane Co., Inc.), there is, at present, no commercial outlet for small-scale microstrainers applicable to flow rates between 1 and 10 liters/min. Thus, it became necessary to use project personnel to fabricate the drum components of the experimental microstrainers; aluminum frames to house the drums were supplied by an outside contractor. Experiences with early designs are detailed in previous reports (45). Discussion is limited here to the design utilized during the *Oscillatoria* and *Micractinium* experiments.

The experimental microstrainers are composed of three primary components: 1) the drum, fabricated of lucite plastic, which continuously rotates during operation and provides support for the straining fabric and containment of the algal culture; 2) the straining fabric, which extends around the drum and retains the particulate matter greater in size than the fabric openings (particulate matter smaller than the fabric openings is removed to a lesser extent after buildup of a filtration matt or "schmutzdecke"), and 3) the frame, drive and backwash system which houses the drum, rotates it and knocks the retained algae from the straining fabric onto the discharge trough.

In Figure 8 are photographs of an experimental unit. Visible are the drum and straining fabric, windscreen (flat surfaces), drive motor, frame and concentrate trough. During operation, pond culture is discharged into the drum, forming a pool 5 to 10 cm deep; the algae are retained on the fabric as it travels through the pool and eventually are knocked off the fabric by the high velocity, low volume backwash spray.

Design of the drum proved very important to the separation efficiency, concentration performance and throughput rate of the strainer. The most effective design incorporates numerous narrow baffles running lengthwise across the drum. These baffles are firmly attached to the straining fabric, thus providing a barrier to prevent algal solids from sliding down the inside of the fabric as it rotates upward. Figure 9 shows the design of the 0.2 m^2 microstrainer drum.

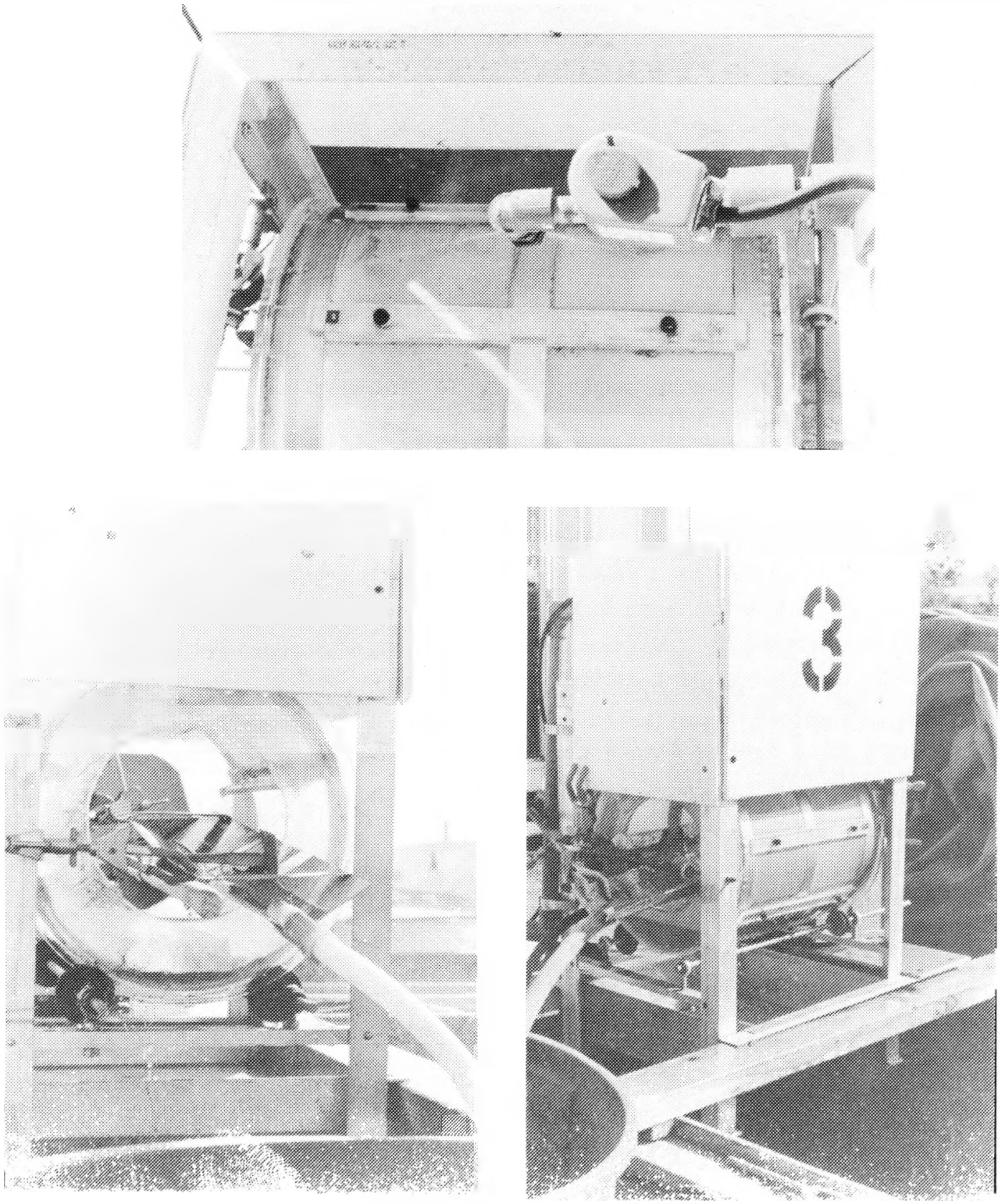


FIGURE 8. EXPERIMENTAL PILOT-SCALE MICROSTRAINER. VIEWS ARE (clockwise starting at top) OF THE SOLENOID VALVE-CONTROLLED BACKWASH SPRAY (looking down on the unit), PERSPECTIVE VIEW OF MICROSTRAINER (note algae on screen and drum pool) AND FACE VIEW SHOWING THE COLLECTION TROUGH (note strained water dripping underneath the drum).

A nylon square mesh industrial quality nylon straining fabric (Nitex brand supplied by Tetko, Inc., Elmsford, New York) was used in all harvesting operations, mostly with 26 μm openings. No problems were encountered with fabric binding, probably because the fabrics were backwashed about 5 minutes beyond the end of harvesting, thus cleansing the fabric. Potable water was used for backwash. No fouling of the fabric was observed.

The microstrainers were placed adjacent to the 3-m^2 ponds (see Figure 3 for the general orientation); inflow was pumped through variable speed flexible impeller pumps at 1-3 liter/min depending on the algal concentrations; strained pond water was pumped from the catch basin to 110-liter graduated plastic containers via small submersible pumps. These same microstrainers are now located atop effluent basins adjacent to the 12 m^2 ponds.

Early experiments (Woodland Oscillatoria inoculations) used a smaller drum (0.1 m^2 straining area) than depicted in Figure 9 . Now installed on the high-rate pond is a two-drum microstrainer with a total of 0.9 m^2 straining area, as shown in Figure 10 . These strainers now remove Scenedesmus from the high-rate pond effluent.

ANALYTICAL METHODS

Suspended Solids Analysis

Total Suspended Matter. Standard Methods (46) 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 and dried at 105°C for one hour in an electric drying oven. Constant weights were found to be attained after one hour 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 disk was weighed; the difference between this weight and the total

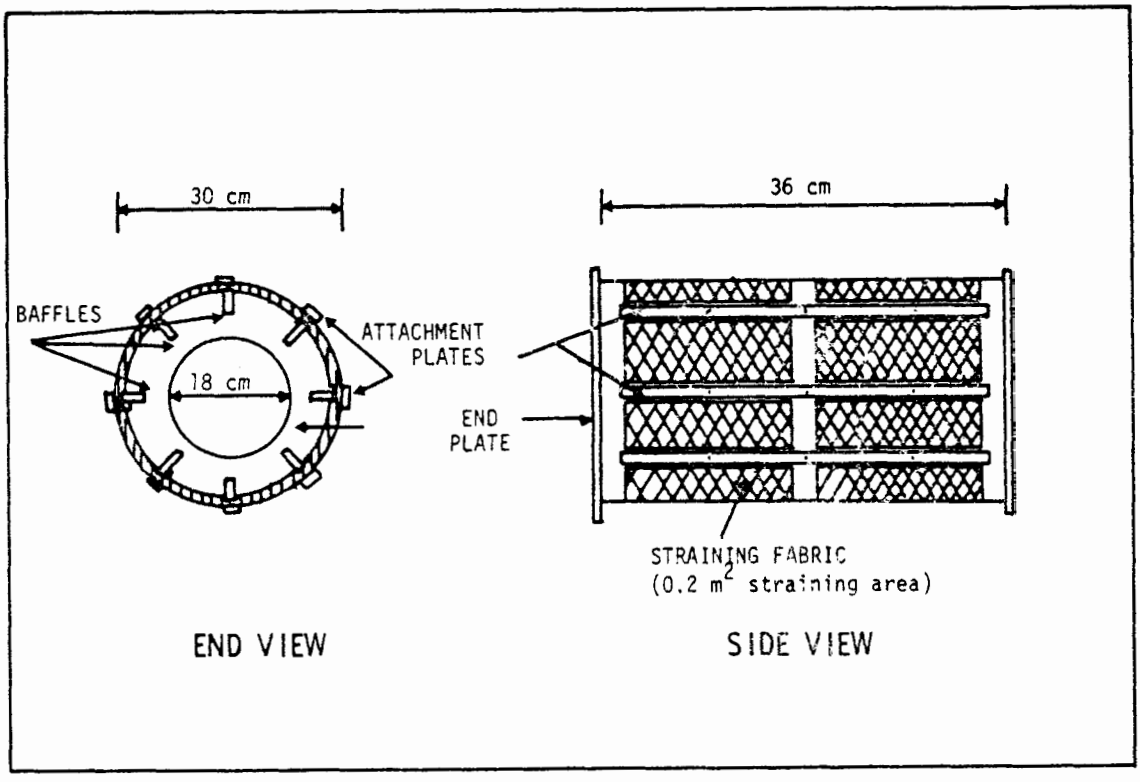


FIGURE 9. DESIGN OF LUCITE DRUM USED IN PILOT-SCALE MICROSTRAINERS

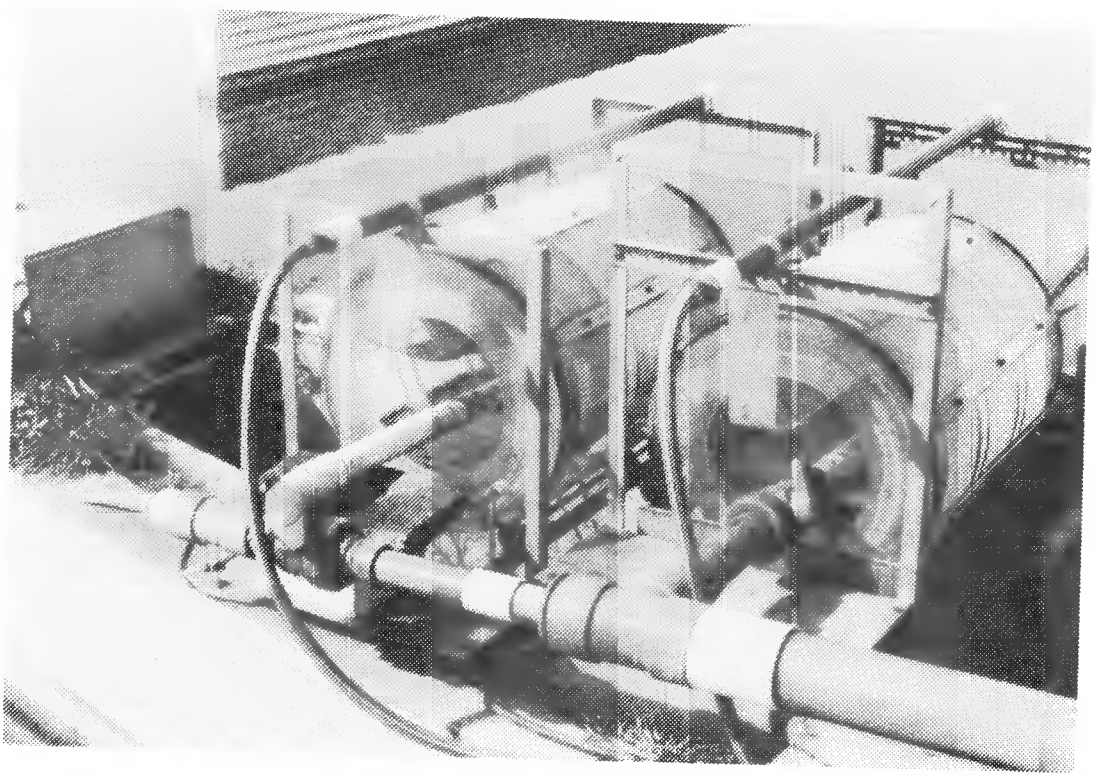


FIGURE 10. HIGH-RATE POND MICROSTRAINING SYSTEM. THE TWO-DRUM UNIT HAS A STRAINING AREA OF 0.9 m^2 AND CAN HARVEST 50 LITERS OF POND CULTURE PER MINUTE.

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 (47) 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; 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 and 650 μm, using a Hitachi Model 100-60 double-beam spectrophotometer. A reading at 750 μm was used to subtract out the effect of turbidity from the other absorbances. As in all light absorbtion 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.

$$\text{Chlorophyll } a \text{ was calculated in mg/l by } (16.5\ D_{665} - 8.30_{650}) \frac{\text{ml MeOH}}{\text{ml sample}}$$

where D = optical density and ml MeOH = final volume of methanol used minus the volume of the filter disk.

Kletts

A Klett-Summerson photoelectric colorimeter with a #66 filter was used to monitor daily changes in pond productivity. The method has the advantage of extreme rapidity and ease of operation by direct measurement of algal concentrations in Klett-units.

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 (46) 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 pre-

pared 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.

Chemical Oxygen Demand (COD)

The dichromate reflux method from Standard Methods (46) 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 4 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 $\frac{(a - G)\ N \times 8000}{\text{ml sample}}$ 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 $Fe(NH_4)_2(SO_4)_2$.

Total Kjeldahl Nitrogen and Ammonia Nitrogen

The determination of total Kjeladahl nitrogen basically followed that of Standard Methods (46), 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 (48). 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 Kjeldahl 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 Kjelkahl 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 500 ml quantities of 50% NaOH were added. The mixtures were immediately distilled into receiving flasks containing 200 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 tri-sodium 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 sulfat standards were prepared and run along with the samples. Ammonia values of the samples were then calculated through construction of a standard curve.

Nitrate Plus Nitrite

The hydrazine sulfate reduction method (NO_3 to NO_2) followed by the diazotization colorimetric determination of NO_2 was used.(49) 5 ml aliquots of filtered samples were measured into test tubes and heated to 37°C in a water bath. The following reagents were added with mixing and allowed to react for 30 minutes at 37°C. 0.2 ml of phenate buffer (1.8 g phenol, 16 ml 1 N NaOH, H_2O to 100 ml); 0.1 ml of hydrazine solution (1.2 g hydrazine sufate to 250 ml H_2O) plus copper sulfate (0.08 g $CuSO_4 \cdot 5H_2O$ to 1000 ml H_2O) in a 1:1 ratio.

The samples were removed from the water bath and 0.10 ml of sulfanilmide - NED solution (5.0 g sulfanilamide, 50 ml conc. HCl, 0.50 g N - (1 - napthyl) - ethylenediame hydrochloride, H_2O to 500 ml) was added. After 30 minutes, the absorbances were read at 543 µm. Sodium nitrate standards were prepared and run along with the samples. Nitrate plus nitrite values were than calculated through construction of a standard curve.

Phosphorus

Filtrable (soluble) orthophosphate was determined by direct colorimetry after filtration with a 0.45 µm membrane filter. Total phosphate was determined in the same manner but preceded by oxidative digestion of the sample.(50)

Orthophosphate (filtrable). A series of five samples were set up at a time, plus a reagent blank and a standard phosphate solution. 20 ml aliquots of samples were measured into 100 ml volumetric flasks and diluted to about 80 ml. 3 ml acid molybdate solution (50 g ammonium molybdate to 200 ml distilled water, 310 ml concentrated H_2SO_4 to 400 ml H_2O; cooled, added together and diluted with H_2O to 1 liter) was added to the first sample. After 30 seconds,

4 ml ANS reducing agent (0.5 g 1 - amino - 2 - napthol - 4 - sulfuric acid in 5 ml of 20% sodium sulfite; dissolved in 200 ml of a 15% sodium metabisulfite solution) was added. It was diluted to 100 ml with H_2O and mixed. At one minute, the procedure was begun with the second sample and so forth. The absorbance of each sample was read at a wavelength of 630 nm exactly 6 minutes after the introduction of the ANS reducing agent. Orthophosphate was calculated in mg P/l by O-PO_4 standard in mg P/absorbance of standard x absorbance of sample x 1000/sample volume.

Total Phosphate. 20 ml aliquots of samples with 1 ml of 5% $MgCl_2 \cdot 5H_2O$, were placed into evaporating dishes and evaporated to dryness on a steam table. Dishes were then placed in a muffle furnace for 1 hour at 800°C. After cooling, 3 ml acid molybdate reagent was added and dishes were placed on the steam table for an additional 30 minutes. The residues were poured with several rinsings into 100 ml volumetric flasks. From this point, the procedure was the same as in the orthophosphate determination.

Microscopy

Microscopic observations were made on fresh samples taken from each pond immediately before harvesting began. A sample was taken in a four-liter beaker from the region of turbulence immediately behind each paddlewheel, and then was subsampled by submerging a 50 ml plastic bottle into the stirred beaker. The samples were counted using a haemacytometer: at least four fields or portions of fields were counted for each sample and usually 1000 or more colonies or discrete cells were enumerated.

The algal cells counted were divided into specific species, or when unidentified algae were present, they were grouped into categories according to gross morphology. Size measurements were also taken so that algal volume concentrations and size distributions could be derived from each count. A typical algal count data sheet is presented in Figure B-3 (see Appendix B).

POND OPERATIONS METHODOLOGY

Harvesting and Dilution Procedures

Summer and fall operations of the 3-m^2 ponds and harvesting systems

were semi-continuous. The schedule of harvesting varied depending on the experimental dilution rate (greater dilution rates required longer harvest times) and on the size of the microstrainer drums used. Early experiments (WO 1 through WO 6) required between 3 and 4 hours for harvesting and dilution, whereas the later experiments utilizing larger strainers required less time (1.5 to 2 hours). The relatively short duration of harvest was necessary because the experimental microstrainers could not be effectively operated at inflow rates less than 1 liter per minute (due to the necessity of maintaining a drum pool during operation).

Microstrainers smaller in size than the units tested are not practical because their fabrication is more difficult and their operation more troublesome. At the detention periods used, the semi-continuous operation should not have significantly affected the experimental results. However, it is likely that production rates were somewhat lower than achievable with continuous daytime dilution.

In experiments WO 1 through WO 6, sewage was added to the ponds during harvesting so that the liquid depth remained constant at 25 cm. Thus, a portion of the pond culture reaching the microstrainer was composed of freshly added sewage; this portion depended on the harvest rate and duration. (Considering a harvest rate of 1.8 liters per minute and a daily dilution of 0.33, the pond culture would be 10% fresh sewage after 60 minutes of dilution, 19% after 120 minutes, and 31% at the end of the 3.5 hrs needed to harvest 330 liters of the 1000-liter pond.) This mode of operation was modified after experiment WO 6 so that no sewage was added to the ponds until harvesting was complete. In all experiments where biomass recycle was practiced, the chosen fraction of harvested algae was returned in one batch to the pond after the harvest was complete.

Sampling

Immediately before the initiation of harvesting, a surface grab sample (using a 4-liter beaker) was taken in the turbulent region behind the paddle wheel. From this sample, subsamples were taken for pH, algal counts, and pond density determinations (Klett, dry weights, and chlorophyll *a*). Klett and pH were measured immediately; the other subsamples were refrigerated until analysis later in the day.

pH was measured using a Beckman "Expandomatic" pH meter (standardized to pH 8.0 buffer); fresh samples (less than 10 minutes removed from ponds) were always used for pH measurements. Temperature was measured *in situ*. Temperature and pH were recorded at 8:30 am before harvesting each day.

Effluent (the strained pond culture) was collected in graduated 110-liter plastic vessels. As each vessel filled (2.25 vessels required for 250 liters harvested), a sample was taken of the stirred vessel's contents; these were combined to give a composite sample, which was refrigerated until analyses were made. The concentrated algal product from the entire harvest was collected, its volume measured using eight-liter containers, and a small sample taken. A sample of the sewage inflow was taken at 15-minute intervals during the inflow period and added to the refrigerated sewage composite.

Data Analysis

A. Definitions of Terms.

1. Harvestability. Harvestability, in the context of this report, refers to the proportion of algae in the pond culture which was retained by straining fabric having 26-μm openings. Determination of this term was made using pond and microstrainer effluent density measurements where

$$\text{Harvestability, \%} = \frac{\text{Pond Density - Effluent Density}}{\text{Pond Density}} \times 100 \quad (7)$$

Harvestability, measured in this manner, is numerically equal to "algal-removal efficiency". The best determination of algal-removal efficiency is made when densities are available as chlorophyll concentrations. Chlorophyll concentration is, however, a poor measure of biomass levels because of its variability with insolation and pond optical density. Therefore, ash-free dry weights were commonly used to calculate algal removals.

Extensive use was made in this project of optical density measurements by the Klett-Summerson Meter to calculate algal removals. Moderately close linear correlation of Klett and ash-free dry weight was attained (see later discussion). However,

the relation is not linear at low Klett values, meaning that the Klett measurement overestimates the density of microstrainer effluents. This effect is due to the coloration and colloidal (fine clays) content of the sewage which gives filtered pond water an optical density greater than that of distilled water. Thus, algal removals, calculated using Klett densities, will underestimate true values.

Harvestability can also be determined from algal counts. Algae greater than 26 μm in two (or three) dimensions are classed as "harvestable" as are all filamentous algae. The percent harvestability as determined by algal size (volume) measurements is calculated as follows:

$$\text{Harvestability, \%} = \frac{\text{volume concentration of harvestable algae}}{\text{total algal volume concentration}} \times 100 \quad (8)$$

2. Biomass Recycle. This term refers to the return to the growth unit of a portion of the algal biomass previously removed prior to harvesting. It is measured as a fraction of the total biomass in the pond effluent. Either captured biomass or effluent biomass can be recycled. The nominal recycle fractions for harvested biomass recycle or effluent biomass recycle refer to the quantity of biomass recycled relative to the amount captured in the harvest stream or lost in the separator effluent, respectively. Nominal recycle fractions are related to their respective biomass recycle rates as follows:

$$\begin{matrix}\text{Harvested} \\ \text{Biomass} \\ \text{Recycle}\end{matrix} = \begin{matrix}\text{Nominal} \\ \text{Harvest} \\ \text{Recycle}\end{matrix} \times \text{Harvestability} \quad (9)$$

$$\begin{matrix}\text{Effluent} \\ \text{Biomass} \\ \text{Recycle}\end{matrix} = \begin{matrix}\text{Nominal} \\ \text{Effluent} \\ \text{Recycle}\end{matrix} \times (1-\text{Harvestability}) \quad (10)$$

3. Production. Production is defined as the areal rate of biomass withdrawal from a pond. Harvestable production is the quantity of captured biomass not returned to the pond (i.e. the net yield); total production is the sum of the net yield and the biomass which escapes with the strainer underflow. The total production depends on the pond algal density and the dilution rate; therefore, the production at the start of the experiment depends to a large extent on the initial density. This artifact of the start-up conditions declines rapidly at first but still influences, to a smaller extent, production values after two or more detention periods have passed. In order to arrive at a closer estimate of the true production rate achieved, a third production term, gross production, is calculated by adjusting the average total production for the change in pond density over the course of the experiment. This relationship is shown in the following equation:

$$\text{Gross Production} = \text{Total Production} + \frac{\left(\text{Final Pond Density} - \text{Initial Pond Density}\right) \times \text{Pond Volume}}{\text{Pond Area} \times \text{Experiment Duration}} \qquad (11)$$

The greatest number of density measurements were made using the Klett Summerson meter which measures optical density at approximately 660-700 nm (using a red filter). Thus, for most of the experiments, the daily production rates are given in terms of Klett-liters x 10^3 meter^{-2} day^{-1}. In order to allow estimation of the production rate in grams ash-free dry weight meter^{-2} day^{-1}, these two terms were correlated using a linear regression program on 102 data pairs taken from all but the Micractinium experiments. The following relationship was obtained:

$$\text{Production, g m}^{-2}\text{ day}^{-1} = 1.6 \times \text{Production, Klett-liters} \times 10^3\ \text{m}^{-2}\text{ day}^{-1} + 1.5 \qquad (12)$$

with r, the correlation coefficient, equal to 0.87, indicating a strong correlation.

The slope obtained corresponds quite well with previous experience in using the Klett-Summerson meter and dry weights as simultaneous

measures of culture densities. The positive-valued intercept is to be expected because the background colloidal turbidity (which shows up in Klett measurements but not in dry weights) causes the Klett values to increase more slowly (for a given increase in dry weight) at the lower range of algal densities than at higher ranges. Since distilled water was used to set the zero point on the Klett meter, it was known that the point (0,0) should be on the regression line. In order to force the line through the point (0,0), a line of slope 1.77 was drawn between the points (0,0) and (10, 17.7). The latter point was chosen to represent the higher end of the range of production values encountered. The adjusted line is at the upper boundary of the 95% confidence interval for slope (1.43 - 1.80). Refer to Figure 11 for a graphical comparison of the two lines.

Explanation of Figures

As explained previously, the harvestability of the cultivated algae was measured using two techniques: microscopic observations and monitoring of microstrainer performance. Results of these measurements are plotted simultaneously using variously shaded bars to represent the contribution of the different algal species to the harvestable population and line-connected points to show the calculated algal removal based on the density of influent and underflow streams of the microstrainer. Thus, for experiment SC1 on October 5, Figure 30 would be interpreted as follows: Microscopic observation showed that of the total algal volume concentration, 60% was composed either of filamentous algae or algal cells or colonies larger than 26 μm in two or three dimensions. Furthermore, harvestable Micractinium, Scenedesmus, and Actinastrum made up 54%, 1%, and 5%, respectively, of the total algal population. On this same day, an algal removal efficiency of 48% was achieved when the pond culture was harvested by microstraining.

Production is graphically divided into harvestable and total portions according to the aforementioned definitions. Harvestable and total productions for experiments SC1 and SC2 (Figure 31) illustrate typical results. As indicated by the legend, the shaded region of each bar represents harvestable production and the white portion represents algal biomass lost in the strainer underflow. The overall bar height corresponds to total production. In

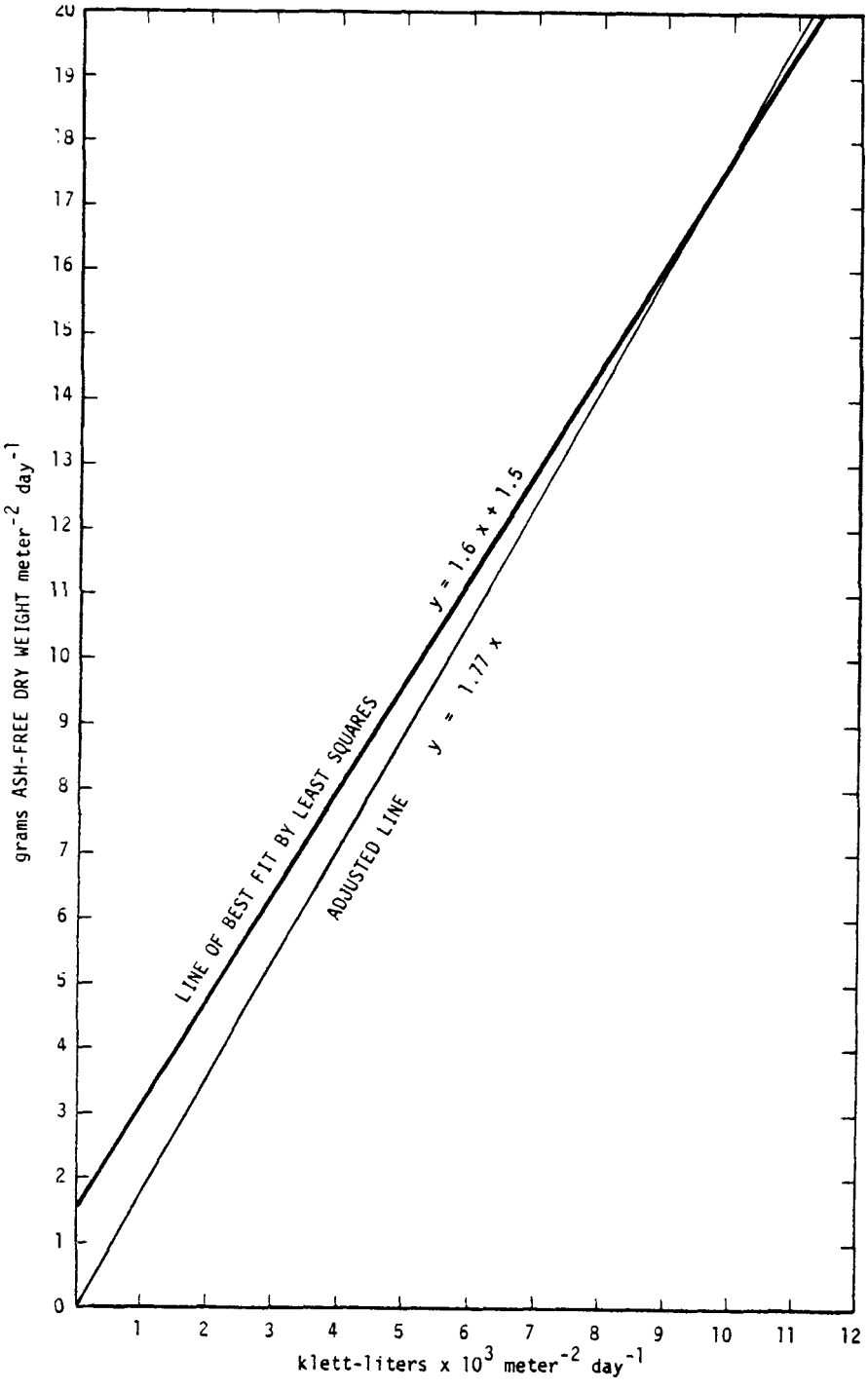


FIGURE 11. CORRELATION OF KLETT-BASED AND ASH-FREE DRY WEIGHT-BASED PRODUCTION VALULS.

experiments where no biomass was recycled, the ratio of harvestable to total production should be equal to the algal-removal efficiency. For example, on October 13, experiment SC2, harvestable production was 4.0 and total production 6.5, giving a ratio of harvestable to total production of 62% which is slightly less than the algal-removal efficiency of 64% observed that day (see SC2 results following). The reason for the discrepancy in the results is that production values and algal-removal efficiencies are calculated from independent measures: harvestable production from the product of concentrate density and volume; non-harvestable production from the product of effluent volume and density; and algal-removal efficiency from the pond culture and separator effluent densities. Whereas the ratio of harvestable to total production for non-recycle ponds is equal to the algal-removal efficiency of the separation stage (microstrainer), this ratio is also a function of the recycle rate in ponds where a portion of either the harvested biomass or the separator effluent is returned to the pond after each harvest. The functions are developed here in order to clarify this and later discussions on production.

Harvestable production, defined as the quantity of captured biomass not recycled, is operationally calculated according to the following equation:

$$P_H = \frac{C_T - C_R}{A} \tag{13}$$

where P_H = harvestable production

C_T = total rate of biomass capture by the separator

C_R = rate of return of captured biomass to growth unit

A = liquid free surface area if growth unit

Total production is the sum of the harvestable production and the quantity of biomass in the separator effluent which is not recycled

Total production is the sum of the harvestable production and the quantity of biomass in the separator effluent which is not recycled

$$P_T = P_H + \frac{L_T - L_R}{A} \qquad (14)$$

where P_H = total production

L_T = total rate of biomass loss through separator

L_R = rate of return of lost (non-harvestable) biomass to growth unit

The ratio of harvestable to total production is given by

$$P_H/P_T = \frac{C_T - C_R}{C_T - C_R + L_T - L_R} \qquad (15)$$

It is desirable to express the term P_H/P_T solely in terms of the algal-removal efficiency (E), the harvested biomass recycle rate (R_C), and the effluent biomass recycle rate (R_L); thus, E, R_C, and R_L need to be expressed in the previously defined terms. The Expressions for E, R_C, and R_L can be developed by referring to the definition diagrams of biomass production and harvesting systems where either harvested biomass (Figure 12) or separator effluent (Figure 13) is recycled. In Figure 12 the flows into the pond are sewage (Q) and recycled biomass (q) from the harvest stream; thus, the flow out of the pond and into the separator is the sum of Q and q. Because the flow volumes were measured on the microstrainer effluents during the experiments, the influent sewage volume in experiments where harvested biomass was recycled was always slightly less (by the ratio $q/(Q + q)$ than the set dilution rate would call for; however, the recycle flow volume was always small (less than 5%) relative to the influent sewage flow making the operational error insignificant.

The microstrainers were operated so that the underflow or effluent flow rate very closely approximated the inflow rate: the volume of the algae removed was always negligible. Therefore, in Figure 12, the inflow and

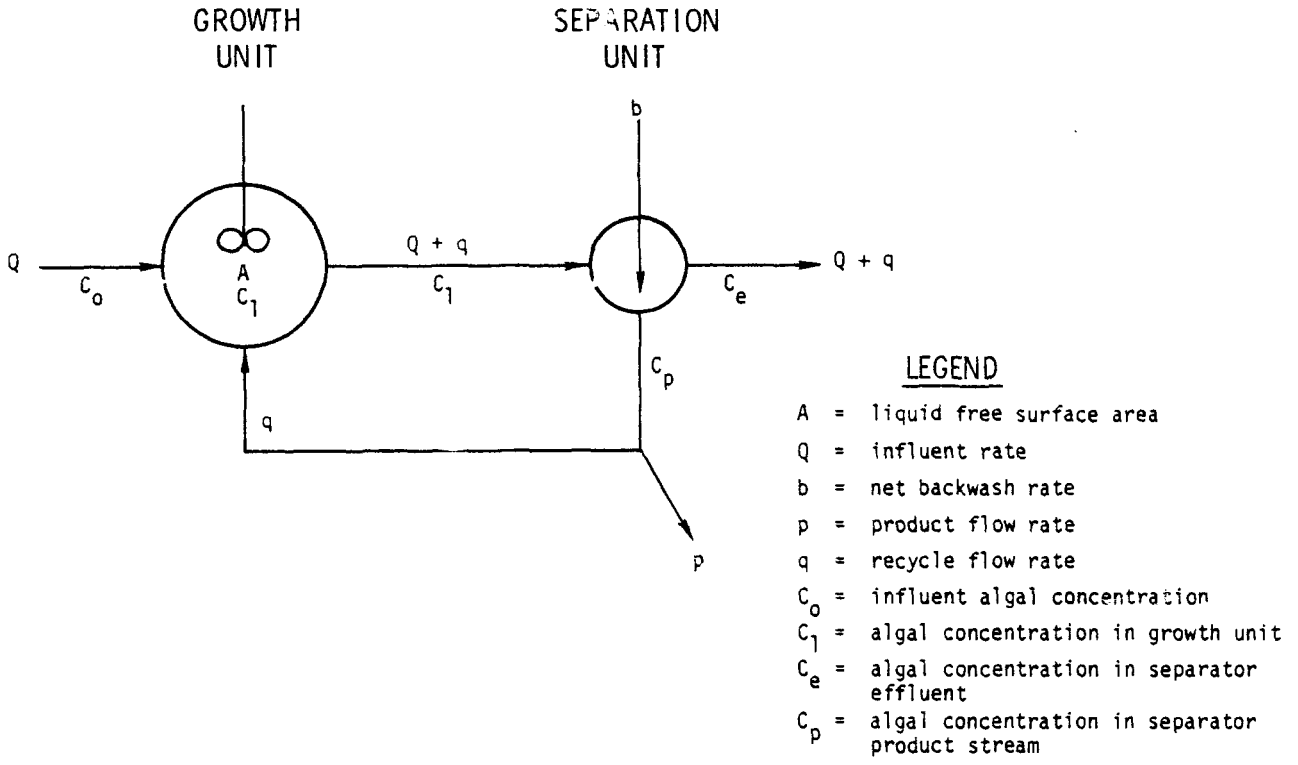


FIGURE 12. DEFINITION DIAGRAM OF AN ALGAL BIOMASS PRODUCTION AND HARVESTING SYSTEM WHERE HARVESTED BIOMASS IS RECYCLED TO THE GROWTH UNIT.

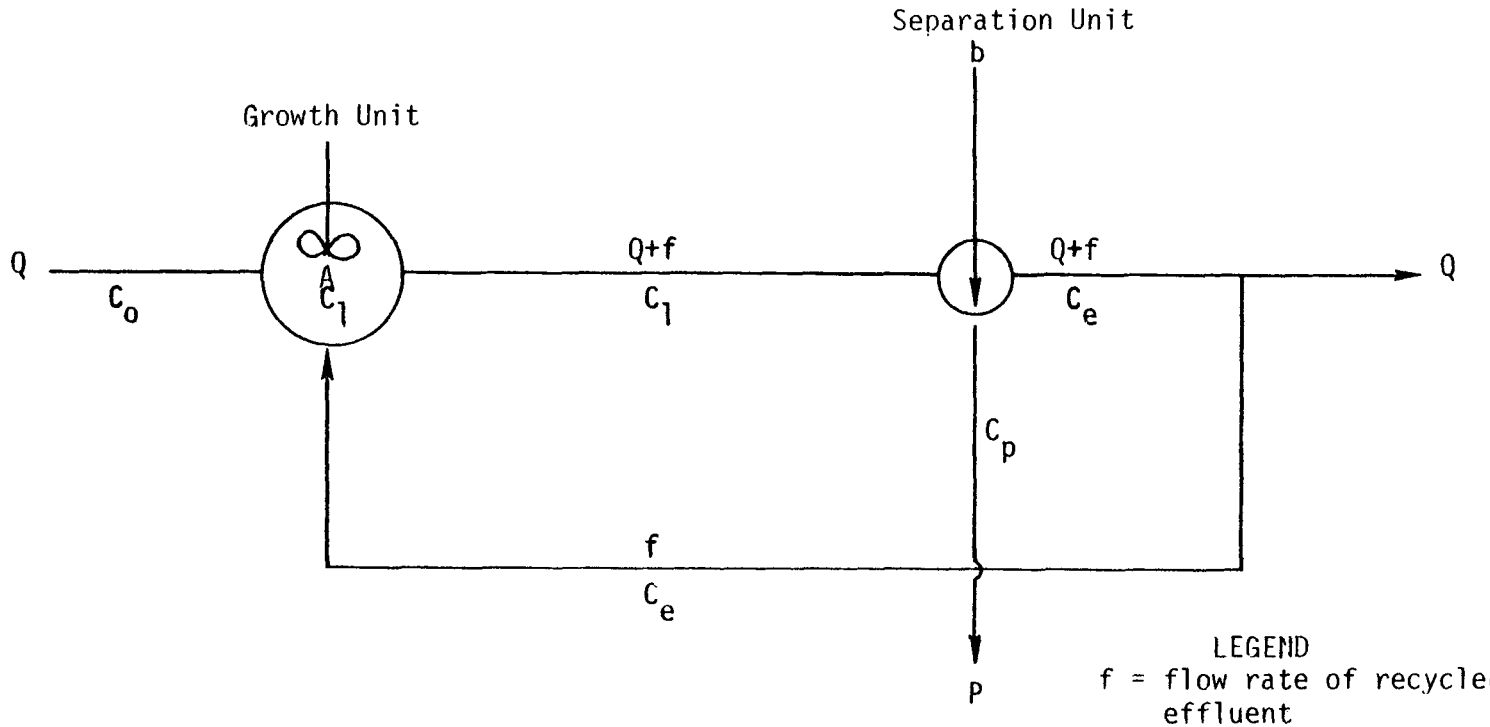


FIGURE 13. DEFINITION OF AN ALGAL BIOMASS PRODUCTION AND HARVESTING SYSTEM INCORPORATING SEPARATOR EFFLUENT RECYCLE

effluent flow rates are identical. Most of the liquid volume (q + p) in the microstrainer product stream derives from the net backwash spray (b); thus, these flow rates are also taken as equal. (Net backwash spray refers only to that portion of the spray incident on the straining fabric.)

The rate of biomass inflow to the separator is $(Q + q)\ C_1$, which is equal to the sum of the outflows $(Q + q)\ C_e$ and bc_p. The term $(Q + q)\ C_e$ corresponds to the rate at which biomass is lost in the microstrainer effluent (L_T) and $(Q + q)\ C_1$ is equal to the sum of the captured biomass (C_T) and the effluent biomass (L_T). Using these expressions for rates of biomass into and out of the separator, and recalling the original definition of algal-removal efficiency:

$$C_T/(C_T + L_T) = [(Q + q)\ C_1 - (Q + q)C_e]/(Q + q)\ C_1 = (C_1 = C_e)/C_1 \equiv E \quad (16)$$

Similarly:

$$C_R/(C_T + L_T) = qC_p/[(Q + q)(C_1 - C_e) + (Q + q)C_e] = qC_p/(Q + q)C_1 \equiv R_c \quad (17)$$

It follows that

$$R_c/E = C_R/C_T \equiv r_c \quad (18)$$

The case where separator effluent is recycled to the growth unit and R_c is zero is described by Figure 13. There are two differences between the two cases: (1) outflow from the system described by Figure 13 is exactly equal to the sewage inflow (neglecting evaporation) and (2) the flow out of the growth unit is equal to the sum of Q, the sewage inflow, and f, the flow rate of effluent being recycled.

Referrring to Figure 13 and using the relevant expressions for biomass rates:

$$L_R/(C_T + L_T) = fC_e/[(Q + f)\ (C_1 - C_e) + (Q + f)C_e] = fC_e/(Q + f)C_1 \equiv R_L \quad (19)$$

and

$$R_L/(1 - E) = R_L(C_r + L_r)/L_T = L_R/L_T \equiv r_L \quad (20)$$

Finally, after dividing the numerator and denominator of equation 15 by $(C_T + L_T)$ and substituting from equations (16), (17), and (19):

$$P_H/P_T = (E - R_c)/(1 - R_c - R_L) \quad (21)$$

$$= \begin{cases} (E - R_c)/(1 - R_c), \text{ if } R_L = 0 & (22) \\ E/(1 - R_L), \text{ if } R_c = 0 & (23) \\ E \quad , \text{ if } R_c = R_L = 0 & (24) \end{cases}$$

Equations (22) and (23) can be rewritten in terms of the nominal recycle variables using equations (18) and (20):

$$P_H/P_T = E(1 - r_c)/(1 - r_c E); \; r_L = 0 \quad (25)$$

$$P_H/P_T = E/[1 - r_L (1 - E)]; \; r_c = 0 \quad (26)$$

The production values for October 13, experiment SC1 (Figure 31) can now be referred to. On that day the harvestable production was 3.1 and the total 5.4 (Klett-liters x 10^3 m^{-2} day^{-1}), giving a ratio of 57%. E for this day was 74% and r_c was set at 50%. Based on E and r_c, P_H/P_T should be 59%. Again, the difference between the two ratios is attributable to the independence of the measurements upon which they are based.

Mic 1 was the experiment conducted incorporating the recycle effluent (r_L = 50%). Referring to a typical day's results on September 11 (Figure 25), harvestable and total productions of 3.1 and 3.8 Klett-liters x 10^3 m^{-2} day^{-1} respectively, are found yielding a P_H/P_T of 82%. The P_H/P_T based on r_L = 50% and E = 74% and, calculated according to Equation (26), is 85%. Thus, it is apparent that P_H/P_T values will always be less than E for harvested biomass recycle and greater than E for ponds where effluent is recycled. This observation is confirmed by plots of Equations (22) and (20) in Figures 14 and 15, respectively. This analysis demonstrates the effect of recycling on harvestable algal productivity.

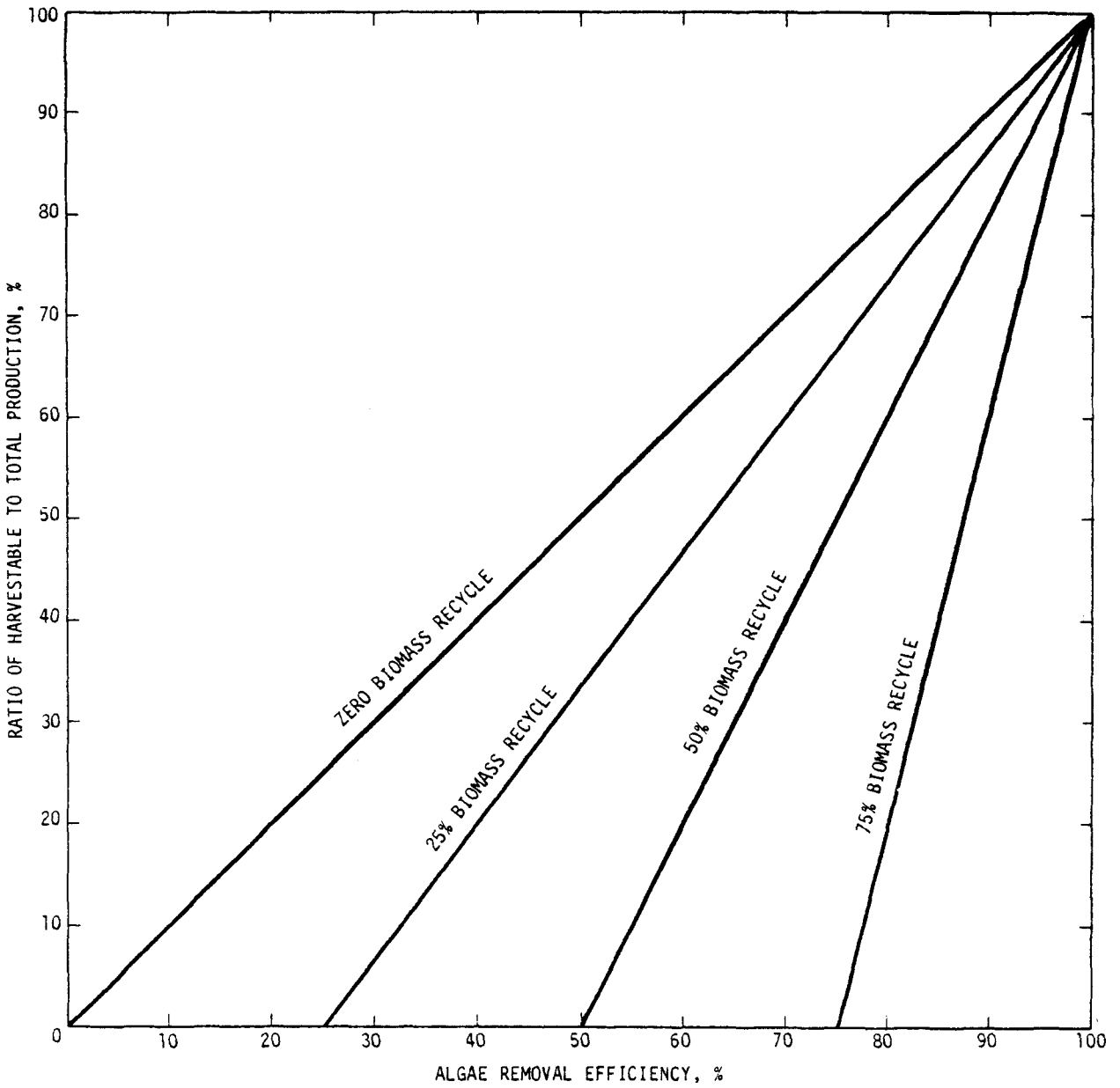


FIGURE 14. RELATIONSHIPS BETWEEN ALGAL-REMOVAL EFFICIENCIES (E) AND RATIOS OF HARVESTABLE TO TOTAL PRODUCTION (P_H/P_T) FOR VARIOUS RATES OF HARVESTED BIOMASS RECYCLE (R_C). THIS FIGURE IS ESSENTIALLY A PLOT OF EQUATION (21) FOR THE CASE OF $R_L = 0$.

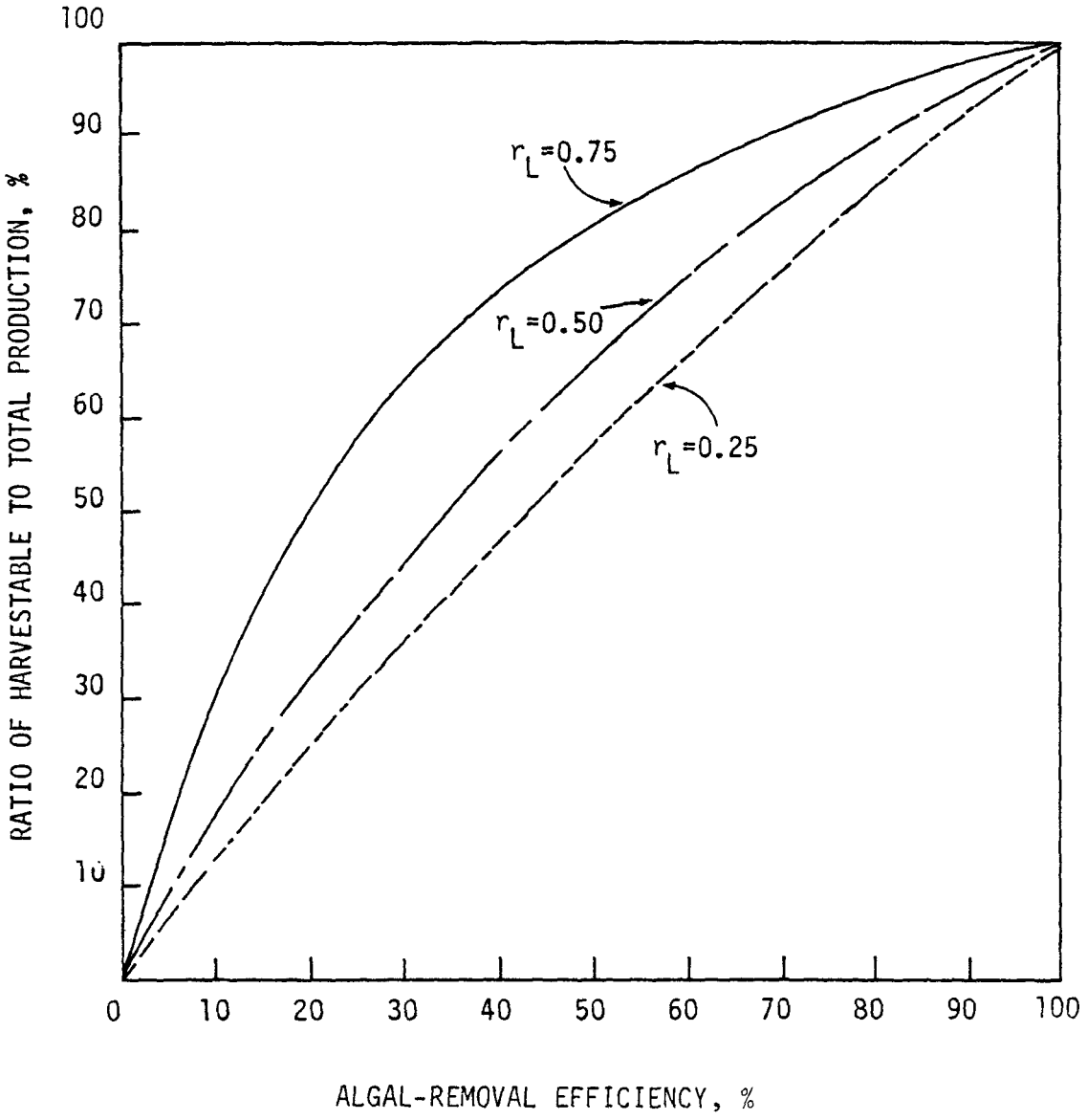


FIGURE 15. RELATIONSHIPS BETWEEN ALGAL REMOVAL EFFICIENCIES AND RATIOS OF HARVESTABLE TO TOTAL PRODUCTION FOR VARIOUS RATES OF SEPARATOR EFFLUENT RECYCLE (r_L). THIS FIGURE IS ESSENTIALLY A PLOT OF EQUATION (26) FOR THE CASE OF $r_c = 0$.

Photosynthetic Efficiencies

Continuous record of insolation was obtained using an Eppley pyranometer and dual-sensitivity range strip chart recorder. The recorder readout was integrated by an area approximation method to give daily insolation values in Langleys. Photosynthetic efficiencies based on total and photosynthetically available (43% of total) insolation were calculated according to the following equations:

$$S.C.E. = \frac{P h_c}{S} \qquad (26)$$

$$P.C.E. = \frac{P h_c}{0.43\ S} \qquad (27)$$

where S.C.E. = solar conversion efficiency

P.C.E. = photosynthetic conversion efficiency

P = production, grams ash-free dry weight per square meter per day

h_c = heat of combustion of algal biomass, taken as 5,500 gm cal per gram ash-free dry weight

S = incident solar energy, gram calories per square meter per day

In Table 5 is given maximum and minimum algal productivity at Latitude 37°N (Richmond, California) as a function of visible light conversion efficiency. The climatic conditions applicable to Richmond, California would average somewhat above the minimum of the table. The insolation values obtained during these experiments were within the limits set by the table.

WOODLAND *Oscillatoria* INOCULATIONS

Source and preparation of inoculum. *Oscillatoria* present in a sample from Pond 3 of the Strong Area facultative pond system, Woodland, California, was isolated and cultured on Allen and Arnon blue-green algal media (51). Several liters of the laboratory culture were transferred outside into a shallow (15 cm deep) vessel. The outdoor culture was partially shaded, bubbled with air, and (through additions of laboratory-grown culture) built

TABLE 5

MAXIMUM AND MINIMUM
ALGAL PRODUCTIVITY OF LATITUDE 37°N
AS A FUNCTION OF MONTH AND VISIBLE
LIGHT CONVERSION EFFICIENCY
(in grms m^{-2} day^{-1})

Month	Condition	FOR ASSUMED VISIBLE SUNLIGHT ENERGY CONVERSION EFFICIENCY									
		1	2	3	4	5	6	7	8	9	10
JAN.	MAX	1.76	3.52	5.28	7.04	8.80	10.56	12.32	14.08	15.84	17.6
	MIN	.79	1.58	2.37	3.16	3.95	4.74	5.53	6.32	7.11	7.90
FEB.	MAX	2.61	5.22	7.83	10.4	13.0	15.6	18.3	20.8	23.5	26.1
	MIN	1.19	2.38	3.57	4.76	5.95	7.14	8.33	9.52	10.7	11.9
MAR.	MAX	3.49	6.98	10.5	13.9	17.4	20.9	24.4	27.9	31.4	34.9
	MIN	1.94	3.88	5.82	7.76	9.70	11.64	13.6	15.5	17.5	19.4
APR.	MAX	4.45	8.90	13.3	17.8	22.2	26.7	31.1	35.6	40.0	44.5
	MIN	2.41	4.82	7.23	9.64	12.0	14.5	16.8	19.3	21.7	24.1
MAY.	MAX	5.22	10.44	15.6	20.8	26.1	31.3	36.5	41.7	46.9	52.2
	MIN	3.06	6.12	9.2	12.2	15.3	18.4	21.4	24.5	27.5	30.6
JUN.	MAX	5.43	10.8	16.3	21.7	27.1	32.6	38.0	43.4	48.8	54.3
	MIN	3.09	6.18	9.27	12.4	15.4	18.5	21.6	24.7	27.8	30.9
JUL.	MAX	5.23	10.4	15.7	20.9	26.1	31.4	36.6	41.8	47.0	52.3
	MIN	3.16	6.32	9.48	12.6	15.8	18.9	22.1	25.3	28.4	31.6
AUG.	MAX	4.74	9.48	14.2	18.9	23.7	28.4	33.1	37.9	42.6	47.4
	MIN	2.77	5.54	8.31	11.1	13.8	16.6	19.4	22.2	24.9	27.7
SEP.	MAX	3.83	7.66	11.49	15.3	19.1	22.9	26.8	30.6	34.4	38.3
	MIN	2.22	4.44	6.66	8.88	11.1	13.3	15.5	17.7	19.9	22.2
OCT.	MAX	2.98	5.96	8.94	11.9	14.9	17.8	20.8	23.8	26.8	29.8
	MIN	1.53	3.06	4.59	6.12	7.65	9.18	10.7	12.2	13.7	15.3
NOV.	MAX	2.02	4.04	6.06	8.08	10.1	12.1	14.1	16.2	18.2	20.2
	MIN	1.02	2.04	3.06	4.08	5.10	6.12	7.14	8.16	9.18	10.2
DEC.	MAX	1.53	3.06	4.59	6.12	7.65	9.18	10.7	12.2	13.7	15.3
	MIN	0.68	1.36	2.04	2.72	3.40	4.08	4.76	5.44	6.12	6.8

up in volume to about 100 liters. At this point the culture was transferred to a rectangular, paddle wheel mixed pond, and later to a 3-m^2 growth unit. During this period, no significant problems were encountered with competing algal species. Once in the 3-m^2 pond, the culture was built up in volume by additions of settled sewage, along with proportionate feedings of HCO_3^- and Fe^{+++}. When the culture volume reached the pond capacity (1000 liters), indicated daily harvesting and dilution procedures were initiated; this was experiment WO1. Two experiments, WO2 and WO3, were inoculated with algae (predominantly Oscillatoria) harvested with microstrainers from the WO1 pond. Later experiments, WO3-5, were, in turn, inoculated with algae harvested from WO2.

Because of a bloom of Micractinium, which quickly established themselves as the predominant algae, inoculums for the last three experiments (WO6, WO9, W)10) involving Oscillatoria were obtained by straining pond culture from a field-scale facultative pond (Strong Pond No. 3 at Woodland) which sustained a significant Oscillatoria population throughout the summer and fall of 1976. The straining operations took about one-half day each. On the same day, the concentrated pond algae (mostly Oscillatoria) were inoculated into ponds filled with a 50% settled sewage and 50% sunlight - dechlorinated potable water mixture.

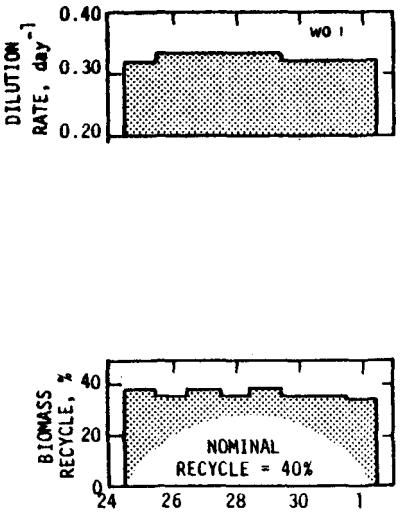
Experimental designs for the Woodland Oscillatoria experiments are given in Table 6. Discussion of the results in this section covers the data summarized in Figures 16 through 23 and in Figures A-1 through A-4 in Appendix A. Further remarks on photosynthetic efficiencies and nutrient removals as well as interpretation of these results follow in the Discussion Section.

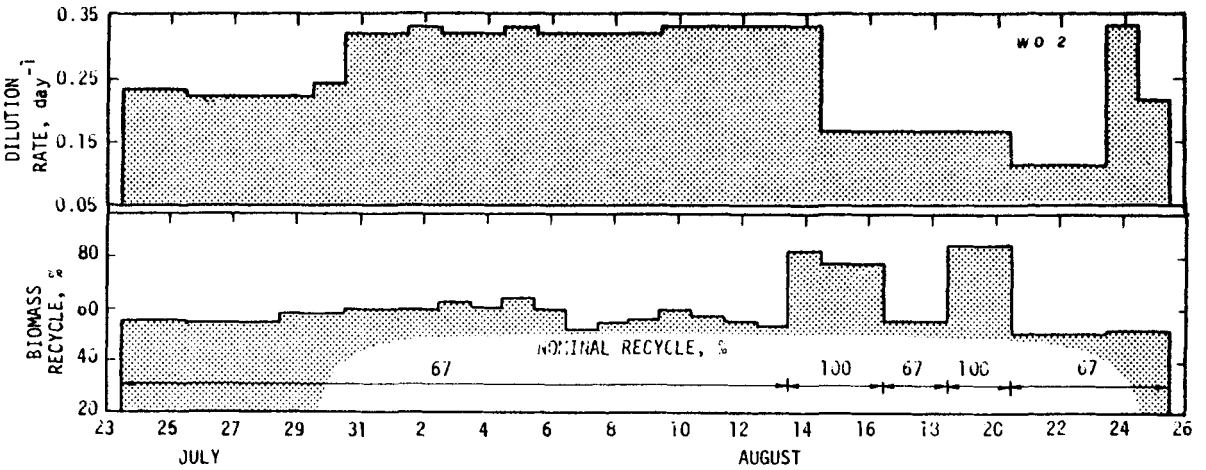
Exp. WO1 (Figures 16-17). As explained above, this experiment consisted of the dilution and harvest biomass recycle of an outdoor culture (consisting primarily of Oscillatoria) which had been grown as a batch culture over the previous two weeks. The culture was diluted daily with 330 liters fresh, settled sewage, and 40% of the harvested biomass was recycled. Over the course of the dilution phase, the culture harvested well (87 + %), but the relative proportion of Oscillatoria declined from 87% initially to 60%

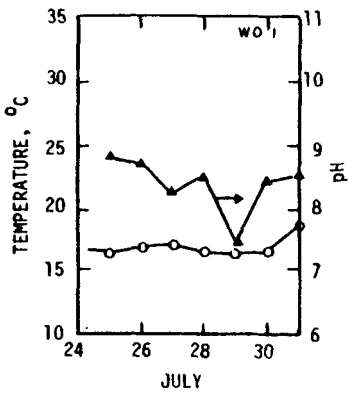
TABLE 6.

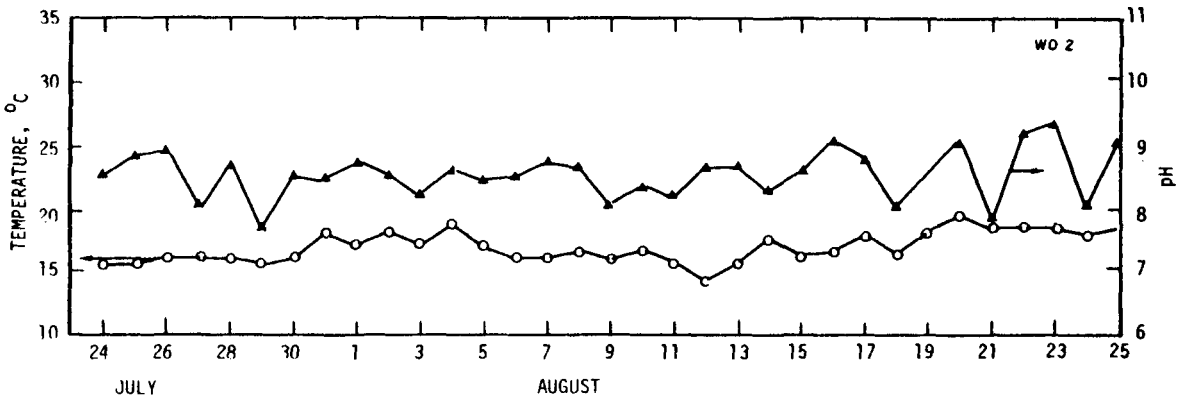
EXPERIMENT DESIGNS FOR WOODLAND
Oscillatoria INOCULATIONS

Experiment Code	Innoculum Source	Nominal Biomass Recycle,% (Averaged)	Dilution Rate, day^{-1} (Averaged)	Figures
W01	Laboratory-grown W. *Oscillatoria*	40	0.33	16-17
W02	W01 Harvest	67	0.31	16-17
W03	W01 Harvest	50	0.32	18-19
W04	W02 Harvest	-0-	0.17	18-19
W05	W02 Harvest	78	0.15	20-21
W06	Field-collected W. *Oscillatoria*	50	0.25	20-21
W09	Field-collected W. *Oscillatoria*	-0-	0.20	22-23
W010	Field-collected W. *Oscillatoria*	67	0.22	22-23


DILUTION RATE, day⁻¹
0.40
0.30
0.20
WO 1
BIOMASS RECYCLE, %
40
20
0
NOMINAL RECYCLE = 40%
24
26
28
30
1


DILUTION RATE, day⁻¹
0.35
0.25
0.15
0.05
WO 2
BIOMASS RECYCLE, %
80
60
40
20
NOMINAL RECYCLE, %
67
100
67
100
67
23
25
27
29
31
2
4
6
8
10
12
14
16
18
20
22
24
26
JULY
AUGUST


TEMPERATURE, °C
35
30
25
20
15
10
pH
11
10
9
8
7
6
WO 1
24
26
28
30
JULY


TEMPERATURE, °C
35
30
25
20
15
10
pH
11
10
9
8
7
6
WO 2
24
26
28
30
1
3
5
7
9
11
13
15
17
19
21
23
25
JULY
AUGUST

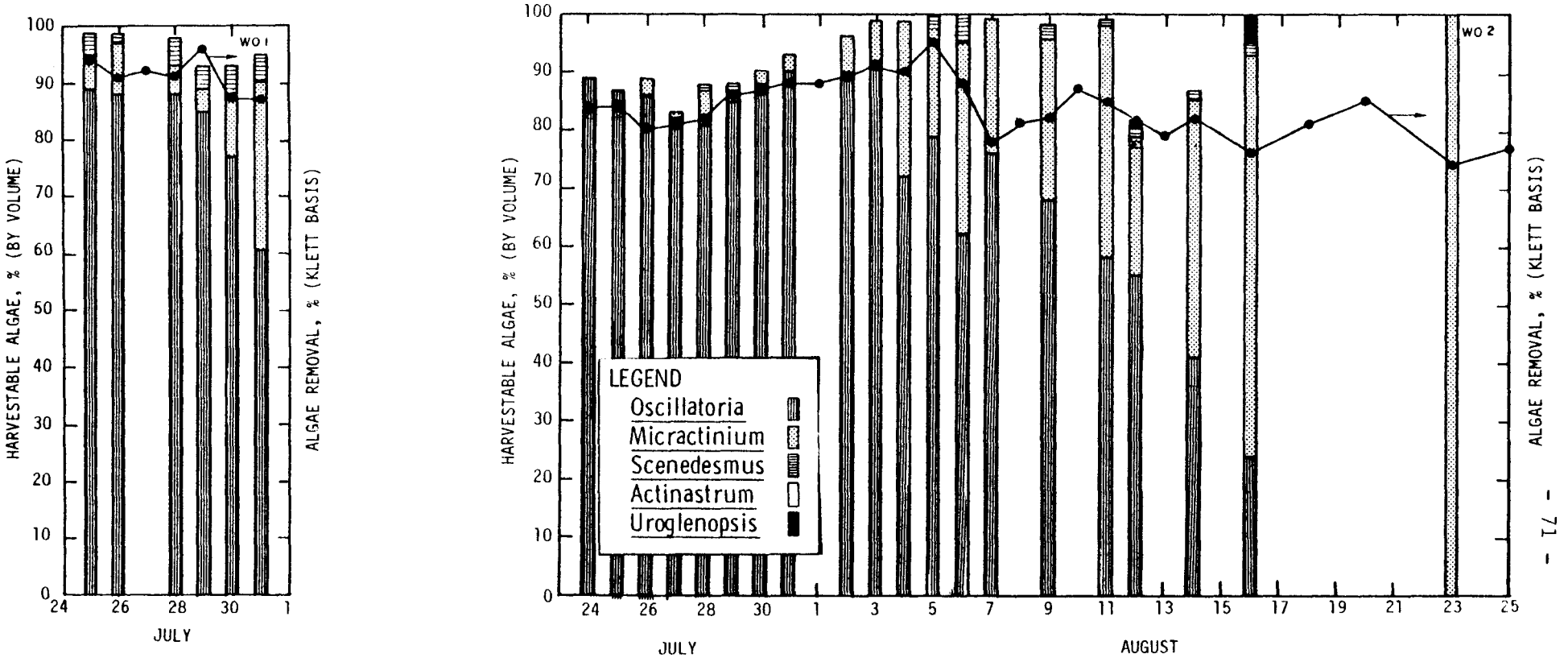


FIGURE 16. OPERATIONAL SCHEDULES, TEMPERATURES, pH, HARVESTABLE ALGAL FRACTIONS, AND ALGAL-REMOVAL EFFICIENCIES FOR EXPERIMENTS WO1 AND WO2

at the experiment's termination when the Oscillatoria started to be washed out at a rate faster than the combined rates of growth and biomass recycle. The concentrations of harvestable Micractinium increased during the experiment (from 0 to 42%) while Scenedesmus stayed fairly constant.

The algal-removal efficiencies achieved by microstraining with 26 μm fabric correlated well with microscopically-determined harvestabilities; they showed the same slightly declining trend but at a lower average level. Microstrainer effluent densities increased slightly during the experiment from Klett 10 initially to Klett 15.

Production values declined with time in similar fashion to the decline of pond density. The averages of 6.6 and 7.9 (Klett-liters x 10^3 meter^{-2} day^{-1}) for harvestable and total production, respectively, are partially an artifact of the high initial pond density on the first day. (This was occasioned by a previously lower dilution rate and higher biomass recycle during the buildup of the culture.) Therefore, gross total production was only 6.4. The average ratio of net to total production was quite favorable, 84%, and stayed consistently high.

The 9 a.m. (PDT) pond temperature varied between 16°C and 19°C throughout the experiment; pH decreased slightly from about 8.8 initially to 8.5 at termination. Insolation for the period was moderate (generally lightly overcast until noon and then clearing) and likely averaged between 600 and 700 Langleys. Quantitative records of the insolation levels were not made.

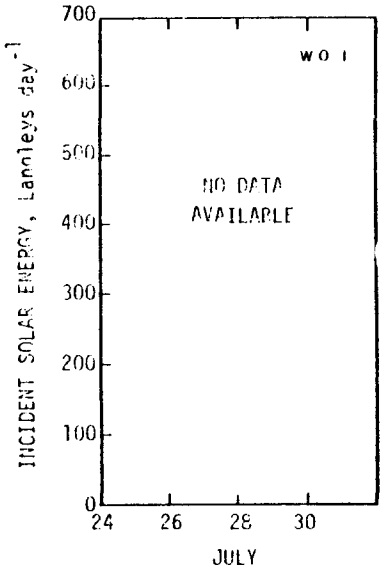
Exp. WO2 (Figures 16-17). Excess harvested algae from Exp. WO1 was added to a second pond along with a 50% sewage-50% water mixture. Beginning July 24 the pond was diluted and harvested daily at 220 to 240 liters per day and 67% of the harvested algae was recycled. Because of an increasing trend in pond density, the dilution rate was increased to 0.32 day^{-1} starting July 31. During the early phase of the experiment (dilution rate = 0.23 day^{-1}), the volume concentration of Oscillatoria increased slightly (from 770 to 1050 μm^3 x 10^6 ml^{-1}). At the faster dilution rate, the Oscillatoria began a slow decline, accompanied by a rapid increase in the Micractinium

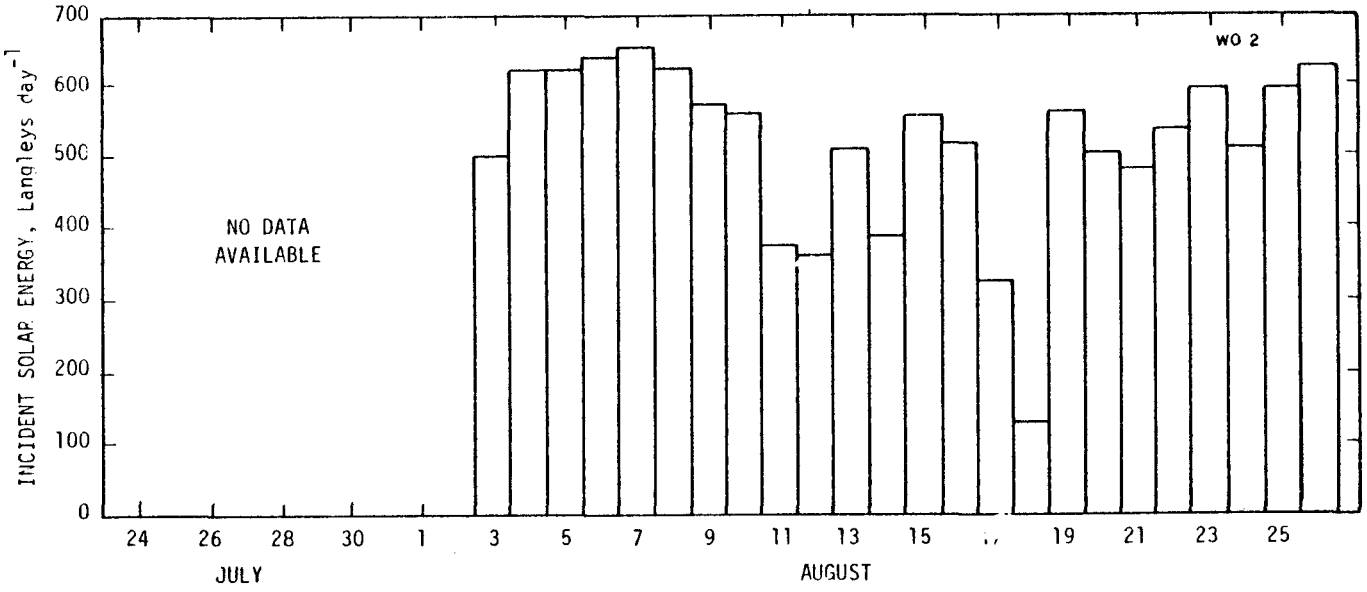
concentration. After several days of cloudy weather, the operating schedule was changed again so that 330 liters were harvested every other day and 100% of the harvest was recycled; thereupon, the pond density stopped its decline. The Oscillatoria and Micractinium, however, continued their respective declining and increasing trends until Micractinium became the predominant algae about August 13.

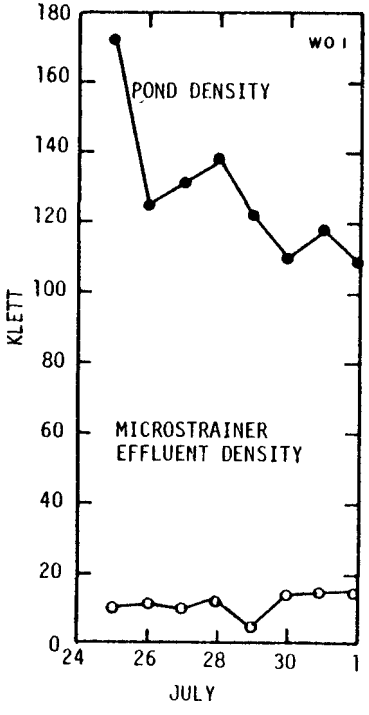
Algal-removal efficiencies achieved with microstraining increased from 84% initially to a peak of 95% on August 5 when Oscillatoria composed 79%, harvestable Micractinium made up 20%, and harvestable Scenedesmus accounted for 1% of the total algal concentration. After this date, the algal removals decreased slightly (microscopically-determined harvestabilities remained high). At the termination date, Micractinium made up almost 100% of the algal population. Throughout the experiment microstrainer effluent densities averaged a Klett of about 20, reaching a maximum of 30 and a minimum of 8. The trend of algal-removal efficiencies, increasing at first and then declining, appears to be partially an artifact of the pond density values (Figures 16 and 17) as the effluent densities showed no long-term trends. The morning temperature gradually rose from 15.5°C on July 24 to 18°C on August 25. Culture pH varied between 7.6 and 9.2 but mostly stayed close to 8.5.

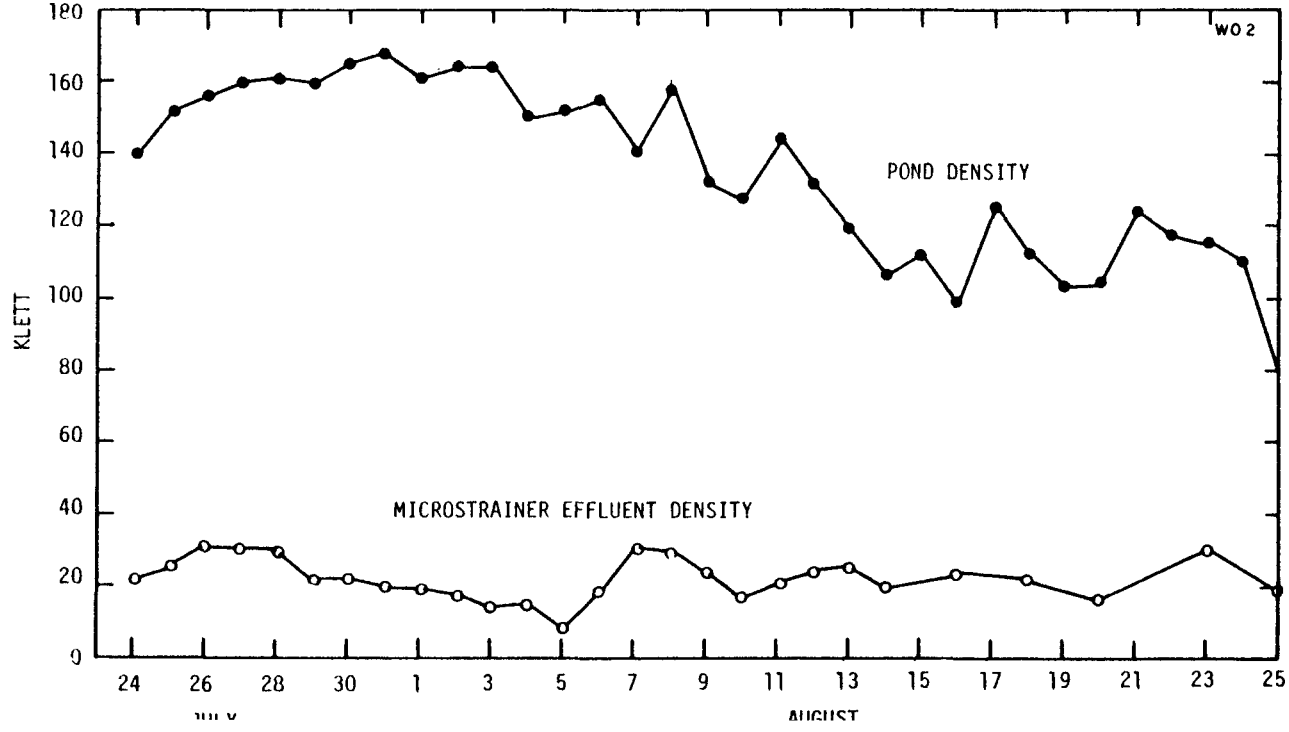
Production values paralleled the initial increasing trend in pond density but remained at a high level for two weeks after pond density began falling. Beginning August 12 production quickly fell to a lower level, about 1/3 of the highest value. This fall corresponded to a period of overcast weather which reduced insolation by about 40%. Low production in the later phase of the experiment is partially attributable to the low (0.16 day^{-1}) dilution rate employed.

Because of the bad weather extending from August 14 through 18, production data beginning on the 14th and extending through the end of the experiment was not considered in the averaging process. Net production averaged 3.9 and total production 6.2 (Klett-liters x 10^3 $meter^{-2}$ day^{-1}) giving a ratio of net to harvestable production of 63%. The relatively low level of this ratio is due to the higher effluent densities obtained and to the greater degree of recycling as compared to WO1.


WO 1
NO DATA
AVAILABLE
INCIDENT SOLAR ENERGY, Langleys day^{-1}
700
600
500
400
300
200
100
0
24
26
28
30
JULY


WO 2
NO DATA
AVAILABLE
INCIDENT SOLAR ENERGY, Langleys day^{-1}
700
600
500
400
300
200
100
0
24
26
28
30
1
3
5
7
9
11
13
15
19
21
23
25
JULY
AUGUST


WO 1
POND DENSITY
MICROSTRAINER
EFFLUENT DENSITY
KLETT
180
160
140
120
100
80
60
40
20
0
24
26
28
30
1
JULY


WO 2
POND DENSITY
MICROSTRAINER EFFLUENT DENSITY
KLETT
180
160
140
120
100
80
60
40
20
0
24
26
28
30
1
3
5
7
9
11
13
15
17
19
21
23
25
AUGUST

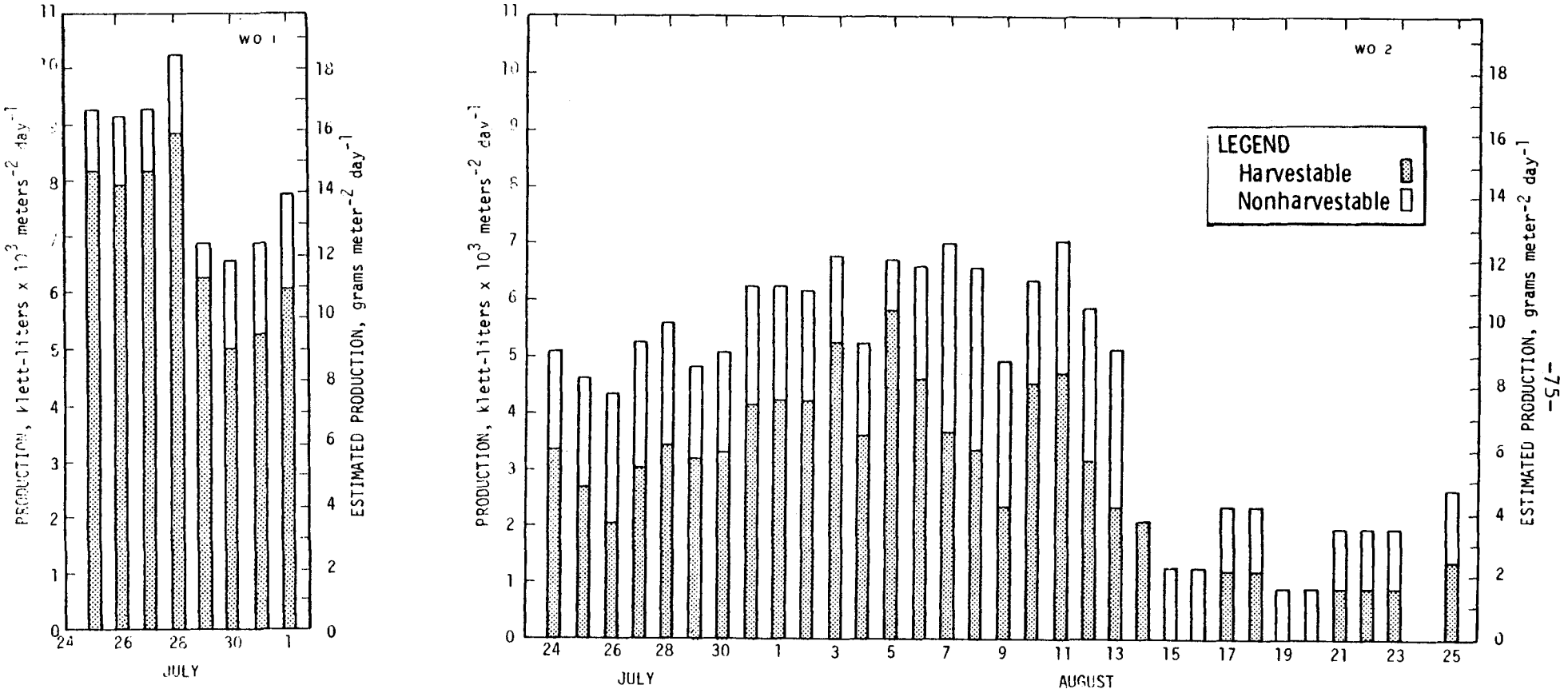


FIGURE 17. INCIDENT SOLAR ENERGIES; POND AND MICROSTRAINER EFFLUENT DENSITIES; AND HARVESTABLE AND TOTAL PRODUCTIONS FOR EXPERIMENTS WO1 AND WO2

Exp. WO3-5 (Figures 18-21). These experiments were initiated in the period when Micractinium was increasing in relative concentrations in Experiments WO1 and WO2. WO3 was inoculated from WO1, then WO4 and WO5 were inoculated from WO2. Later inoculations contained higher relative Micractinium concentrations. WO3 was started at a moderate dilution rate (0.24 day^{-1}) but the rate was increased to 0.38 day^{-1} after four days. Dilution rate was lowered (to an averaged 0.19 day^{-1}) following poor weather. The nominal recycle was set at 50%. WO4 involved no recycle and its dilution rate was decreased from 0.25 day^{-1} initially to 0.125 day^{-1} (i.e. 250 liters harvested on alternate days) when weather conditions deteriorated. Experiment WO5 received a high degree of recycle (75-100%) and was run at low (0.1-0.2 day^{-1}) dilution rates.

Initially, in experiment WO3, Oscillatoria accounted for 84% of the total algal concentration. The quantity of Oscillatoria, both in terms of percent and algal volume concentration, decreased rapidly after August 4 and did not show up in microscopic observations after August 13. The initial decline of these algae corresponded to the incresed dilution rate. Algal counts showed that Micractinium increased steadily until August 8 and then stayed at a fairly constant level. The harvestability of the culture as determined from microscopic observations ranged between 67% and 88% for most of the experiment; the decrease in Oscillatoria was matched by an increase in harvestable Micractinium. Only one variation from this pattern appeared: algal counts showed a significant breakup of Micractinium colonies on August 11 and 13. Klett-based algal-removal efficiencies correlated well in pattern (although they showed microscopically determined harvestabilities to be consistently high) to algal volume-based harvestabilities. Microstrainer effluent densities were usually around a Klett of 20 except during the Micractinium breakup period when the density reached Klett 47.

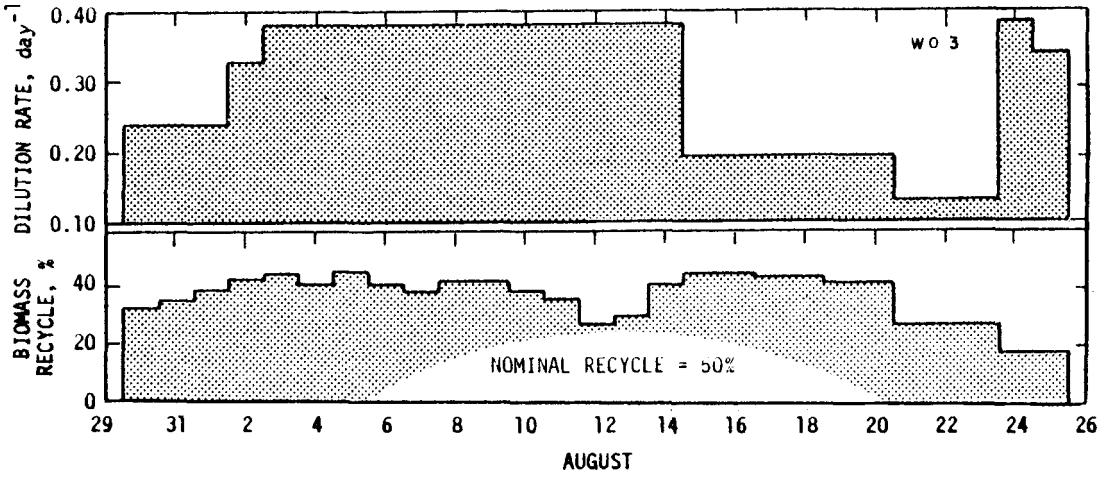
Production Values. Both harvestable and total production values exhibited an increasing trend up to August 10. After this date the harvestable production fell sharply (corresponding to the period of poor algal-removals) and eventually both harvestable and total production stabilized at relatively low levels. It appears that the period of lower-than-normal insolation was very quickly followed by a decrease in harvestability. The harvestability

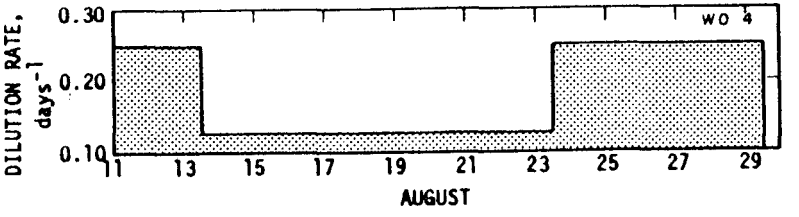
was restored by decreasing the dilution rate but at a sacrifice in production. Average net and total productions were 3.2 and 5.5 (Klett-liters x 10^3 meters^{-2} day^{-1}), giving a ratio of net to total production of 58%. Temperature varied between 15°C and 19°C during the experiment except when it rained one day and the temperature fell to 14°C. pH values seemed to oscillate about the 8.5 value, reaching as high as 9.2 and as low as 7.5 (excluding the first day).

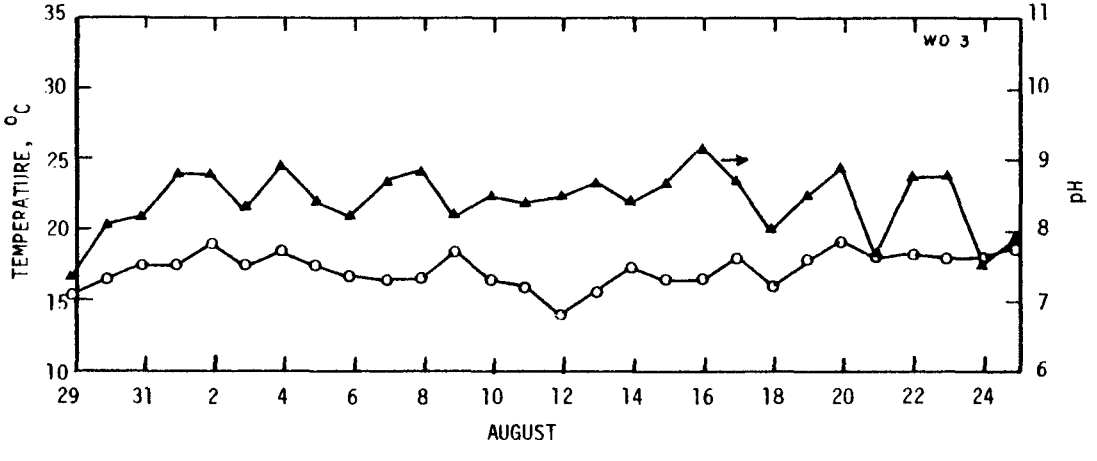
Production values for WO4 showed an initial decreasing trend which reversed itself in the latter third of the experiment. This trend approximately paralleled the changes in pond density. Ratios of net to total production were very good, up to August 23 (82%-94%) but became poorer due to lower achieved algal removals. The beginning of the trend toward lower harvestabilities but higher production (both harvestable and total) coincided with an increase in dilution rate from 0.12 day^{-1} to 0.25 day^{-1}.

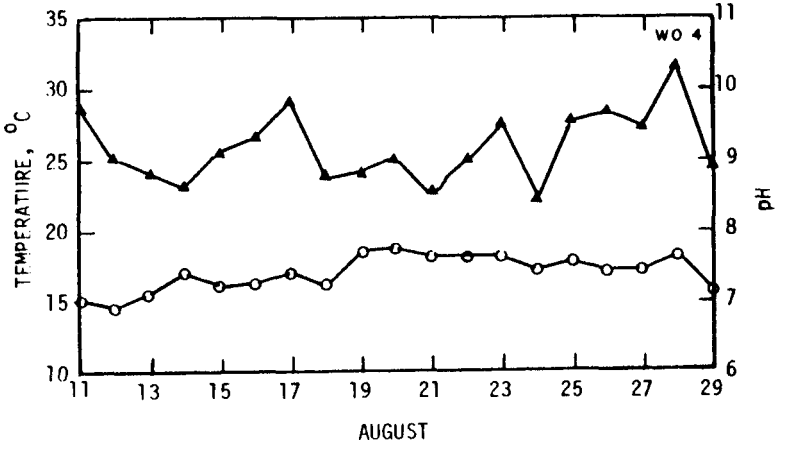
Experiment WO4 was started with a low level of Oscillatoria (35%). Its concentration steadily decreased until August 17 when it comprised 25% of the total algal concentration; after this date, the Oscillatoria population apparently crashed (possibly due to the decreased sunlight) as filaments did not again show up in the microscopic observations. Micractinium stayed at a fairly constant level, but, because of the decreasing contribution of Oscillatoria to the total algal concentration, its relative proportion increased until it made up over 90% of the algal population. By August 21 harvestable Micractinium comprised 94% of total algae and Uroglenopsis, 1%. However, the abrupt increase in dilution rate on August 24 had a detrimental effect on the algae, apparently causing the Micractinium colonies to break up. Harvestable Micractinium accounted for 68% of all algae on August 29, but the trend of algal removals indicates that this proportion could have fallen even lower on the days immediately preceding the August 29 algal count.

Microstrainer effluent densities, after having dropped steadily from Klett 19 initially to Klett 4 on August 21, rose sharply at the increase in dilution rate and reached a peak of 61 on August 29. Interestingly enough, pond densities also began to rise on August 21, perhaps indicating that the algal growth had been limited by the low organic loading rate. The pond culture, therefore, may have been in the process of adapting to the increased loading when the experiment was terminated, as evidenced by the steady


WO 3
DILUTION RATE, day^{-1}
0.40
0.30
0.20
0.10
BIOMASS RECYCLE, %
40
20
0
NOMINAL RECYCLE = 50%
29 31 2 4 6 8 10 12 14 16 18 20 22 24 26
AUGUST


WO 4
DILUTION RATE, days^{-1}
0.30
0.20
0.10
11 13 15 17 19 21 23 25 27 29
AUGUST


WO 3
TEMPERATIRE, °C
35
30
25
20
15
10
pH
11
10
9
8
7
6
29 31 2 4 6 8 10 12 14 16 18 20 22 24
AUGUST


WO 4
TEMPERATIRE, °C
35
30
25
20
15
10
pH
11
10
9
8
7
6
11 13 15 17 19 21 23 25 27 29
AUGUST

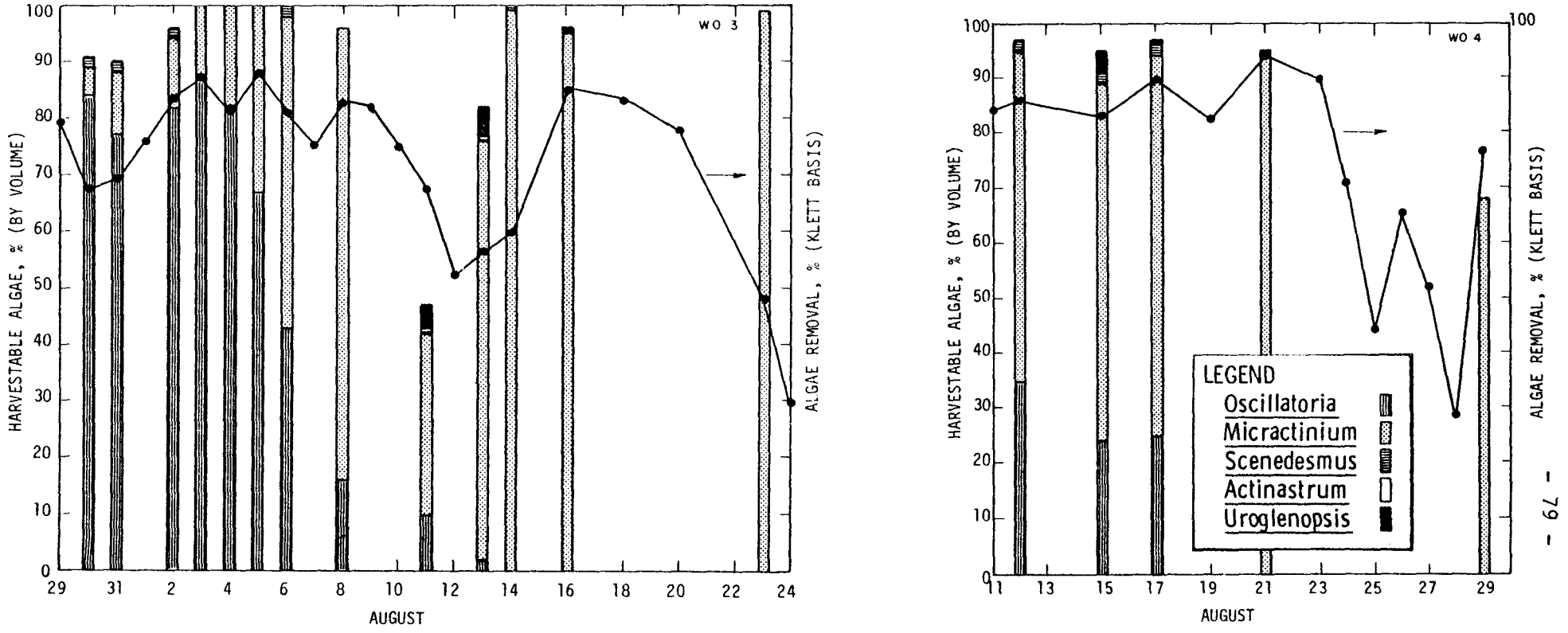
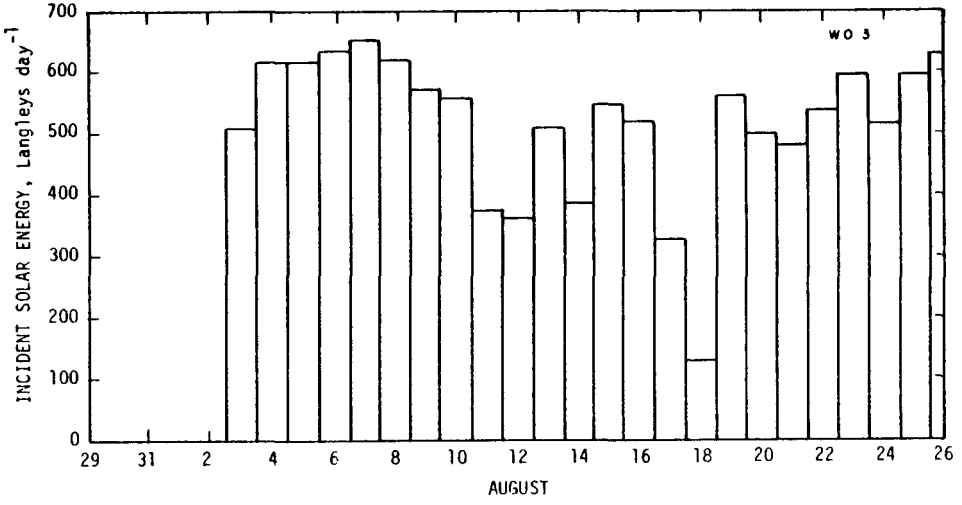
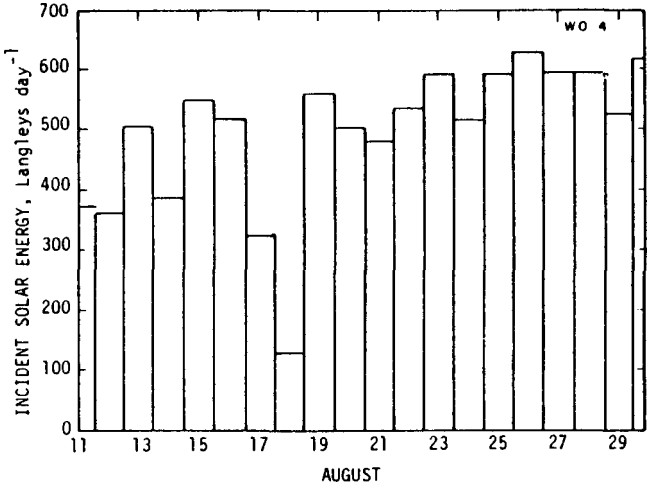
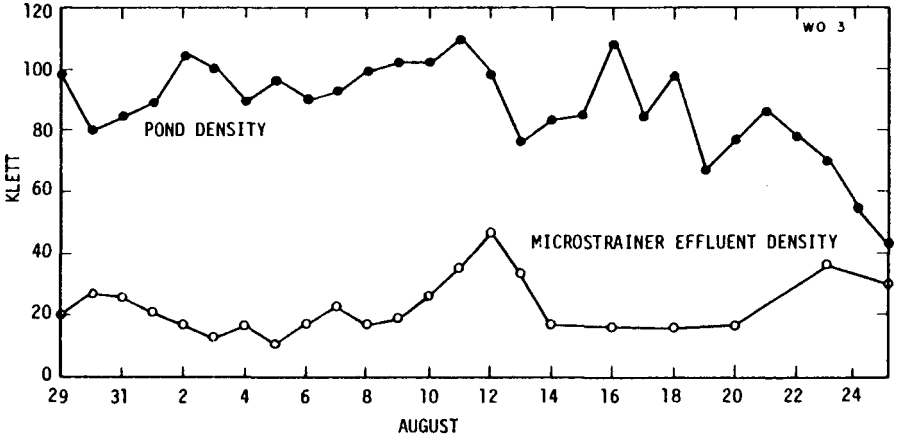
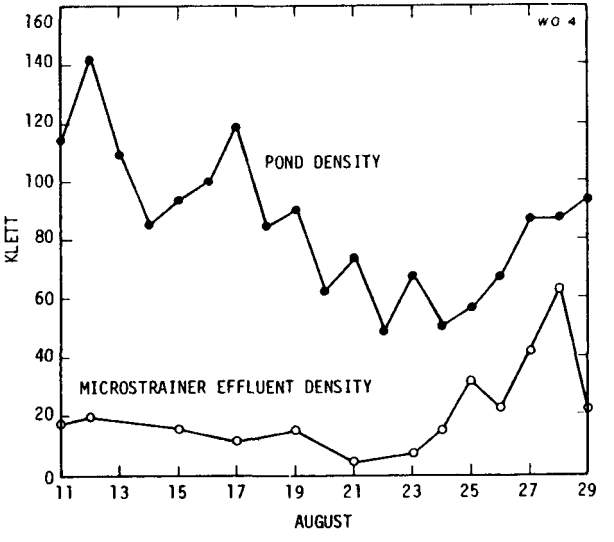


FIGURE 18. OPERATIONAL SCHEDULES, TEMPERATURE, pH, HARVESTABLE ALGAL FRACTIONS, AND ALGAL REMOVAL EFFICIENCIES FOR EXPERIMENTS WO3 AND WO4.


WO 3
INCIDENT SOLAR ENERGY, Langleys day^{-1}
0
100
200
300
400
500
600
700
29 31 2 4 6 8 10 12 14 16 18 20 22 24 26
AUGUST


WO 4
INCIDENT SOLAR ENERGY, Langleys day^{-1}
0
100
200
300
400
500
600
700
11 13 15 17 19 21 23 25 27 29
AUGUST


WO 3
POND DENSITY
MICROSTRAINER EFFLUENT DENSITY
KLETT
0
20
40
60
80
100
120
29 31 2 4 6 8 10 12 14 16 18 20 22 24
AUGUST


WO 4
POND DENSITY
MICROSTRAINER EFFLUENT DENSITY
KLETT
0
20
40
60
80
100
120
140
160
11 13 15 17 19 21 23 25 27 29
AUGUST

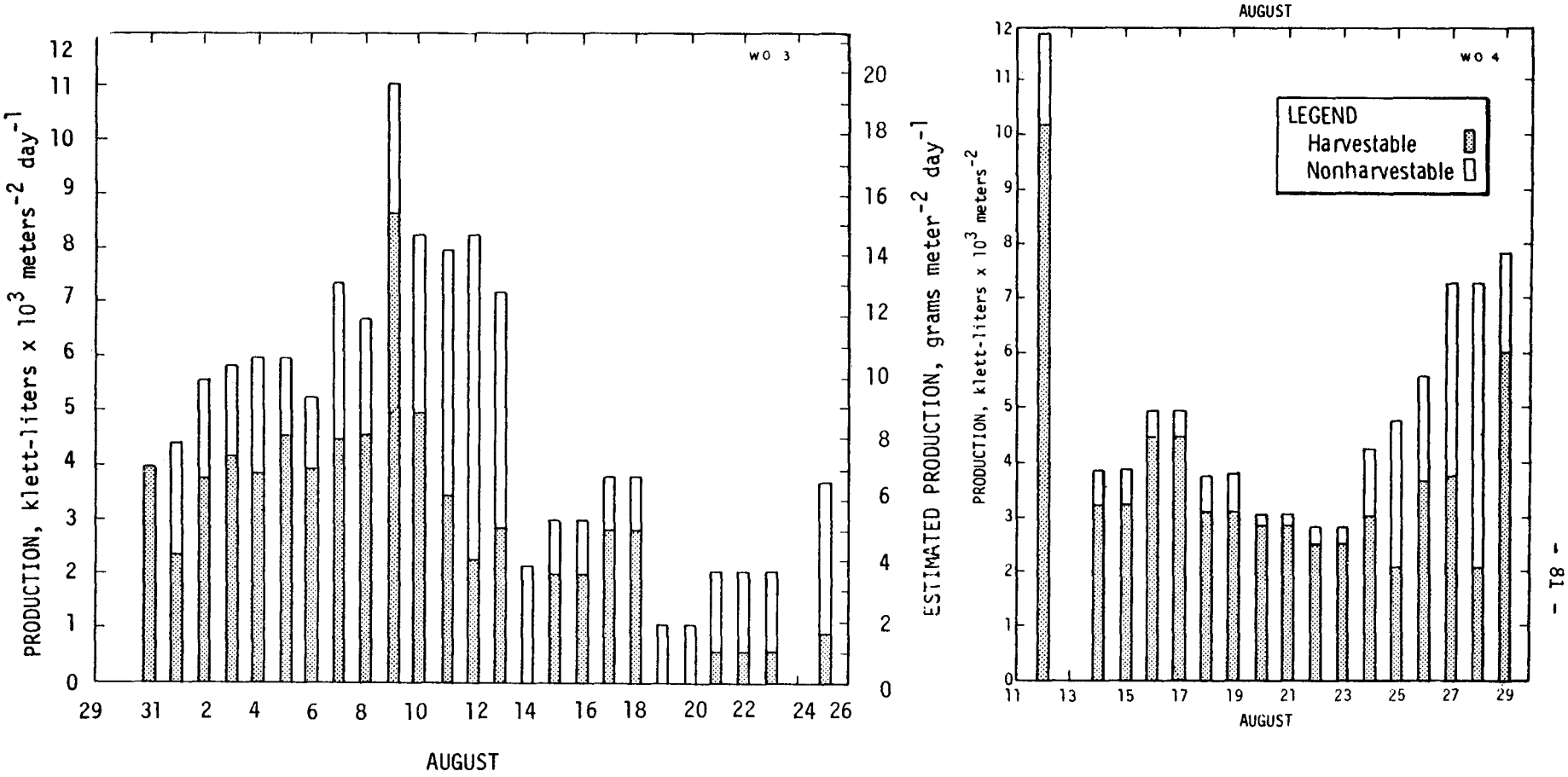


FIGURE 19. INCIDENT SOLAR ENERGIES, POND AND MICROSTRAINER EFFLUENT DENSITIES, AND HARVESTABLE AND TOTAL PRODUCTIONS FOR EXPERIMENTS WO 3 AND WO 4

increasing pond densities and the apparent increase in culture harvestability on August 29.

Experiment WO5. This experiment was run at low dilution rates (0.10-0.20 day^{-1}) and high nominal recycle rates (75-100%). The pond steadily decreased in density. No definite influences of either dilution rate or recycle rate on pond density were apparent. However, as with previous experiments, microstrainer effluent densities increased with faster dilution. As could be expected with the low dilution rate employed, production values were low. Ratios of harvestable to total production varied with dilution rate; at 0.20 day^{-1} the average ratio was 59% whereas at 0.10 day^{-1} the average ratio was 67%.

This pond was started with inoculum high in both Oscillatoria and Micractinium, along with significant numbers of Chlorella (grouped in the "other" category in algal counts). As in all previous experiments except WO2 and WO3, the Oscillatoria declined slowly at first and were then evidently washed out (not showing up in counts after August 21). Micractinium remained at initial levels. By August 17 most of the harvestable algae were Micractinium; however, the harvestability of the Micractinium culture declined somewhat when the dilution rate was doubled.

The morning temperature ranged between 15°C and 20°C. pH became quite variable from day-to-day towards the experiment's end, generally staying between 8 and 9.5 but jumping as high as 10.4 on August 25.

Exp. WO6 (Figures 20-21). After the demise of Oscillatoria in WO5, it was decided that the next effort to grow this algae should exclude Micractinium. In order to achieve this goal, a portable microstrainer was brought to the Woodland Pond System (old treatment plant site) and several hundred liters of concentrated algae were strained from a pond. Subsequent examination under a microscope revealed the concentrate to be composed primarily of Oscillatoria with a small percentage of Chlorella.

The culture was slowly brought to 1000 liter volume by periodic additions of settled sewage over a period of six days; during this time, the culture was 50% shaded. An algal count on August 5 showed that Oscillatoria comprised 90%

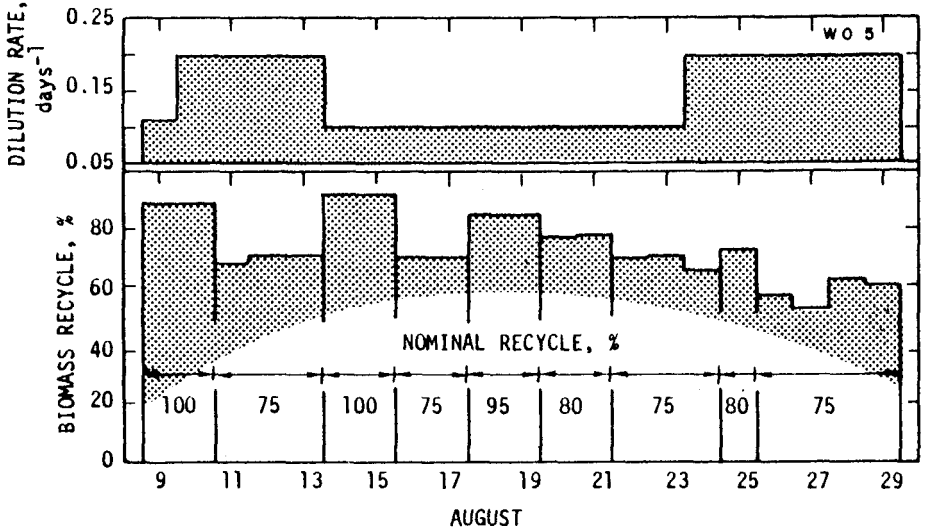
of the total algae and were the only harvestable species present. Observed algal-removal efficiencies closely followed a decline in the percentage of Oscillatoria falling from about 80% initially to 50%. The population of non-harvestable algae steadily increased during the experiment (from 100 to 200 $\mu m^3 \times 10^6 ml^{-1}$); this population was made up of Chlorella, desmids, Ankistrodesmus, and numerous other species.

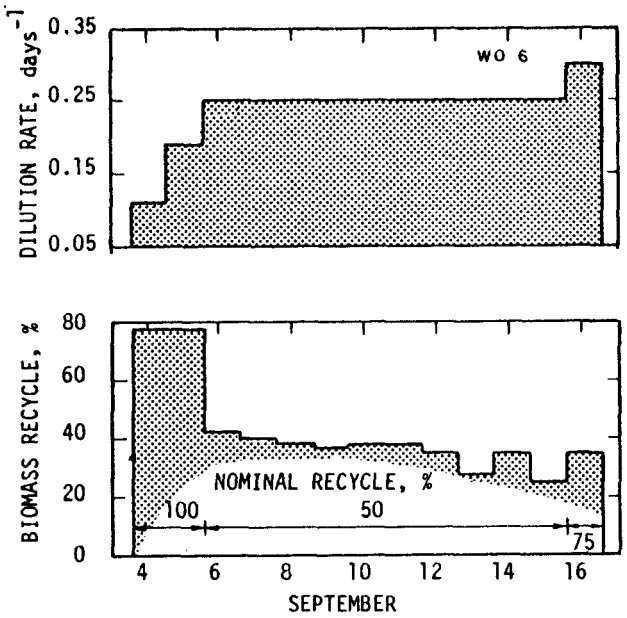
Pond density began at Klett 166 and climbed to Klett 178 during the initial phase when small dilutions and high recycle were used. Once the dilution rate was raised to 0.25 day^{-1}, the pond density dropped sharply to a lower steady-state level for several days and then began a gradual decreasing trend. Microstrainer effluent densities were fairly constant over the first week of the experiment but then climbed up to a Klett of 60 at experiment termination. The rise in effluent densities corresponds to the fast decrease in Oscillatoria and increase in single-celled algal species.

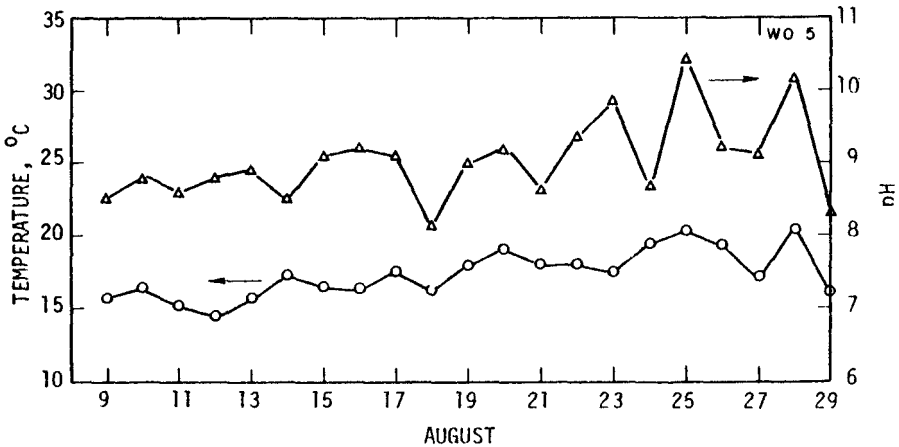
Total production throughout the experiment was uniformly high; however, a trend of decreasing values for harvestable production is clearly evident so that by the experiment's end, the ratio of harvestable to total production attained was only 18%, compared to a ratio of 64% on September 8. Temperatures remained around 15°C except for the September 8-10 period when they rose to as high as 20°C. Interestingly enough, pH values were also fairly steady between 8.5 and 9 except for the period after the warming trend when they dropped to a low of 8.

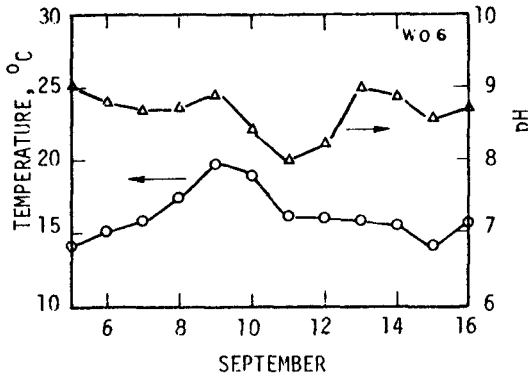
Exp. W09-W010 (Figures 22-23). Although W04 was set up as a control-type experiment in which no biomass recycling was done, there still existed a need to run two identically operated ponds inoculated with Oscillatoria, one with recycle and one without, in order to gain a clearer picture of the effect of biomass recycling on the population dynamics of the algae. The W06 culture was completely harvested and the concentrate obtained was used to inoculate two ponds. Also, a portion of a batch culture containing Oscillatoria and Micractinium was used. In order to ensure uniform inoculation, the two ponds were intermixed.

As seen in the graphs of algal counts (Figure 22), the concentrations of the various algae at the experiment's start were closely alike. It is


WO 5
DILUTION RATE, days^{-1}
0.25
0.15
0.05
BIOMASS RECYCLE, %
80
60
40
20
0
NOMINAL RECYCLE, %
100
75
100
75
95
80
75
80
75
9
11
13
15
17
19
21
23
25
27
29
AUGUST


WO 6
DILUTION RATE, days^{-1}
0.35
0.25
0.15
0.05
BIOMASS RECYCLE, %
80
60
40
20
0
NOMINAL RECYCLE, %
100
50
75
4
6
8
10
12
14
16
SEPTEMBER


WO 5
TEMPERATURE, °C
35
30
25
20
15
10
pH
11
10
9
8
7
6
9
11
13
15
17
19
21
23
25
27
29
AUGUST


WO 6
TEMPERATURE, °C
30
25
20
15
10
pH
10
9
8
7
6
6
8
10
12
14
16
SEPTEMBER

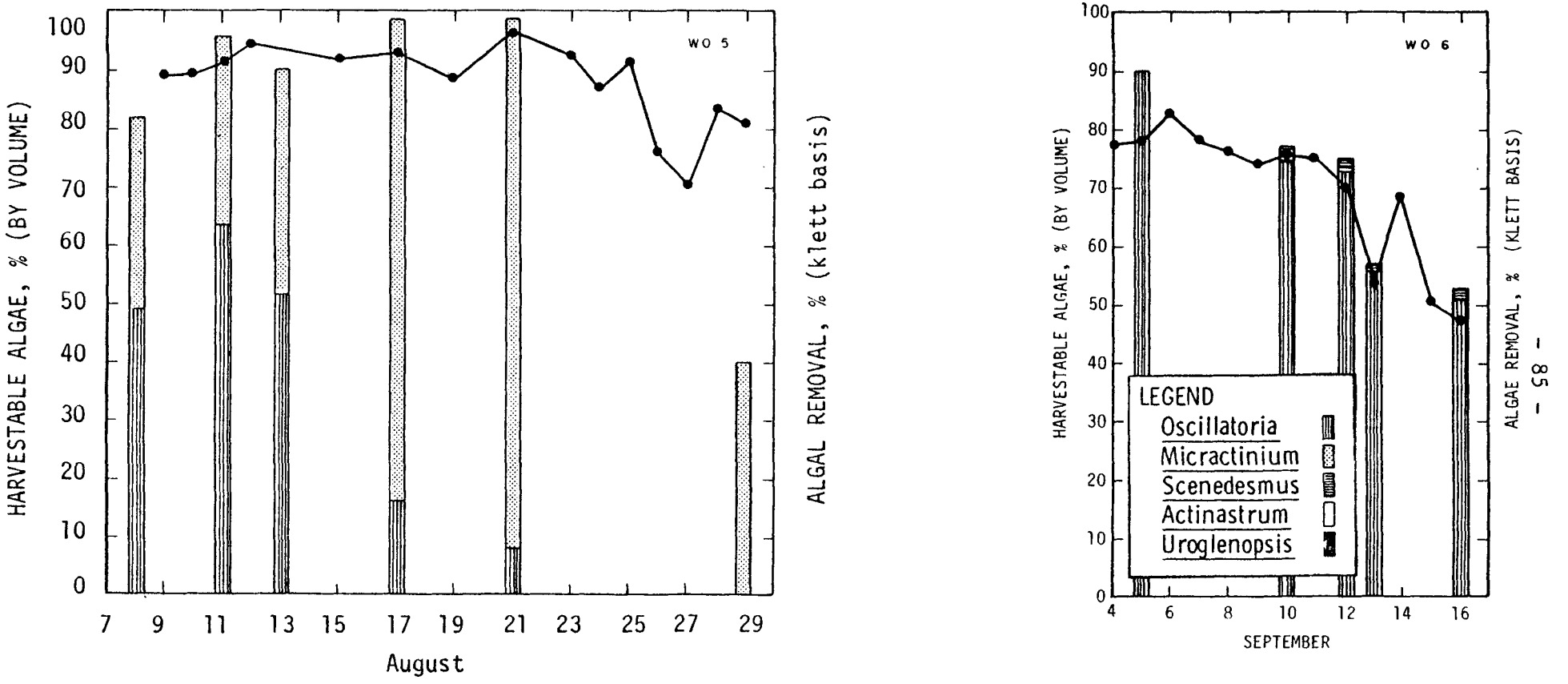
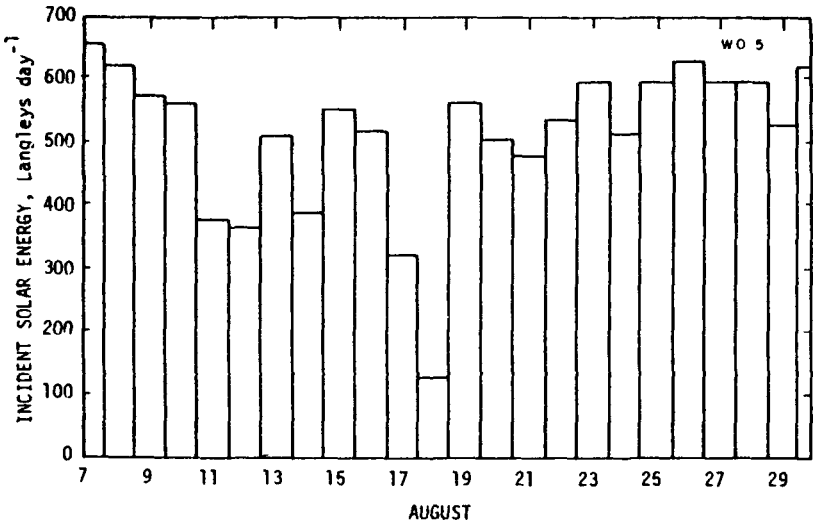
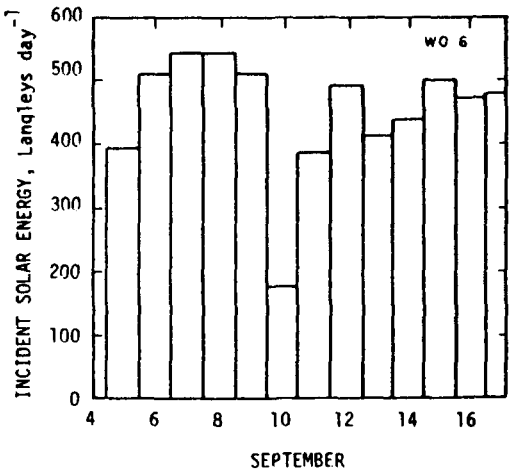
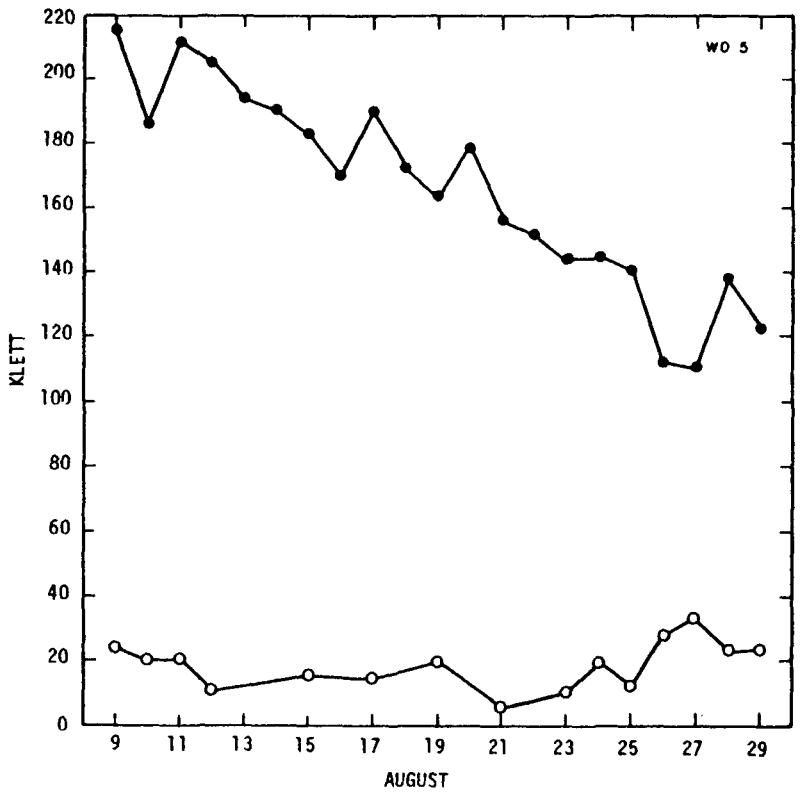
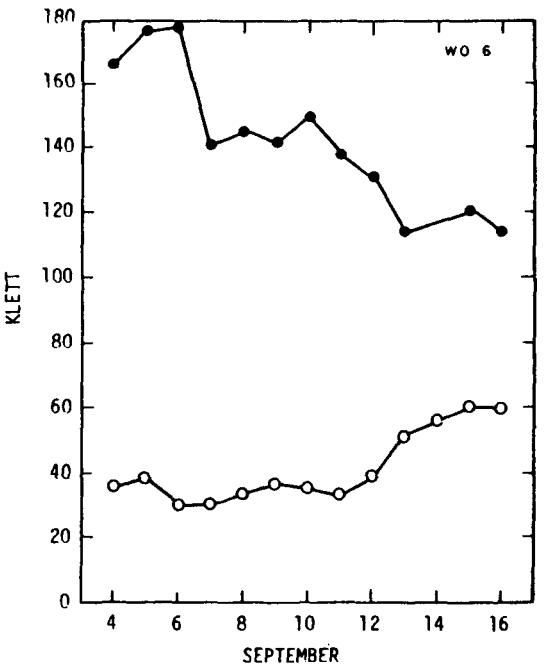


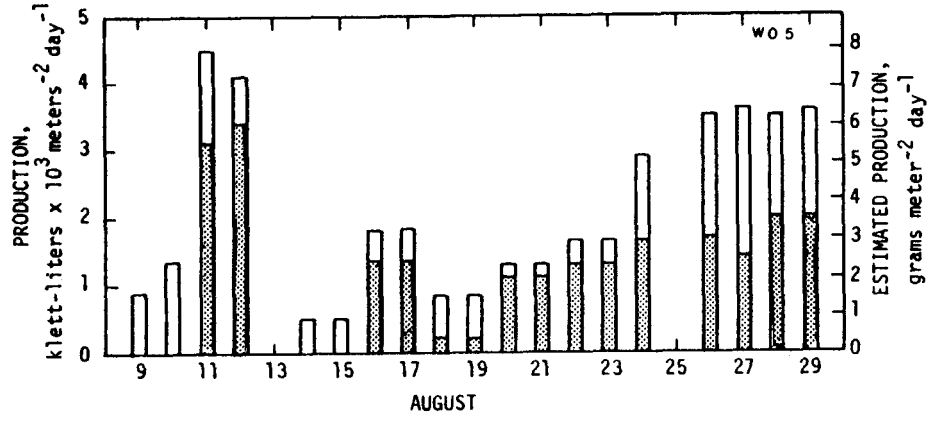
FIGURE 20. OPERATIONAL SCHEDULES, TEMPERATURES, pH, HARVESTABLE ALGAL FRACTIONS, AND ALGAL-REMOVAL EFFICIENCIES FOR EXPERIMENTS WO 5 AND WO 6


WO 5
INCIDENT SOLAR ENERGY, Langleys day^{-1}
700
600
500
400
300
200
100
0
7
9
11
13
15
17
19
21
23
25
27
29
AUGUST


WO 6
INCIDENT SOLAR ENERGY, Langleys day^{-1}
600
500
400
300
200
100
0
4
6
8
10
12
14
16
SEPTEMBER


WO 5
KLETT
220
200
180
160
140
120
100
80
60
40
20
0
9
11
13
15
17
19
21
23
25
27
29
AUGUST


WO 6
KLETT
180
160
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
SEPTEMBER



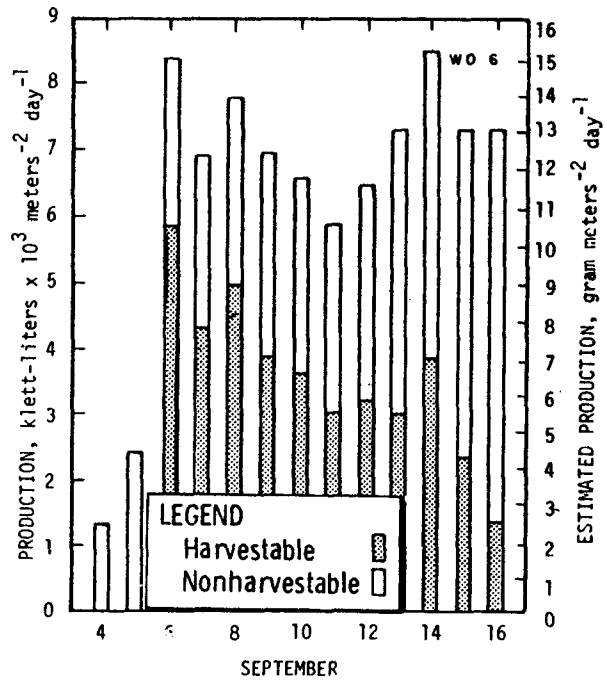
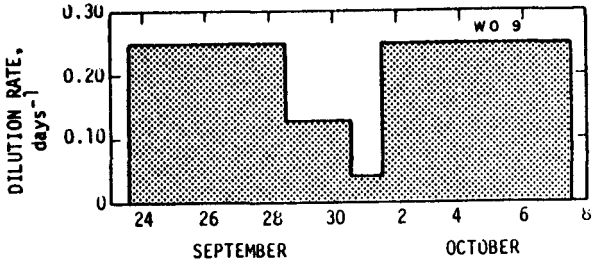
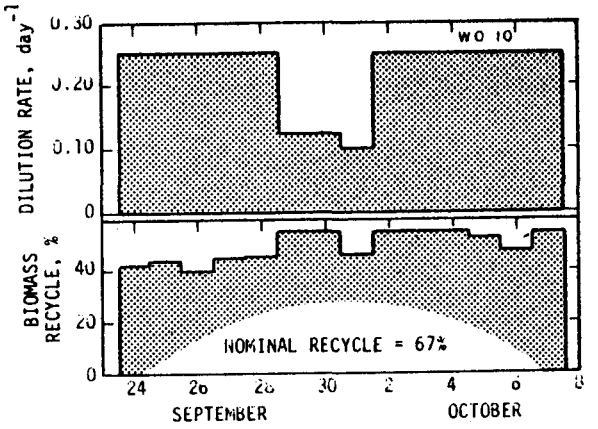
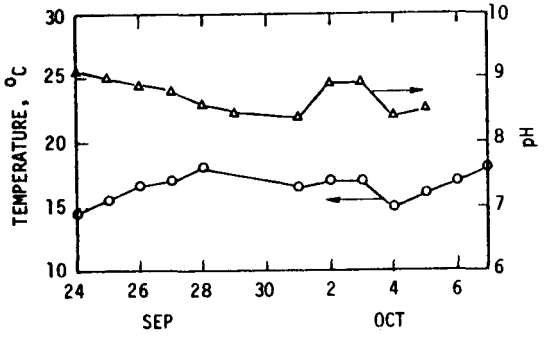
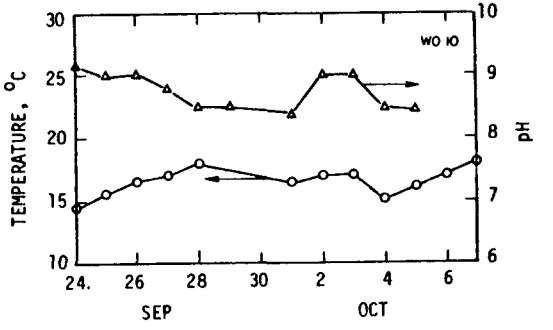


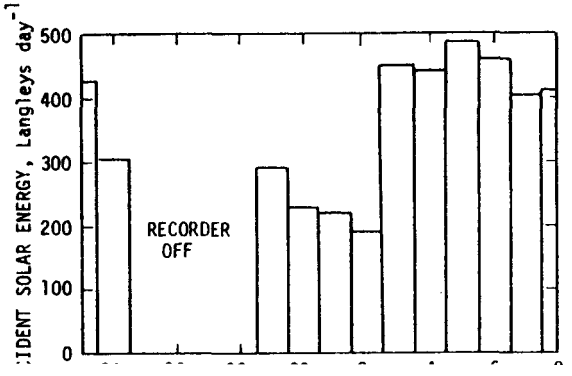
FIGURE 21. INCIDENT SOLAR ENERGIES; POND AND MICROSTRAINER EFFLUENT DENSITIES; AND HARVESTABLE AND TOTAL PRODUCTIONS FOR EXPERIMENTS WO5 AND WO6.

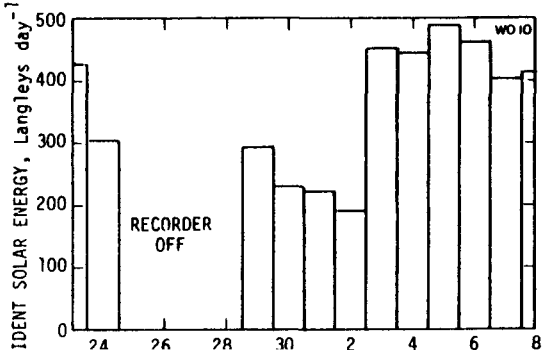

WO 9
DILUTION RATE, days^{-1}
0.30
0.20
0.10
0
24
26
28
30
2
4
6
8
SEPTEMBER
OCTOBER

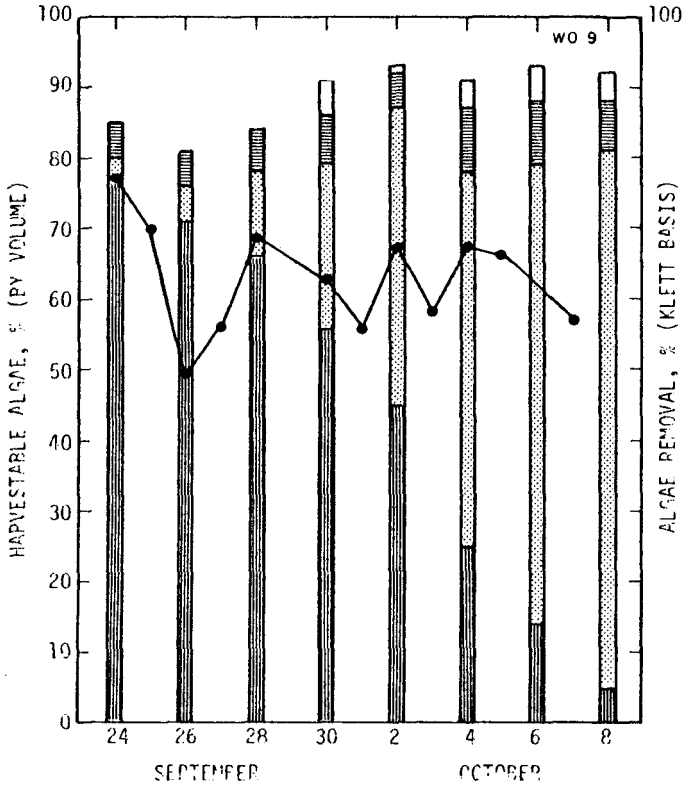

WO 10
DILUTION RATE, day^{-1}
0.30
0.20
0.10
0
BIOMASS RECYCLE, %
40
20
0
NOMINAL RECYCLE = 67%
24
26
28
30
2
4
6
8
SEPTEMBER
OCTOBER


TEMPERATURE, °C
30
25
20
15
10
pH
10
9
8
7
6
24
26
28
30
2
4
6
SEP
OCT


WO 10
TEMPERATURE, °C
30
25
20
15
10
pH
10
9
8
7
6
24
26
28
30
2
4
6
SEP
OCT


IDENT SOLAR ENERGY, Langleys day^{-1}
500
400
300
200
100
0
RECORDER OFF


WO 10
IDENT SOLAR ENERGY, Langleys day^{-1}
500
400
300
200
100
0
RECORDER OFF
24
26
28
30
2
4
6
8



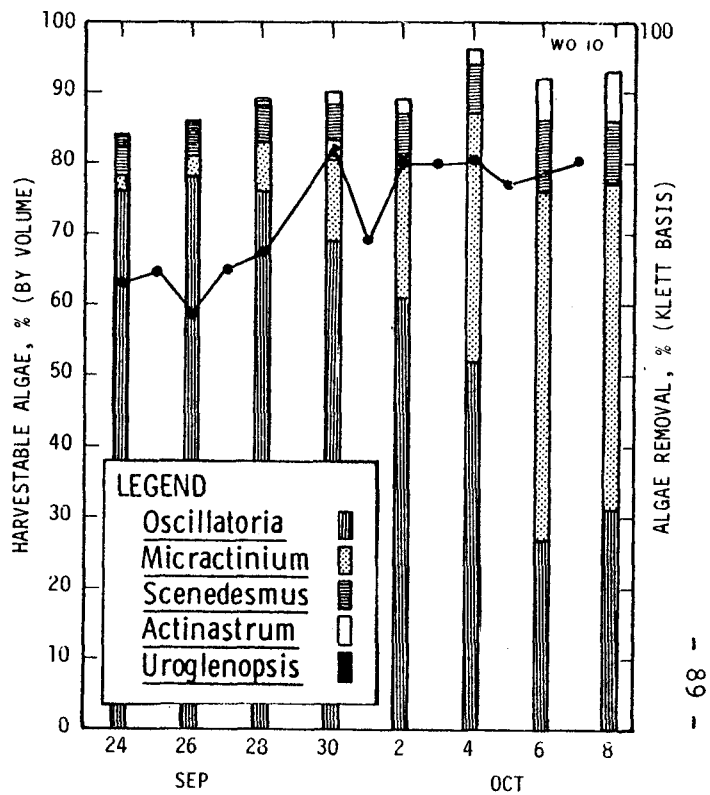
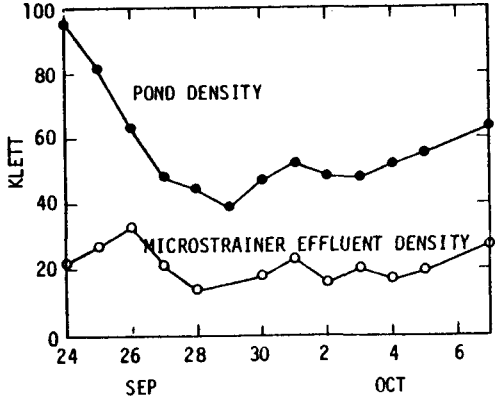
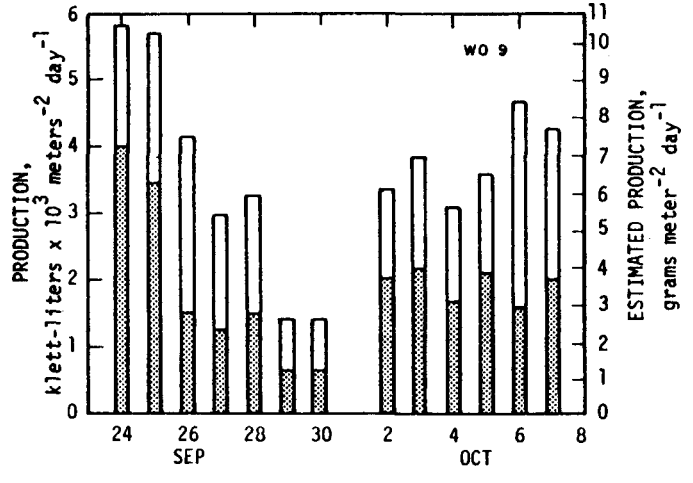
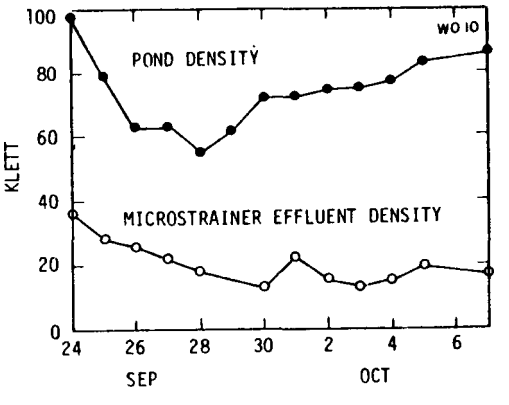


FIGURE 22. OPERATIONAL SCHEDULES, TEMPERATURES, pH, INCIDENT SOLAR ENERGIES, HARVESTABLE ALGAL FRACTIONS, AND ALGAL-REMOVAL EFFICIENCIES FOR EXPERIMENTS WO9 AND WO 10







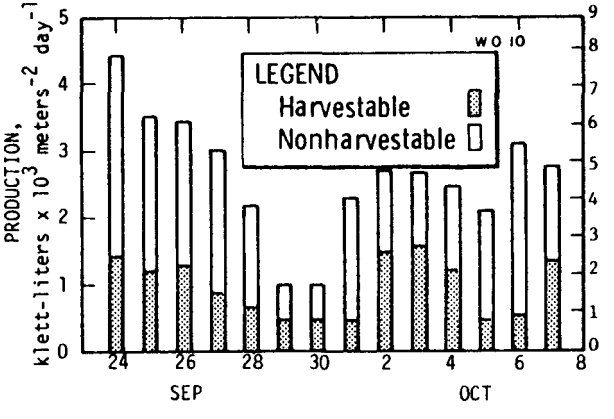


FIGURE 23. POND DENSITIES, MICROSTRAINERS EFFLUENT DENSITIES, HARVESTABLE PRODUCTIONS AND TOTAL PRODUCTIONS FOR EXPERIMENTS WO 9 AND WO 10.

apparent that in WO10 which received biomass recycle the rate of Oscillatoria decline is slower than in WO9. However, dynamics of the other species do not show clear-cut differences between the recycle and non-recycle ponds. Observed algal removals were better in the pond being recycled (74% for WO10 versus 60% for WO9): part of the difference can be attributed to the higher pond density in WO10 but a portion is actually due to lower microstrainer effluent densities for WO10 (average Klett = 19) than for WO9 (average Klett = 22). WO9 and WO10 were the first experiments where Scenedesmus made up a significant portion of the harvestable algal population (as much as 10%). Actinastrum also showed up in the algal counts as a harvestable form. The fairly wide discrepancies between harvestabilities predicted on the basis of algal volume concentrations and observed algal removal efficiencies is believed to be due in part to the relatively low pond densities (at higher densities a filtration matt tends to form which aids in capturing algae) and to the ability of Scenedesmus or Actinastrum colonies larger than 26 μm in two directions to still slip through the 26 μm x 26 μm openings in the straining fabric (of course, the openings are not perfectly uniform in size).

There were marked disparities in production for the two experiments although values from each experiment followed the same general trend. Experiment WO9 (non-recycle) gave higher average results for both harvestable (1.4) and total (3.0 Klett liters x 10^3 meter^{-2} day^{-1}) production as compared to the results of WO10 (harvestable = 0.9, total = 2.4). Ratios of harvestable to total production for WO9 and WO10 were 47% and 38%, respectively.

The pond density in both experiments declined rapidly at first, paralleling the decline in Oscillatoria, but then began a period of increasing density in response to the increasing concentration of Micractinium. Micractinium were the predominant algae at the end of both experiments, although Oscillatoria were displaced as the predominant algae earlier in the non-recycle pond.

Temperature and pH were very similar between ponds and even displayed the same patterns. The range of temperature was 14.5-18°C while pH varied between 8.4 and 9.1.

Micractinium BLOOM

After the experiences with Micractinium in the previously described

experiments, it was decided to begin a series of experiments where these algae were either predominant initially (Mic 1, Mic 2, and Mic Control) or present at low but identical concentrations between pond-pairs consisting primarily of single-cell algae (SC 1 & SC 2, SC 3 & SC 4). The experimental designs for these sets of experiments are given in Table 7.

Experiments Mic 1, 2, and Control (Figures 24-29 and A5-A7 in Appendix A)

Inoculum was obtained from the W03, 4, and 5 experiments which had, by this time, been taken over by Micractinium. The three ponds involved were intermixed prior to startup. Mic 1 was run at a dilution rate of 0.15 day^{-1} with no recycle of harvested biomass. However, 30% (300 liters) of the pond culture was harvested daily and 150 liters of the microstrainer effluent recycled to the pond. The percent biomass recycled (Figure 24) refers to the quantity of algae present in the 150 liters of microstrainer effluent. When the experiment was designed, it was expected that Micractinium would eventually be disposed of in the culture because of the recycle of non-harvestable algae. However, the concentration of these algae remained almost constant throughout the experiment and there was no net increase in the concentration of non-harvestable species. Micractinium were the only harvestable algae present throughout Mic 1; the "other" species were made up mostly of Chlorella. Observed algal-removal efficiencies were rather poor (average = 74%) although the average microstrainer effluent density (Klett 13) compared quite favorably to the recycled pond (Mic 2-Klett 12) and control pond (Mic Control-Klett 12). Thus, the relatively low observed algal removal efficiencies are seen to be an artifact of the low pond density.

Production from Mic 1 was high (average harvestable = 3.7, average total = 4.3, Klett-liters x 10^3 $meters^{-2}$ day^{-1}) with a favorable ratio of harvestable to total production (86%). Production in terms of grams ash-free dry weight $meter^{-2}$ day^{-1} are also graphed; pond and microstrainer effluent densities are plotted in mg solids $liter^{-1}$, and algal removals were calculated from dry weights. The most important difference between Kletts and dry weights is that algal removals calculated on the basis of dry weights (85%) are higher than those based on Klett (74%).

Mic 2 was run at 0.25 day^{-1} dilution with the nominal recycle rate set at 50%. Because of the great harvestability of this culture, the actual

TABLE 7. EXPERIMENT DESIGNS FOR Micractinium (Mic) AND SINGLE CELL (SC) EXPERIMENTS

Experiment Code	Inoculum Source	Nominal Biomass Recycle,% (averaged)	Dilution Rate, day^{-1} (averaged)	Figure
Mic 1	WO 3 Harvest WO 4 Harvest WO 5 Harvest	0	0.15	24-25
Mic 2	"	50	0.25	26-27
Mic Cont	"	0	0.25	28-29
SC 1	Mic 2 Micro-strainer eff. + Mic Cont.Micro-strainer eff.	100 Initially 50 later	0.25	30-31
SC 2	"	0	0.25	30-31
SC 3	SC 1 Micro-strainer eff. + SC 2 Micro-strainer eff.	0	0.33	32-33
SC 4	"	100 Initially 58 later	0.33	32-33

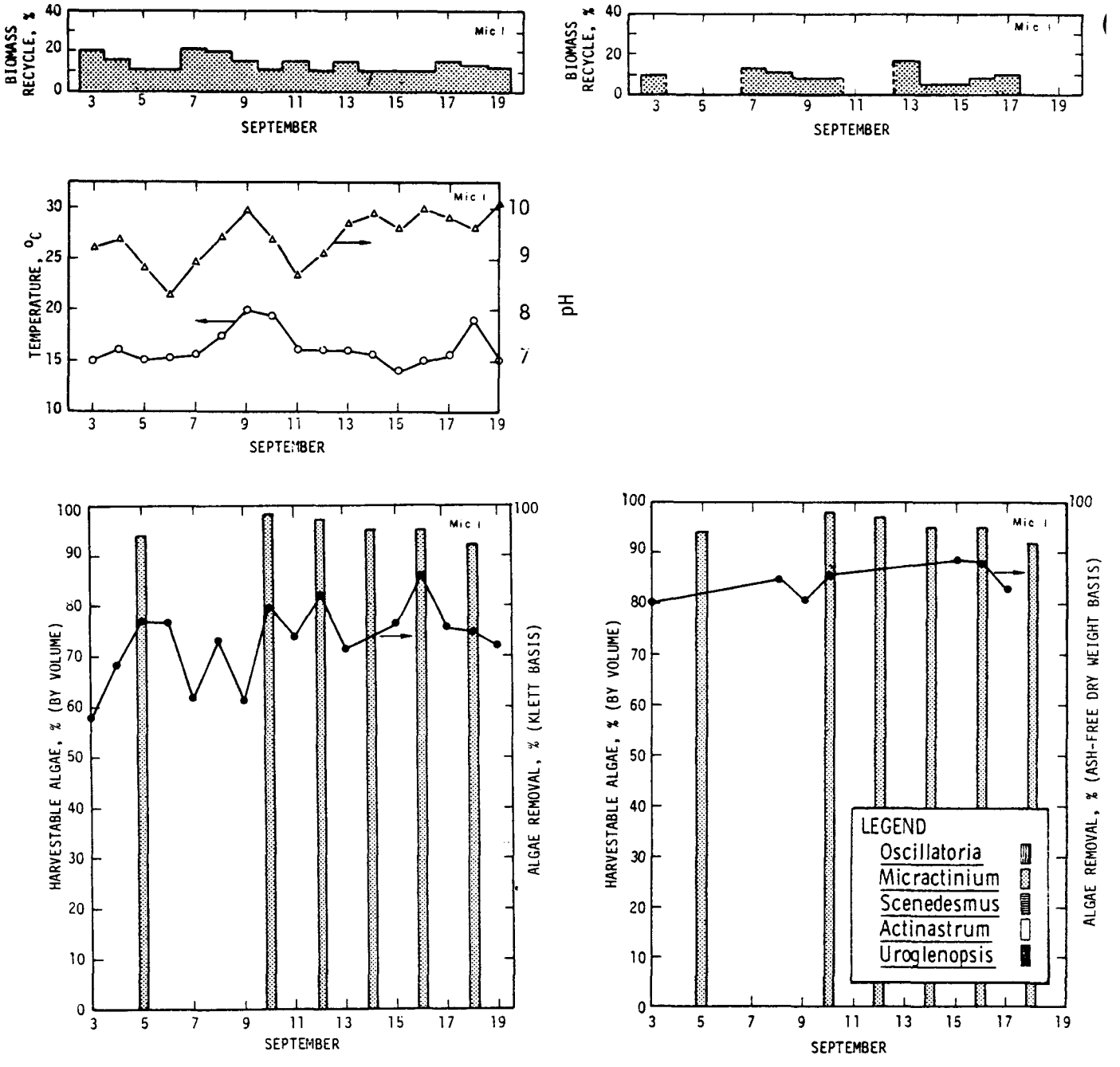


FIGURE 24. BIOMASS RECYCLE RATES AND ALGAL-REMOVAL EFFICIENCIES BASED ON KLETT AND DRY WEIGHTS, TEMPERATURES, pH, AND HARVESTABLE ALGAL FRACTIONS FOR EXPERIMENT MIC 1

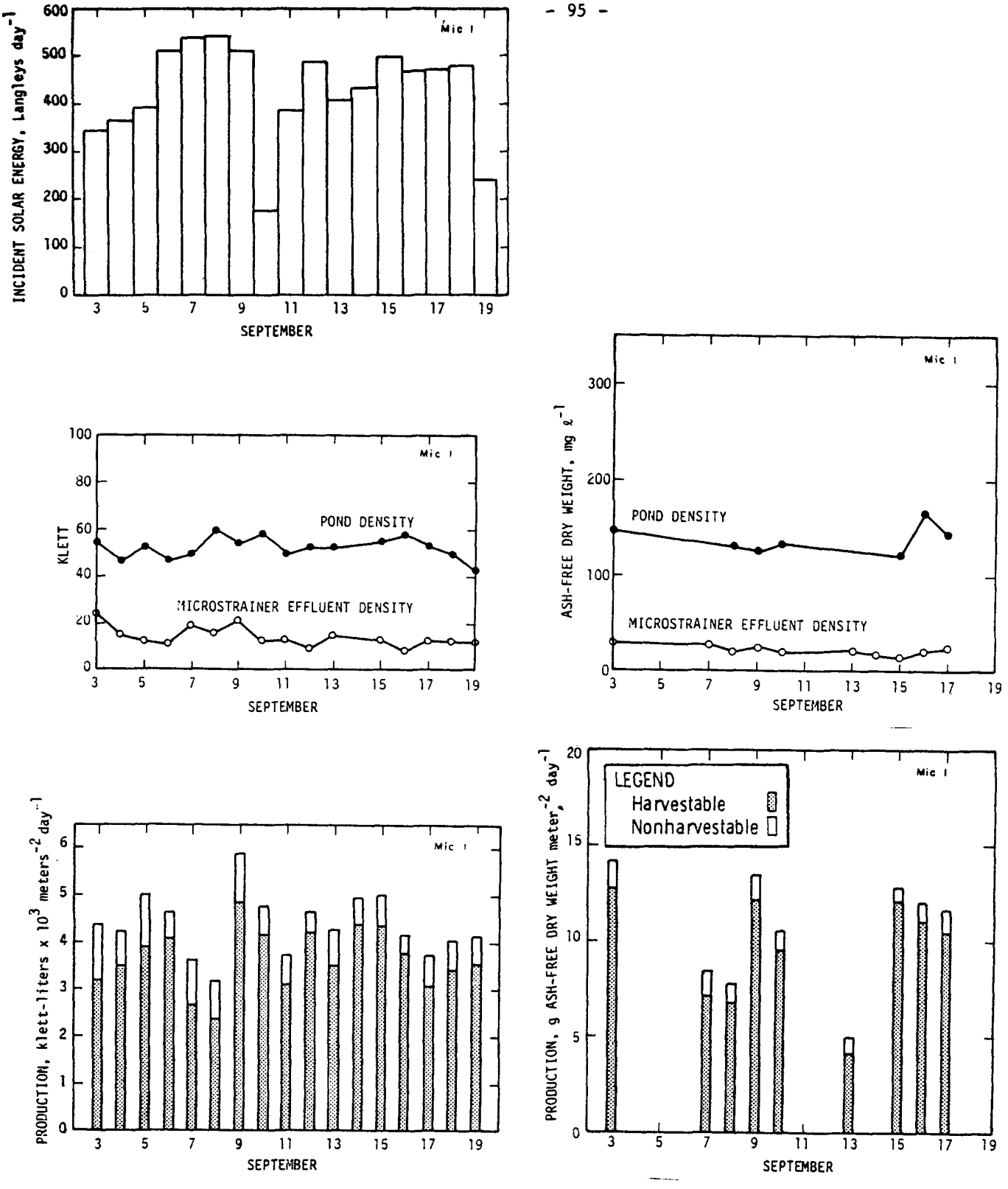
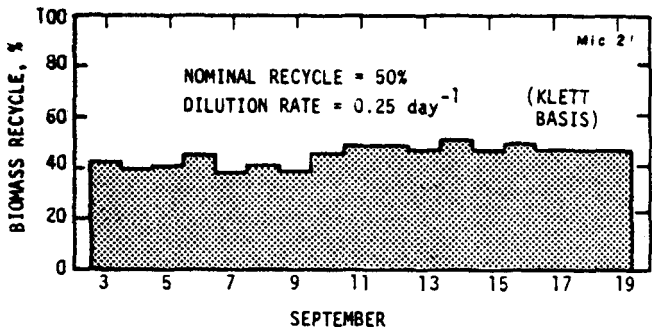
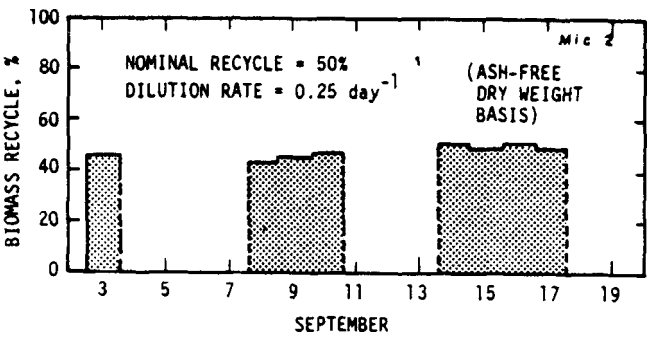
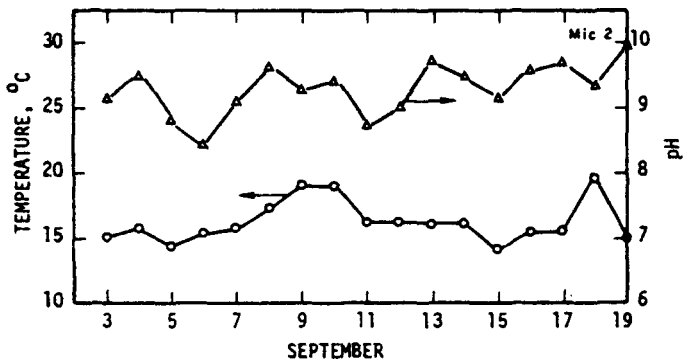
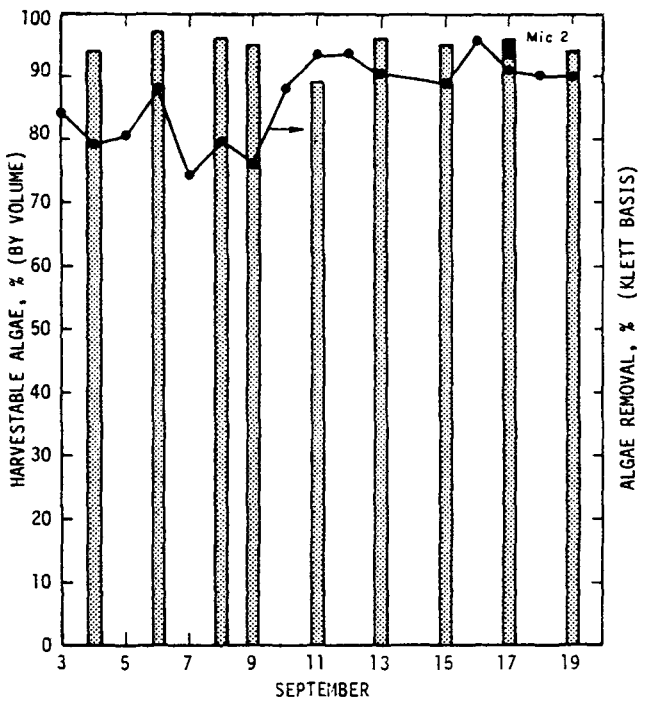


FIGURE 25. INCIDENT SOLAR ENERGIES; KLETT- AND DRY WEIGHTS-BASED POND DENSITIES, MICROSTRAINER EFFLUENT DENSITIES, HARVESTABLE PRODUCTIONS, AND TOTAL PRODUCTIONS FOR EXPERIMENT MIC 1









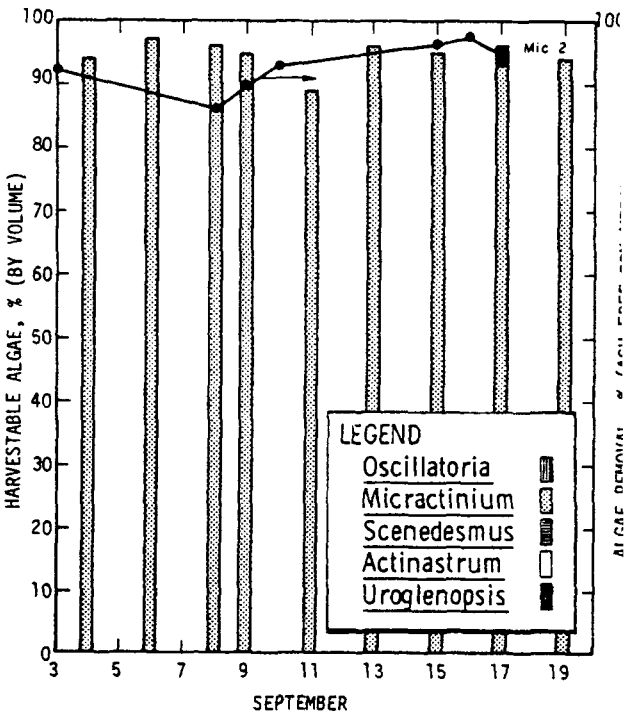


FIGURE 26. KLETT- AND DRY WEIGHTS-BASED BIOMASS RECYCLE RATES AND ALGAL-REMOVAL EFFICIENCIES, TEMPERATURES, pH, AND HARVESTABLE ALGAL FRACTIONS FOR EXPERIMENT MIC 2

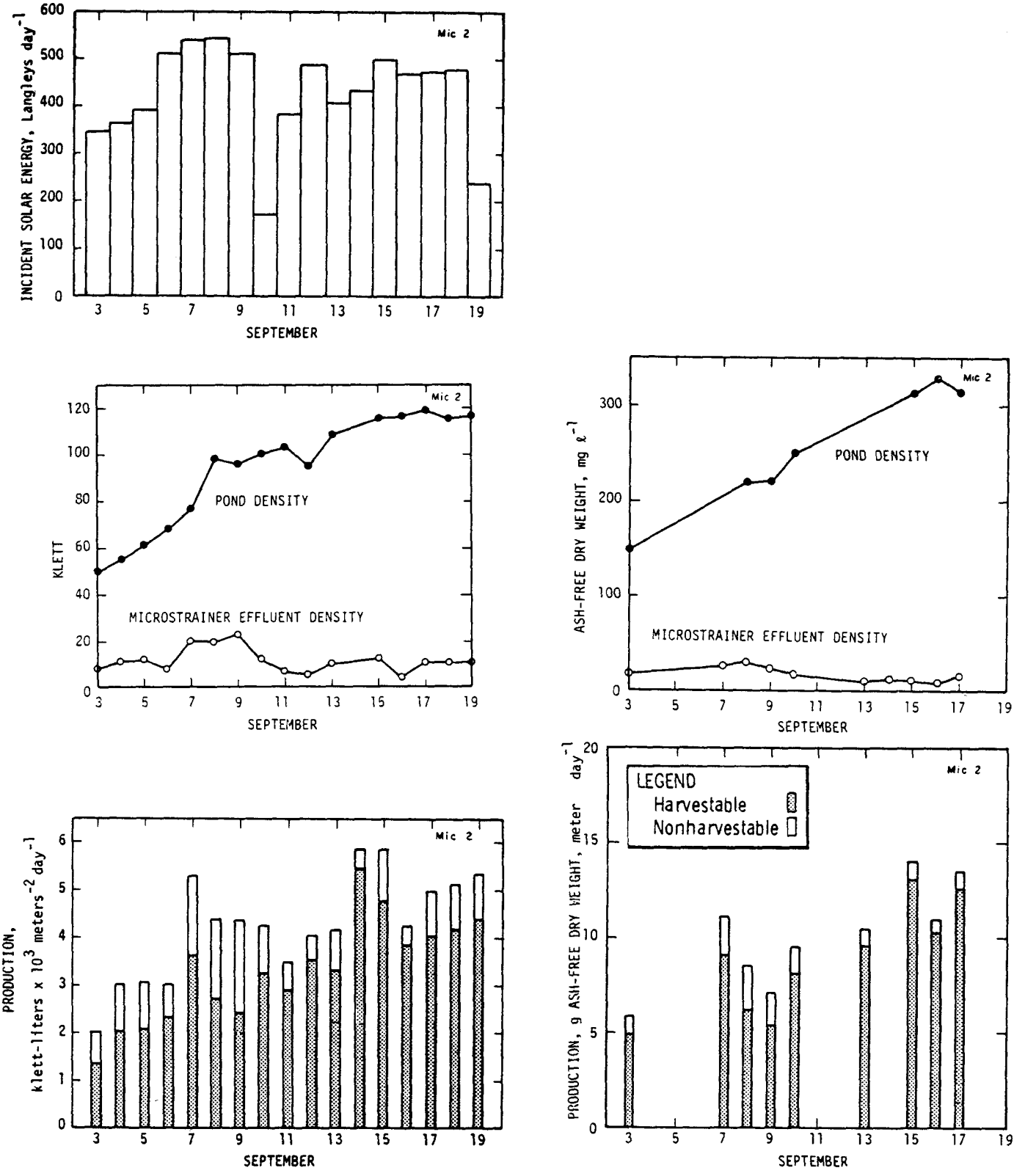


FIGURE 27. INCIDENT SOLAR ENERGIES, KLETT- AND DRY WEIGHTS-BASED POND DENSITIES, MICROSTRAINER EFFLUENT DENSITIES, HARVESTABLE PRODUCTIONS, AND TOTAL PRODUCTIONS FOR EXPERIMENT MIC 2

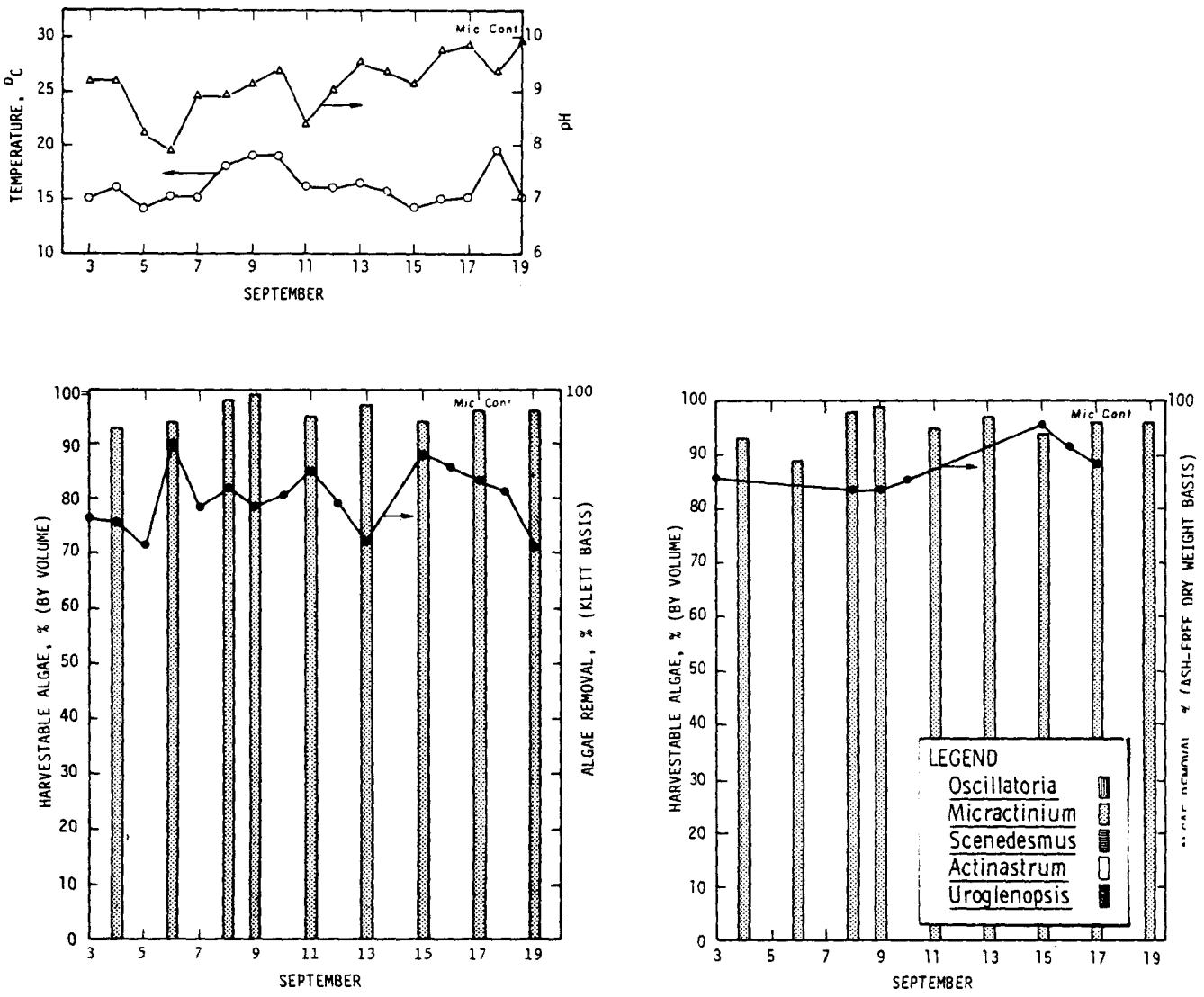


FIGURE 28. TEMPERATURE, pH, HARVESTABLE ALGAL FRACTIONS, AND KLETT- AND DRY WEIGHTS-BASED ALGAL-REMOVAL EFFICIENCIES FOR EXPERIMENT MIC CONTROL

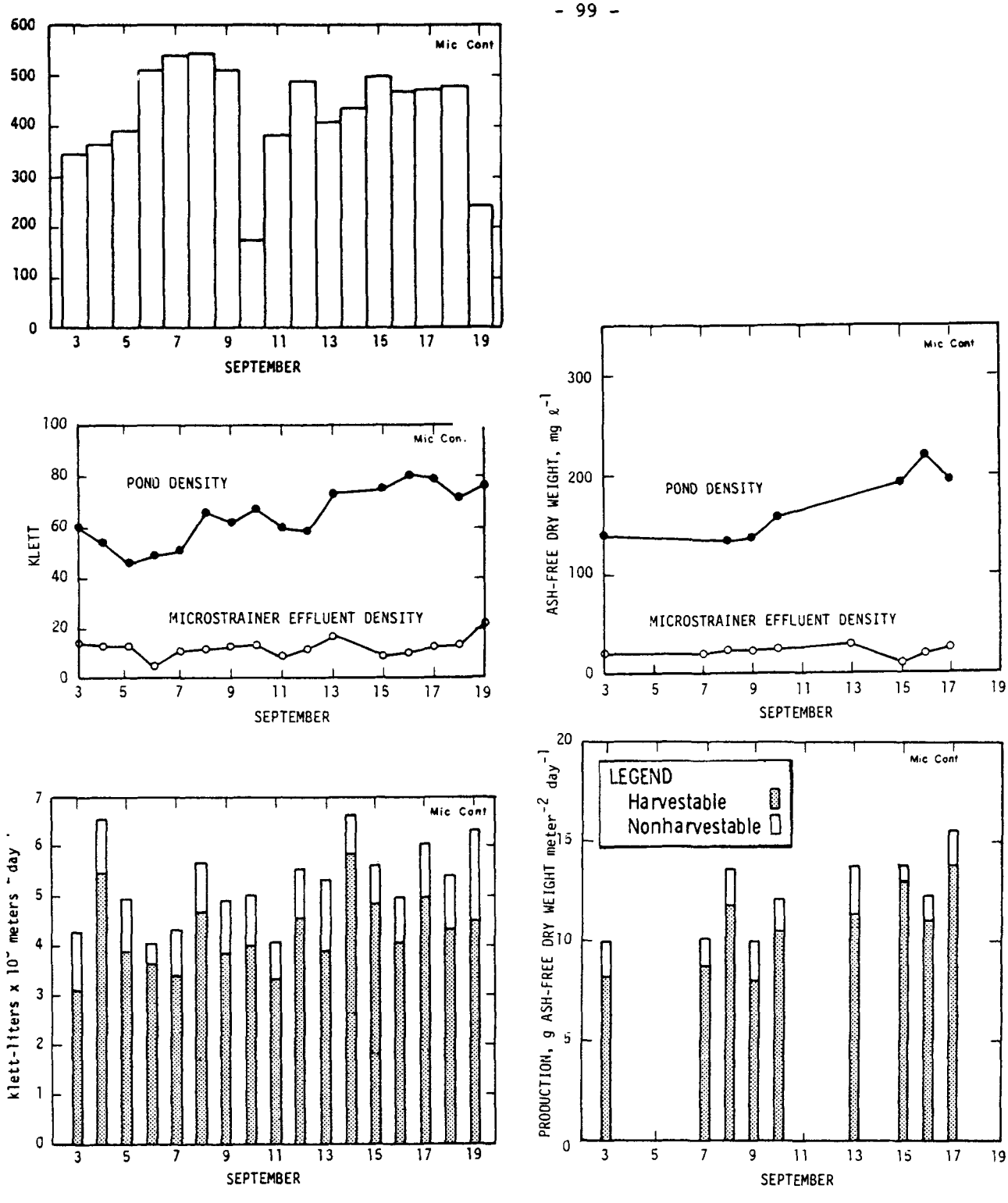


FIGURE 29. INCIDENT SOLAR ENERGIES, KLETT- AND DRY WEIGHTS-BASED POND DENSITIES, MICROSTRAINER EFFLUENT DENSITIES, HARVESTABLE PRODUCTIONS, AND TOTAL PRODUCTIONS FOR EXPERIMENT MIC CONTROL

climbed until the last days when it seemed to reach a steady state at a Klett of about 115-120. This increase was accounted for primarily by a gradual increase in the Micractinium concentration. As with Mic 1, Micractinium predominated throughout the experiment by over one order of magnitude in concentration. The average algal removals were 87% (Klett basis) and 93% (dry weight basis).

Production values increased rather steadily, starting at a relatively low level (2×10^3 Klett liters $\times 10^3$ meter^{-2} day^{-1}) but ending at respectable values for harvestable and total production (3.5 and 4.1, respectively). Average production was 3.4 (harvestable) and 4.4 Klett-liters $\times 10^3$ meter^{-2} day^{-1} (total) for a harvestable to total ratio of 77%.

Experiment Mic Control was designed to allow comparison of the effects of 50% (nominal) and zero biomass recycle for ponds run at identical dilution rates. Pond densities were intermediate of the Mic 1 and Mic 2 experiments, showing a gradual rising trend. As with Mic 1, the only harvestable algae present were Micractinium which greatly dominated the non-harvestable population which was composed primarily of Chlorella. The concentration of Micractinium increased slowly throughout the experiment, paralleling the rise in pond density.

Observed algal removal efficiencies averaged 81% (Klett basis) and 87% (dry weight basis); no definite trend in efficiencies was exhibited. The production values for this experiment were quite consistent and showed a slight rising trend. Average productions based on Klett were 4.1 (harvestable) and 5.2 (total), giving a ratio (harvestable to total) of 79%. The corresponding production averages based on ash-free dry weight were 11.0 and 12.7 grams meter^{-2} day^{-1} for harvestable and total production respectively; in this case, the harvestable to total ratio was 87%. When adjustments are made for the rise in pond density, gross productions of 5.9 Klett liters $\times 10^3$ meter^{-2} day^{-1} and 15.0 grams meter^{-2} day^{-1} were obtained.

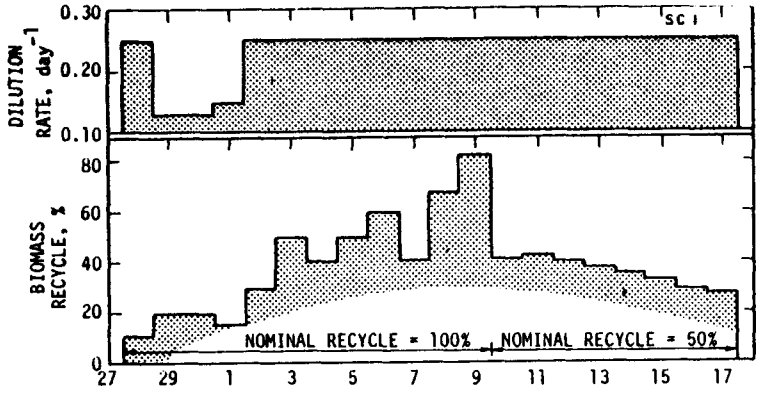
Throughout the three simultaneous experiments, temperatures for all ponds were essentially identical: ranging between 14°C and 20°C and averaging about 15°C. pH values were similar in pattern and magnitudes: the highest morning pH for any pond was 10.0 (observed in Mic 1) and the lowest 7.9

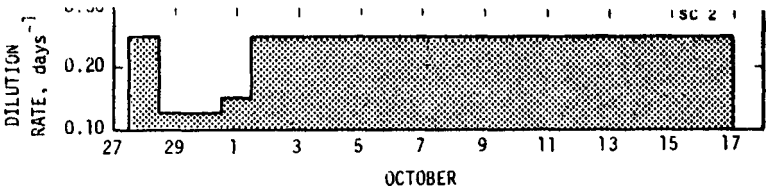
(Mic Control). Mic 1 had the highest pH (generally 0.2-0.4 pH units above the other two experiments) and Mic Control the lowest.

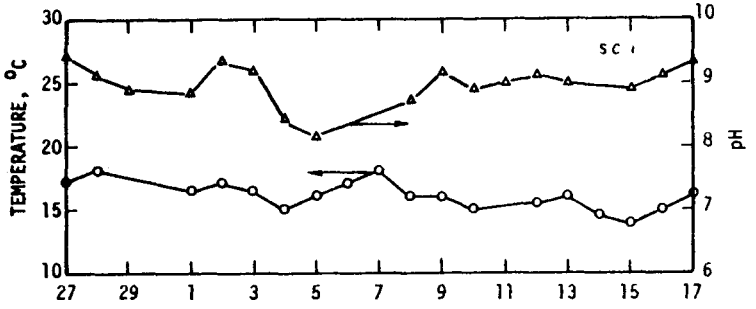
Exp. SC1-2 (Figures 30-31). Throughout the previous experiments, one conclusion became convincingly apparent: for the algae studied, the operational parameters tested and the environmental conditions encountered, the effects of biomass recycling on algal population dynamics in the experimental ponds were too subtle to allow quantification. Therefore, several new aspects in the inoculation phases were incorporated into the designs of the SC1 and SC2 experiments. First, about 75% of each of the Mic 2 and Mic Control cultures were harvested; the remaining culture was pumped to another pond (where it was maintained as an outdoor "stock" Micractinium culture for several weeks) and the microstrainer effluent was returned to the original ponds. Settled sewage was then added incrementally over the next two days to bring the cultures up to 1000-liter volumes. Once at full volume, 20% of each pond was harvested again; the harvest was discarded and the microstrainer effluent returned to the ponds. Finally, the ponds were intermixed to ensure identical starting algal populations.

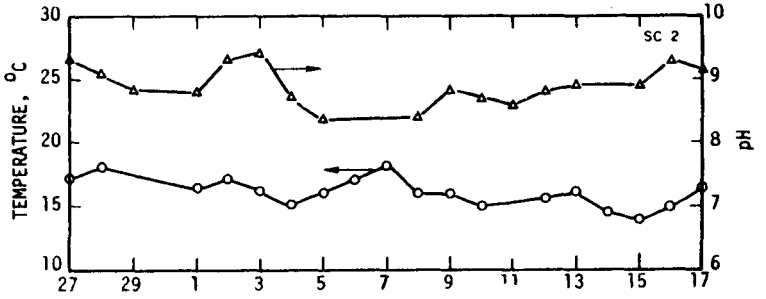
Dilution rates for both ponds were set at 0.25 day^{-1} except for a short cloudy period early in the experiments when the rates were reduced to 0.12 day^{-1}. Initially, SC1 received 100% recycle of the harvest, but since the culture harvested poorly at first, the actual rate of biomass recycle began at 11%. The biomass recycle rate increased to 87% 12 days into the experiment. On the 13th day (October 10), the nominal recycle rate was cut to 50% for the duration of the experiment; actual biomass recycling decreased gradually from 43% on the 10th to 25% at experiment termination. There was no recycle of harvested biomass in SC2.

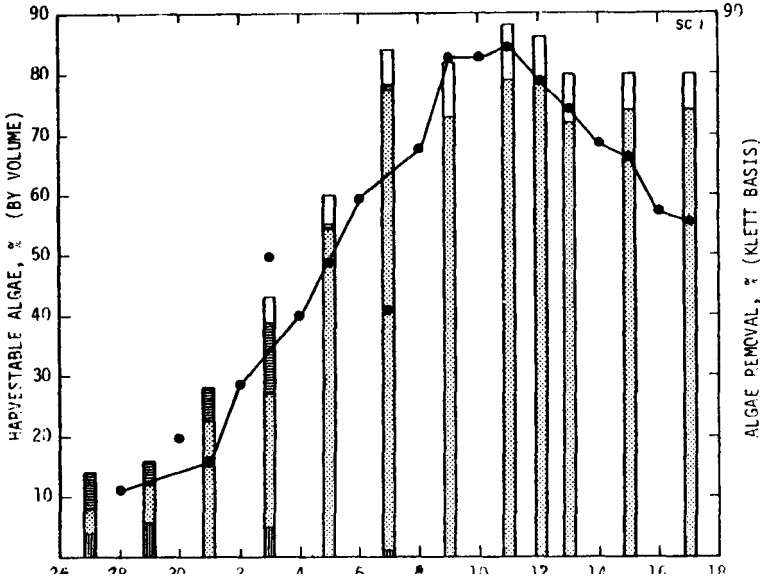
Both cultures were initially dominated by Chlorella and to a lesser extent Scenedesmus. Roughly equal concentrations (10-18 $\mu m^3 \times 10^6 ml^{-1}$) of Oscillatoria, Actinastrum, and Micractinium were present initially. Similar trends in population dynamics developed in both cultures: Micractinium and Actinastrum increased in concentration whereas all other species declined. However, the rates of change in specific algal concentrations differed between cultures: Micractinium increased at a faster rate in SC1, reaching a concentration of over 300 $\mu m^3 \times 10^6 ml^{-1}$, whereas the ending concentration for

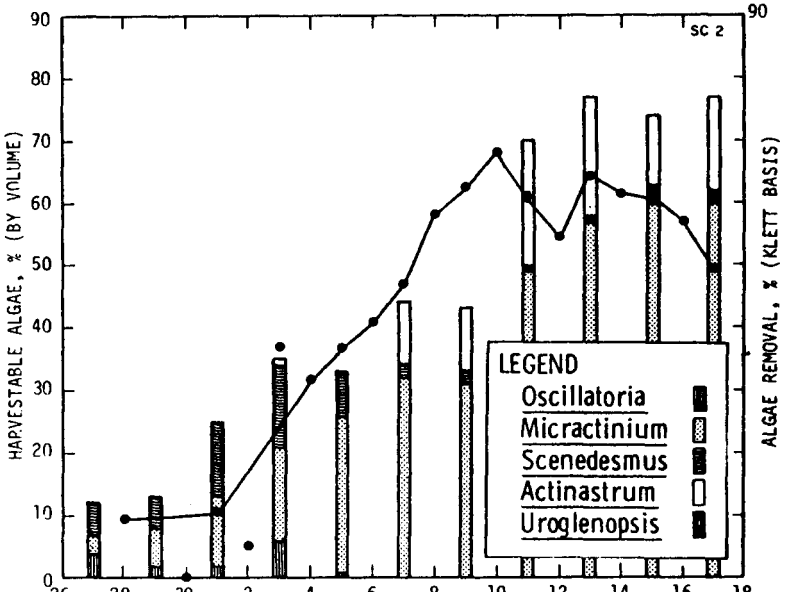

SC 1
DILUTION RATE, day⁻¹
0.30
0.20
0.10
BIOMASS RECYCLE, %
80
60
40
20
0
NOMINAL RECYCLE = 100%
NOMINAL RECYCLE = 50%
27 29 1 3 5 7 9 11 13 15 17

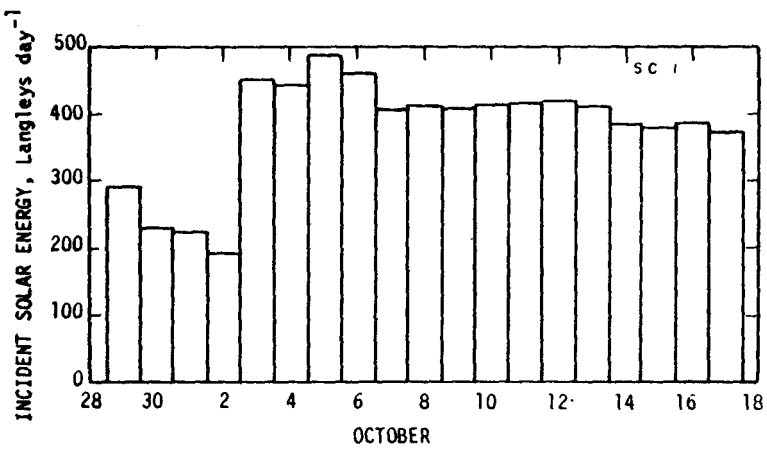

DILUTION RATE, days⁻¹
0.20
0.10
27 29 1 3 5 7 9 11 13 15 17
OCTOBER

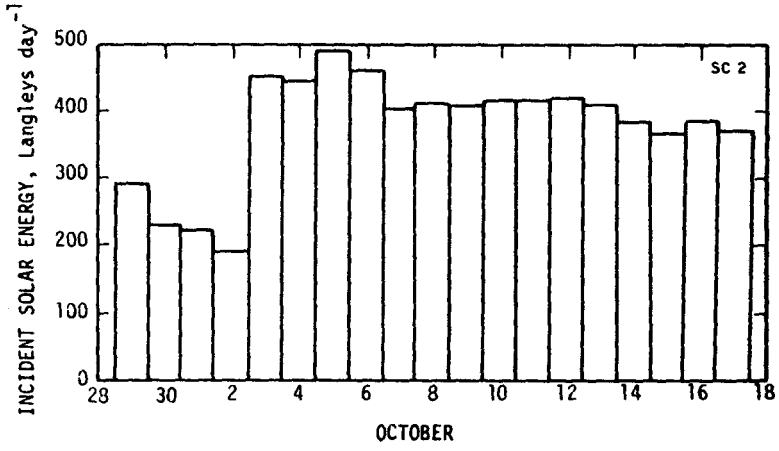

SC 1
TEMPERATURE, °C
30
25
20
15
10
pH
10
9
8
7
6
27 29 1 3 5 7 9 11 13 15 17

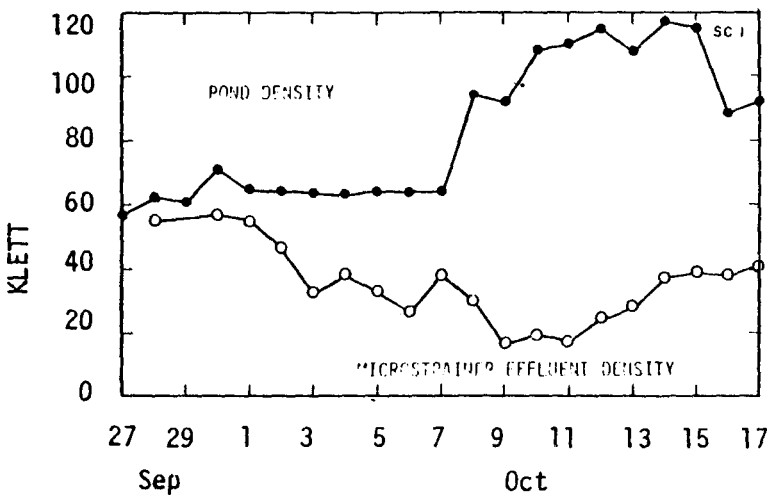

SC 2
TEMPERATURE, °C
30
25
20
15
10
pH
10
9
8
7
6
27 29 1 3 5 7 9 11 13 15 17


SC 1
HARVESTABLE ALGAE, % (BY VOLUME)
ALGAE REMOVAL, % (KLETT BASIS)
90
80
70
60
50
40
30
20
10


SC 2
HARVESTABLE ALGAE, % (BY VOLUME)
ALGAE REMOVAL, % (KLETT BASIS)
90
80
70
60
50
40
30
20
10
0
LEGEND
Oscillatoria
Micractinium
Scenedesmus
Actinastrum
Uroglenopsis







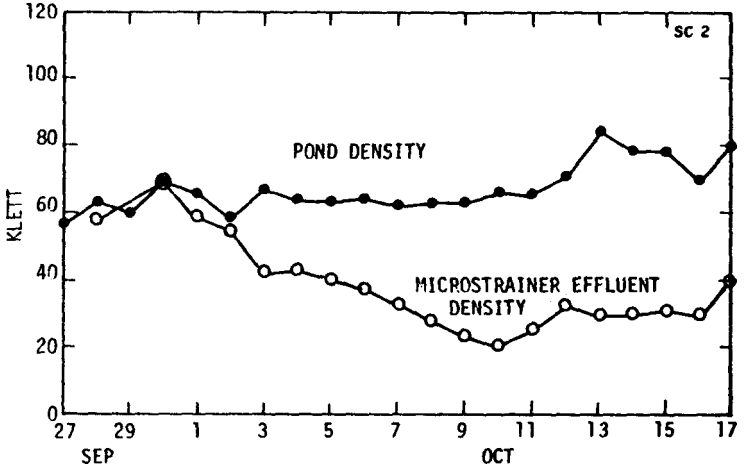


FIGURE 30. OPERATIONAL SCHEDULES, TEMPERATURE, pH, HARVESTABLE ALGAL FRACTIONS, ALGAL-REMOVAL EFFICIENCIES, INCIDENT SOLAR ENERGIES, POND DENSITIES, AND MICROSTRAINER EFFLUENT DENSITIES FOR EXPERIMENTS SC1 AND SC2

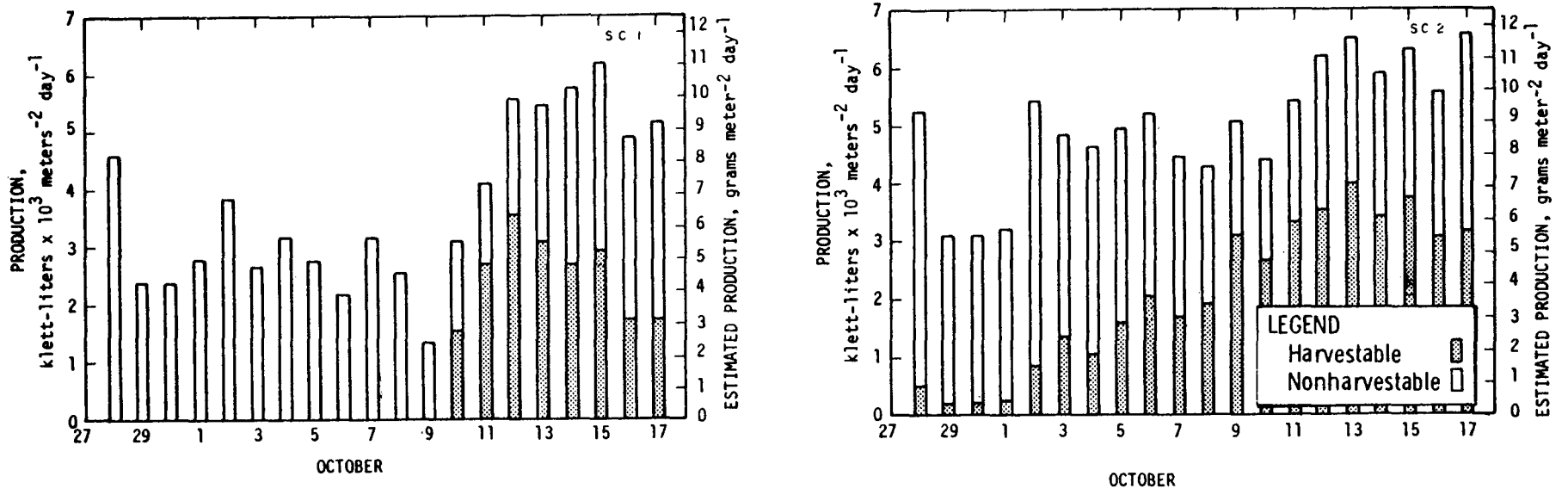


FIGURE 31. HARVESTABLE AND TOTAL PRODUCTIONS FOR EXPERIMENTS SC1 AND SC2

SC2 was about 200. The concentrations of nonharvestable algal species grouped in the "other" category (mostly Chlorella) declined more slowly in the nonrecycle pond (SC2) than in the pond where harvested biomass was recycled. Observed algal removal efficiencies in SC1 increased steadily up to October 11 and closely paralleled the harvestabilities predicted on the basis of algal counts. After the October 11 date, algal removals and, to a lesser extent, predicted harvestabilities decreased.

Pond densities in SC1 remained almost constant throughout the first 11 days, but then increased sharply. Microstrainer effluent densities generally decreased until October 11 after which time they rose slightly. Both the pond and the microstrainer densities were affected by a tear in the straining fabric of the harvesting unit which was discovered and repaired October 8. The jump in pond densities between October 7 and 8 can be attributed to a sharp increase in the amount of biomass captured and thus the amount recycled. This is confirmed by actual biomass recycle rates which rose from 41% on October 7 to 67% on October 8. A less pronounced effect was visible from the trend of microstrainer effluent densities which continued a smooth decline through October 7 and 8, indicating that the tear probably occurred not more than one harvest before it was discovered.

The date at which microstrainer effluent densities began to rise (October 11) corresponded rather closely to the day the nominal recycle rate was decreased from 100% to 50%, thus indicating that the high-rate of recycling had played a beneficial role in making the culture harvestable. However, the SC2 data show the same trend (decreasing microstrainer effluent densities until October 10 at which time densities increased again), although the increase was smaller in magnitude. It is clear, however, that the absolute effluent densities achieved by straining the SC1 culture were lower (generally by 2-4 Klett units) than obtained from SC2.

As was expected, SC2 pond densities were consistently lower than those for SC1. Production conversely showed consistently higher values in the non-recycle pond: averages calculated over the period of 50% recycle in SC1 showed values of 3.0 and 5.4 Klett-liters x 10^3 meter^{-2} day^{-1} for harvestable and total production respectively (ratio - 56%) whereas averages of harvestable and total production for SC2 over the same period gave 3.6

and 6.1 Klett-liters x 10^3 meter^{-2} day^{-1}, respectively (ratio = 59%). Trends in pond densities were similar over this time period for the two experiments.

Temperatures were identical between ponds, ranging between 15°C and 18°C. Closely similar variations in pH were also observed; maximum pH was 9.4 and the minimum 8.1. SC2 showed slightly lower (by 0.1-0.3 pH units) pH than SC 1.

Exp. SC3-4 (Figures 32-33). Similar inoculation procedures to SC1 and SC2 were followed for these experiments: microstrainer effluents from SC1 and SC2 cultures were used to start the ponds. Several hundred liters of an outdoor batch culture containing some Oscillatoria in addition to various other algal species were distributed between the ponds. Finally, the ponds were brought up to 1000-liter volumes by incremental additions of settled sewage. After intermixing, dilution rates were set at 0.33 day^{-1}; SC4 received 100% nominal recycle initially. This rate was later reduced to 50% for about one week but was then increased again, first to 66% and finally to 75%. Of course, the actual rate of biomass recycled varied also with algal removal efficiencies, roughly averaging 60% early in the experiment and 40% later on.

Initial algal populations were essentially identical. First in abundance were a variety of algae in the non-harvestable size range including Chlorella and small pigmented flagellates; tied for second were Micractinium and Scenedesmus, followed by Oscillatoria and Actinastrum. Once dilution began, distinct differences between experiments were seen after about 10 days: Micractinium increased at similar rates in both cultures until this time, then it abruptly crashed in SC3, declining almost to zero by the experiment's end. At the same time, the Micractinium continued increasing in SC4. After 10 days into the experiment, the non-harvestable algal population displaced Micractinium in SC3 and continued to increase in concentration, whereas in SC4 these algae declined to a minimum on October 21 and then increased. It appears that if the experiment had continued, the "other" algae would have also displaced Micractinium in SC4 (See Chapter IV).

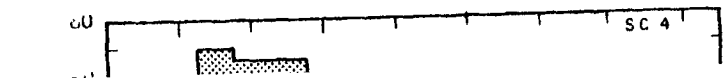
Trends in pond and microstrainer effluent densities were also widely disparate between experiments. Pond densities were higher in the recycle

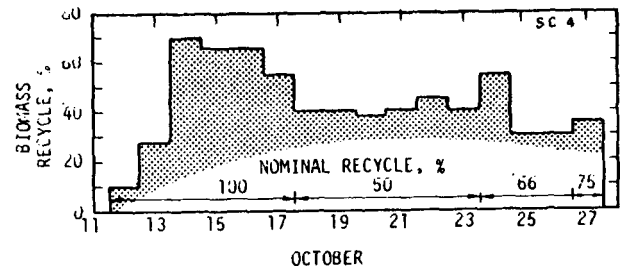
pond. Microstrainer effluent densities in SC3 were never greatly lower than the pond densities, and, in fact, equaled pond densities on two occasions. This behavior was in contrast to effluent densities from SC4 which decreased to a minimum of Klett 18 on October 20 (versus a pond density of 77). These densities did, however, increase again after October 20 until they approached or even surpassed effluent densities from SC3.

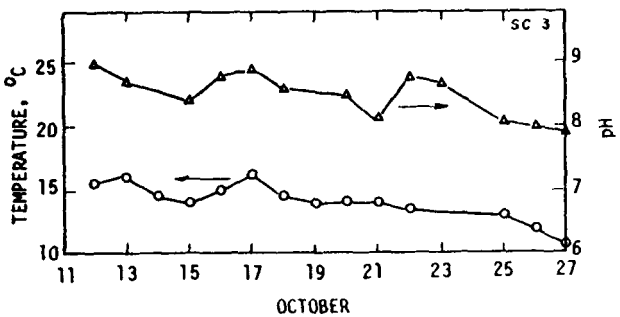
Algal-removal efficiencies observed from SC4 paralleled the rise in Micractinium over the first 11 days but then fell with the decline in proportions of Micractinium as the population of non-harvestable algae increased about 15% and declined to about 10% by the experiment's end. pH declined the last few days. Most of the harvestable population was composed of Micractinium with lesser amounts of Scenedesmus and Actinastrum; Oscillatoria was initially present but eventually declined to only 1%.

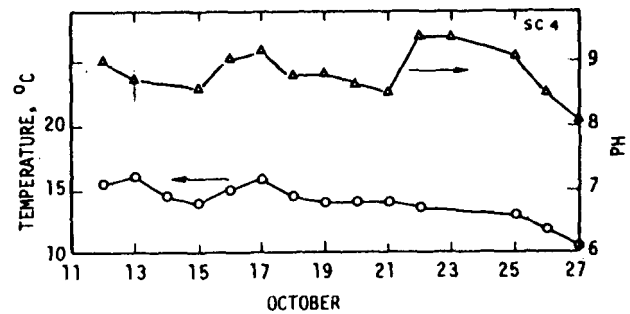
Removal efficiencies were significantly lower than predicted harvestabilities in SC3 except towards the end when both sets of data indicated almost zero harvestability. Except for an initial, short-lived, upward trend, (which paralleled the observed increase in Micractinium during the first days), the algal-removal efficiencies generally decreased throughout the experiment. It is interesting to note that in both experiments the fall in Micractinium concentrations were immediately preceded by a breakup of the colonies into smaller-sized fragments and, more strikingly, a loss of spines by the colonies.

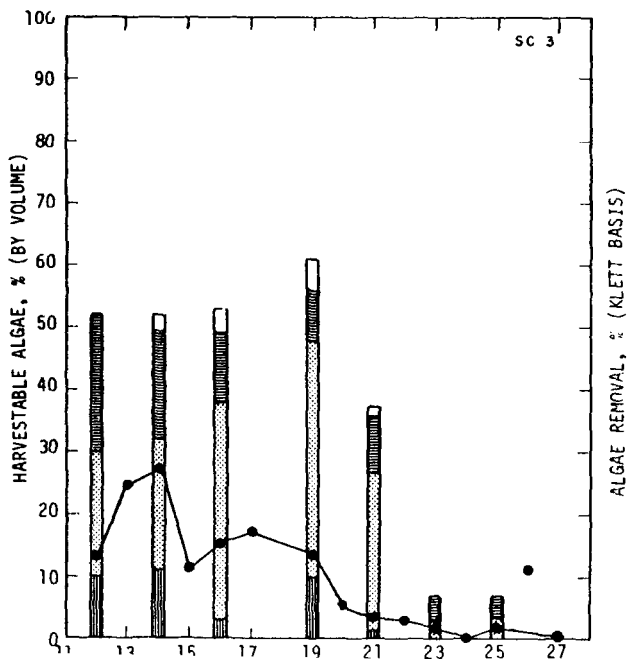
Both ponds were high in total production (as to be expected for the fast dilution rates employed) but low in harvestable production. SC3 showed a gradual decrease in total production (probably related to the decreasing insolation during this period) and a proportionally faster decline in harvestable production. SC4 exhibited declining values of total production until the nominal recycle rate was changed from 100% to 50%; from this point, it increased up to the last day of the experiment. Values of harvestable production, however, fell (except for an initial increase between October 19 and 20 which was due to the transition from two days of operation at a lower dilution rate). The values for harvestable and total production in SC4 averaged over the period when nominal recycles were less than 100% came out to be 1.9 and 6.1 Klett-liters x 10^3 meter^{-2} day^{-1} (ratio = 31%). Values

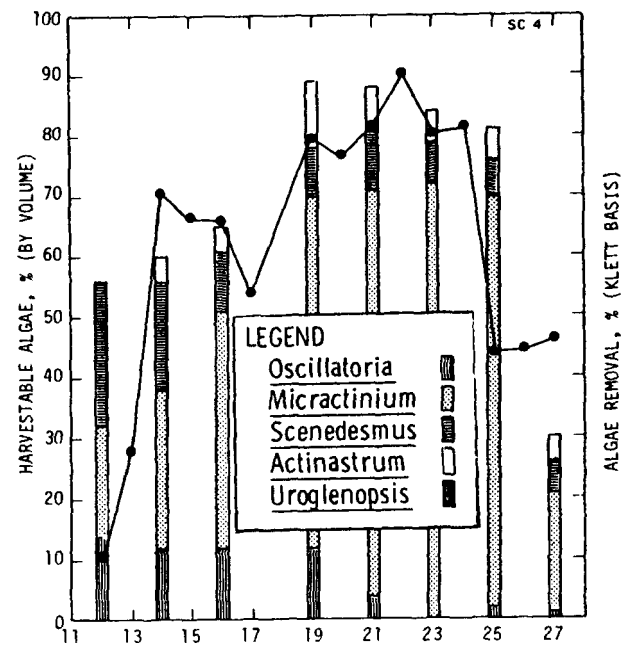

SC 4

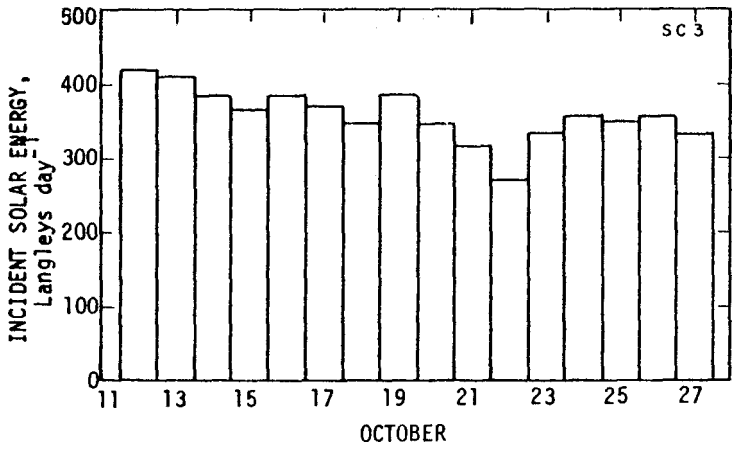

SC 4
BIOMASS RECYCLE, %
NOMINAL RECYCLE, %
100
50
66
75
11 13 15 17 19 21 23 25 27
OCTOBER

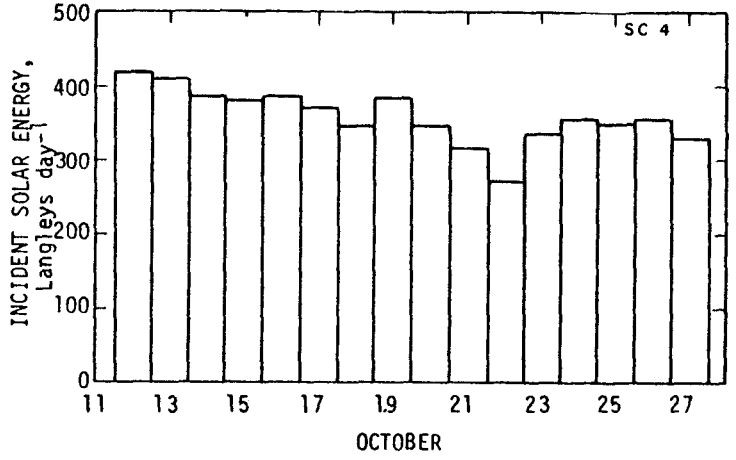

SC 3
TEMPERATURE, °C
pH
10 15 20 25
6 7 8 9
11 13 15 17 19 21 23 25 27
OCTOBER

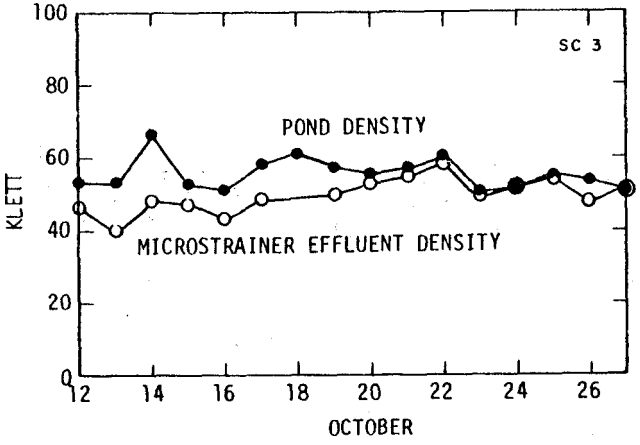

SC 4
TEMPERATURE, °C
pH
10 15 20
6 7 8 9
11 13 15 17 19 21 23 25 27
OCTOBER


SC 3
HARVESTABLE ALGAE, % (BY VOLUME)
ALGAE REMOVAL, % (KLETT BASIS)
0 10 20 30 40 50 60 70 80 90 100
11 13 15 17 19 21 23 25 27


SC 4
HARVESTABLE ALGAE, % (BY VOLUME)
ALGAE REMOVAL, % (KLETT BASIS)
LEGEND
Oscillatoria
Micractinium
Scenedesmus
Actinastrum
Uroglenopsis
0 10 20 30 40 50 60 70 80 90 100
11 13 15 17 19 21 23 25 27







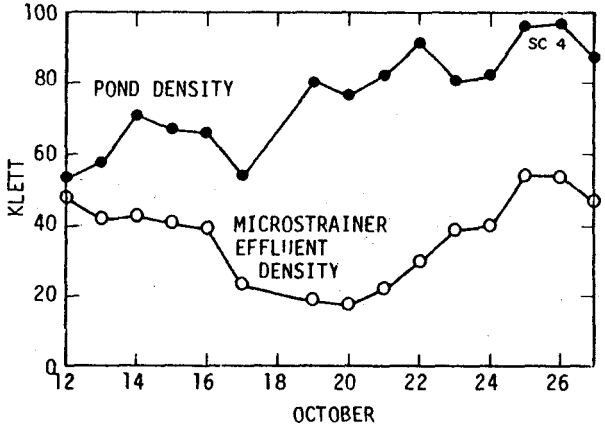
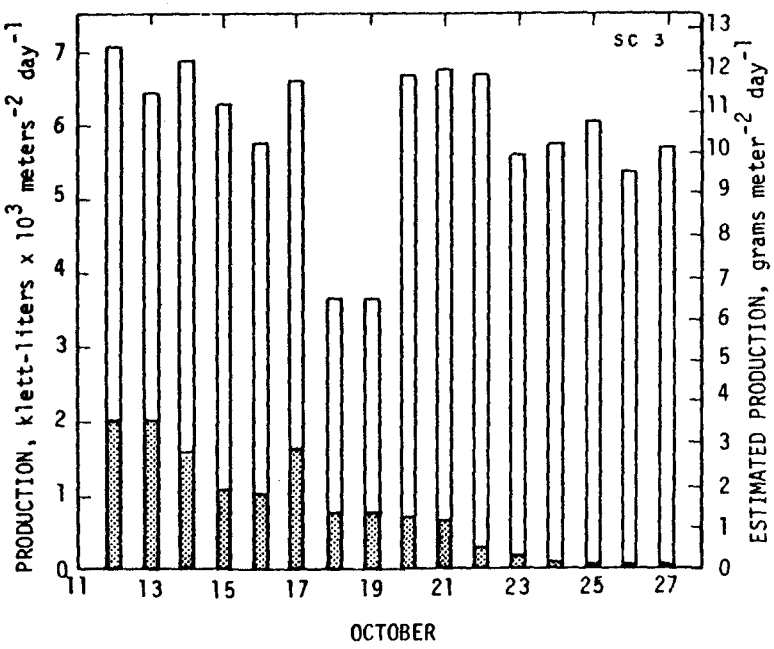


FIGURE 32. OPERATIONAL SCHEDULES, TEMPERATURE, pH, HARVESTABLE ALGAL FRACTIONS, ALGAL-REMOVAL EFFICIENCIES, INCIDENT SOLAR ENERGIES, POND DENSITIES, AND MICROSTRAINER EFFLUENT DENSITIES FOR EXPERIMENTS SC3 and SC4



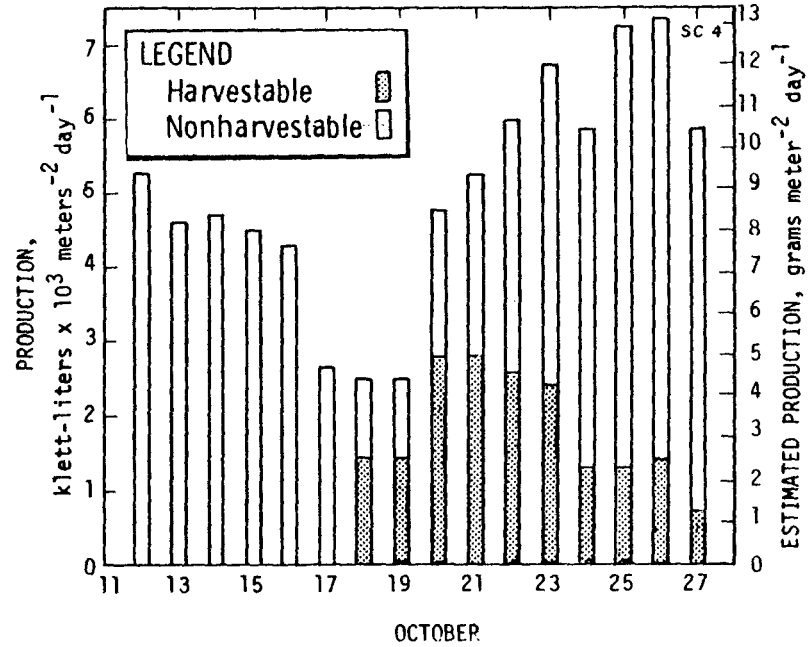


FIGURE 33. HARVESTABLE AND TOTAL PRODUCTIONS FOR EXPERIMENTS SC3 AND SC4

averaged over the same period for SC3 were 0.3 and 6.1 Klett-liters x 10^3 meter^{-2} day^{-1} for harvestable and total production, respectively, giving a ratio of harvestable to total production of 5%. Temperatures began at about 15°C and declined to about 10°C by the experiment's end. pH underwent several cyclic variations in both cultures. SC4 exhibited generally higher values for pH by about 0.2-0.4 units and ranged between pH 8.1 and 9.4. pH in SC3 varied between 7.9 and 9.0.

DISCUSSION OF RESULTS

A summary of the results obtained from operating the Richmond 3-m^2 ponds during summer and fall 1976 is presented in Table 8. All values reported represent average pond and microstrainer performance compiled over two weeks or more of pond operation. In experiments where pond algal populations continued to change over the course of operation, these averages do not reflect true steady-state values; this was a problem inherent in the experimental designs (which were planned to induce transients in algal populations) and thus could not be avoided. Care was taken in the averaging process to exclude values obtained from the initial startup phase of pond experiments where transients were the greatest.

Algal-removal Efficiencies. High harvesting efficiencies attained during the initial phases of the Oscillatoria inoculations confirm the notion that filamentous algae can be readily strained from pond cultures using fabric with 26 μm openings. Higher average efficiencies were achieved in recycled ponds. This difference is partially due to the higher density of the recycled ponds which aids in harvestability of all algae by decreasing the effective pore size of the microstrainer screen by mat formation. Also, higher density ponds will exhibit a better overall harvestability (% removal) because of background turbidity (clay, color, detritus colloids, etc.) and because microstrainers do not appear to remove algae completely. Such approximately constant background has a larger effect on % removal in lower density ponds. In addition to higher cell density, a higher proportion of harvestable algae (e.g. Oscillatoria in the initial experiments and Micractinium in later experiments) also aided in harvesting the smaller algae (again due to

TABLE 8

SUMMARY OF EXPERIMENTAL RESULTS OBTAINED DURING POND OPERATIONS

Exp.	Dilution Rate $days^{-1}$	Nominal Recycle %	Pond Density		Effluent Density		Algal Removal %[c]		Effluent Nutrient Concentrations mg/l			Nutrient Removals %		
			Klett	Dry Wt[a] mg/l	Klett	Dry Wt[a] mg/l	Klett Basis	Dry Wt Basis	COD	NH_3-N	Ortho-P	COD	NH_3-N	Ortho-P
WO1	0.33	40	122	--	12	--	90	--	--	--	--	--	--	--
WO2	0.31	67	113	247	20	34	86	86	146	8.1	7.0	53	70	21
WO3	0.32	50	94	226	22	31	76	84	141	4.9	6.4	54	80	28
WO4	0.17	0	81	188	23	42	70	75	132	2.7	5.1	59	88	43
WO5	0.15	78	142	317	18	27	85	91	155	3.4	5.6	50	85	25
WO6	0.25	50	147	228	45	69	66	69	245	3.4	6.5	29	85	13
WO9	0.20	0	55	--	22	--	60	--	--	--	--	--	--	--
WO10	0.22	67	75	--	19	--	74	--	--	--	--	--	--	--
Mic 1	0.15[b]	0	50	141	13	19	74	85	164	0.9	4.9	53	96	34
Mic 2	0.25	50	104	273	12	17	87	93	160	1.7	5.2	54	92	30
Mic Cont.	0.25	0	65	171	12	19	81	88	154	3.0	5.2	56	87	30
SC1	0.25	50	113	--	29	--	74	--	--	--	--	--	--	--
SC2	0.25	0	75	--	30	--	60	--	--	--	--	--	--	--
SC3	0.31	0	54	--	52	--	3	--	--	--	--	--	--	--
SC4	0.31	60	87	--	38	--	68	--	--	--	--	--	--	--

[a] Ash-free

[b] 30% of this pond was harvested daily, with 50% of the influent composed of sewage and 50% composed of microstrainer effluent.

[c] Algal removal is the average of daily calculated efficiencies.

TABLE 8 (cont'd.)

Exp.	Incident Solar Energy Langleys Day^{-1}	Basis[d]	Production g meter^{-2} day^{-1}			Ratio of Harvestable to Total Productivity %	Solar Conversion Efficiency, %			Photosynthetic Conversion Efficiency %		
			Harvest	Total	Gross		Harvest	Total	Gross	Harvest	Total	Gross
WO1	--	K	11.9	14.2	11.4	84	--	--	--	--	--	--
WO2	540	M	7.1	10.7	8.9	66	0.7	1.1	0.9	1.7	2.6	2.1
WO3	480	M	6.9	9.2	10.5	75	0.8	1.1	1.2	1.8	2.6	2.8
WO4	490	M	9.0	11.7	8.1	77	1.0	1.3	0.9	2.4	3.0	2.1
WO5	500	K	2.3	4.0	1.3	58	0.3	0.4	0.1	0.6	1.0	0.3
WO6	450	M	5.2	11.4	10.7	46	0.6	1.4	1.3	1.5	3.2	3.0
WO9	350	K	2.6	5.5	5.5	47	0.4	0.9	0.9	1.0	2.0	2.0
WO10	350	K	1.6	4.3	5.4	37	0.3	0.7	0.9	0.6	1.6	2.0
Mic 1	430	M	9.1	10.1	10.5	90	1.2	1.3	1.4	2.7	3.0	3.1
Mic 2	430	M	9.2	10.7	14.3	86	1.2	1.4	1.8	2.7	3.2	4.3
Mic Cont	430	M	11.0	12.7	15.0	87	1.4	1.6	1.9	3.3	3.8	4.5
SC1	395	K	5.4	9.7	10.5	56	0.8	1.3	1.5	1.7	3.1	3.4
SC2	395	K	6.5	10.9	12.9	60	0.9	1.5	1.8	2.1	3.5	4.2
SC3	340	M	0.6	11.3	11.3	5	0.1	1.8	1.8	0.2	4.3	4.3
SC4	340	M	4.1	10.3	10.4	40	0.7	1.7	1.7	1.6	3.9	3.9

[d] K = production in g meter^{-2} day^{-1} = 1.8 x production in Klett-liters x 10^3 meter^{-2} day^{-1}.

M = average composed of three or more dry weight measurements.

better mat formation). An example is found in the Micractinium series of experiments, where the recycled ponds had the highest density and exhibited somewhat higher harvesting efficiencies (than did the nonrecycled and effluent recycled ponds). Indeed, in that case, all ponds had the same residual background dry weights and densities after harvesting.

Fall experiments which started with low-level inoculations of Micractinium showed the most drastic effects of biomass recycle on algal-removal efficiencies attained with microstraining. The first experiments, SC1 and SC2, showed average algal-removals of 74% and 60% for the recycle (50% nominal) and nonrecycle ponds, respectively. However, effluent densities were essentially the same, indicating that similar amounts of non-harvestable algae grew in each pond. Experiments SC3 and SC4, run at a faster dilution rate (0.31 day^{-1}), did exhibit widely disparate effluent densities: Klett 38 for the recycle pond (SC4) and Klett 52 for the nonrecycle pond (SC3). These average values fail to show that in the last few days of the experiments the effluent densities for SC4 were rising and were apparently destined to reach levels close to the SC3 pond densities. The ending trend of effluent densities for SC4 does not refute the fact that biomass recycle did exert a positive effect on culture harvestability; however, the transient nature of this effect does not yet allow these results to be extrapolated to large-scale efforts of algal cultivation.

Nutrient Removals. Removals of chemical oxygen demand (COD) from influent sewage were similar in all experiments (50-60%) except WO6 where 29% of influent COD was removed.

The highest removal of NH_3-N was achieved in experiment Mic 1 (96%); the low effluent ammonia level (less than 1 mg/liter as N) indicates that algal growth in this pond may have been nitrogen limited. Of the two Mic experiments run at dilution rates of 0.25 day^{-1}, the recycle pond showed a greater ammonia removal than the nonrecycle pond (92% and 87%). The amounts of NH_3-N removed were greater than could be predicted to occur based on stoichiometric considerations (e.g. for Mic 2 the production of algae was 14.3 $g/m^2/day$ or 82% of the 1.7 g N $removed/m^2/day$). The difference between the predicted and observed NH_3-N removals would be greater if the N content of the algae (not measured) was below 10%. Additional N removal could

partially be due to the deposition of some algae on the pond bottom as a stable sludge and certainly to the outgassing of NH_3. Because the form of ammonia in water is strongly pH dependent, the ponds with higher pH would be expected to lose more ammonia. This expectation correlates with the parallel increases of average pH (refer to previous discussions) and NH_3-N removals.

NH_3-N levels in effluents from Oscillatoria inoculations ranged between 2.7 and 8.1 mg/liter, corresponding to removals of 88% to 70%. The greatest removal was attained with the nonrecycle pond operated at 0.17 day^{-1} dilution; whereas the lowest occurred in experiment W02 (0.31 day^{-1} dilution, 67% nominal biomass recycle).

Removals of orthophosphate for all experiments ranged between 21% and 43%. The relatively high concentrations of orthophosphate present in pond effluents (4.9 mg/liter to 7.0 mg/liter) indicate that this nutrient was at no time limiting to growth.

Production. The most representative series of experiments insofar as biomass production is concerned were the Micractinium series. The experimental series were designed to allow comparison of production between two ponds (Mic 2 and Mic control) diluted at identical rates (0.25 day^{-1}) but with different (50% and zero, respectively) nominal harvest recycle rates. In addition, a third pond was operated so that 30% of its volume was harvested daily, but only one-half of the inflow was made up of sewage; the other 50% was microstrainer effluent recycled to the pond. The greatest production of harvestable algae (11.0 $g/m^2/day$) was achieved in the 0.25 day^{-1} diluted, nonrecycled pond. Total production of both harvestable and non-harvestable algae was 12.7 $g/m^2/day$, yielding a ratio of harvestable to total production of 87%. Ponds Mic 1 and Mic 2 exhibited almost identical harvestable production values (9.1 and 9.2 $g/m^2/day$, respectively).

Maintenance of lower culture densities through effluent recycling can serve to reduce biomass losses through respiration under light-limiting conditions. The higher productions of Mic Control relative to Mic 2 indicate that the higher culture density maintained in Mic 2 may have exerted a detrimental effect on net algal growth. This relationship is less pronounced

when the total production values are adjusted for changes in pond density. The lower productions for Mic 1, in spite of its lower pond density, could indicate that the culture was nutrient limited, most likely by nitrogen.

Absolute values of production in all experiments are of limited applicability to large-scale pond operations; values from experiments WO1 through WO10 are, to a large extent, artifacts of the inoculations, whereas values from experiments SC1 through SC4 are affected by the great transients in algal populations which occurred. Comparison of production values between these experiments, however, does yield several valuable observations: (1) the total productions from identically-diluted ponds in simultaneous experiments were greater in the ponds not receiving recycled harvested biomass, (2) for the same experiments the gross productions were also greater in the nonrecycle ponds, although to a lesser extent, and (3) with the exception of experiments SC3 and SC4, the harvestable productions in the non-recycled ponds were greater as were the ratios of harvestable to total production.

Solar and Photosynthetic Conversion Efficiencies. The highest conversion of total solar energy into harvestable biomass (1.4%) was attained in experiment Mic Control. When the incident solar energy is adjusted to account for the fraction photosynthetically available, a photosynthetic conversion efficiency of 3.3% is obtained. These efficiencies are especially significant because they represent the conversion of solar energy into biomass which can be easily and inexpensively removed from the culture liquid. Of course, when the total biomass produced in the Mic Control growth pond is considered, solar and photosynthetic conversion efficiencies of 1.6% and 3.8%, respectively, are obtained. The Mic 1 and Mic 2 ponds both exhibited harvestable biomass-based solar and photosynthetic conversion efficiencies of 1.2% and 2.7%, respectively. The highest conversion efficiency of solar energy into total biomass (1.8%) was obtained in experiment SC3; however, its gross efficiency was lower than that of Mic Control.

The experiments presented in this chapter achieved their main objective: development of an experimental system suitable for studies of algal species control. The applicable data to species control is reviewed in the next chapter. Future experiments will concentrate on combining biomass

:ecycle with other operational methods likely to lead to growth of harvest-
ıble biomass: higher algal concentrations, longer detention times, carbona-
:ion of diluted sewage, and nitrogen limitations.

IV. SPECIES CONTROL: EXPERIMENTAL

OUTDOOR POND RESULTS

The first algal strain chosen for inoculation was an Oscillatoria strain occurring in the Napa, California oxidation ponds. At the time these algae were harvested (by microstraining) from the Napa ponds, they comprised about 25% of the pond population, most of the other algae being species of Scenedesmus. Although considerable enrichment for Oscillatoria was effected by the microstraining process, about 20% of the harvest consisted of Scenedesmus. This algal harvest from Napa was transported the same day to Richmond and inoculated into a 1000 liter pond containing one-half tap water, one-half clarified sewage and 100 ppm $NaHCO_3$. The pond was mostly shaded (see Fig. 34) to prevent photo-oxidative death of the inoculum. The Scenedesmus contaminants grew much better than the Oscillatoria, and within a few days it became necessary to harvest nearly 100% of the pond to prevent the green algae from totally taking over the pond. Microstraining proved to be an excellent selective separation method for Oscillatoria filaments of moderate length; however, after screening, the harvested Oscillatoria had to be reinoculated into a new batch of diluted sewage; the filaments broke up again, the green algae grew well, and this first experiment became a failure.

Two things were learned from this run: the Oscillatoria grew poorly on Richmond sewage compared to the Scenedesmus and inoculating a small amount of filamentous blue-green algae quickly into unconditioned media can lead to filament breakage. A second inoculation of Napa Oscillatoria was made, this time a much larger one (25-fold). Again, it was found necessary to harvest nearly the entire pond volume to eliminate Scenedesmus, and again, the Oscillatoria did

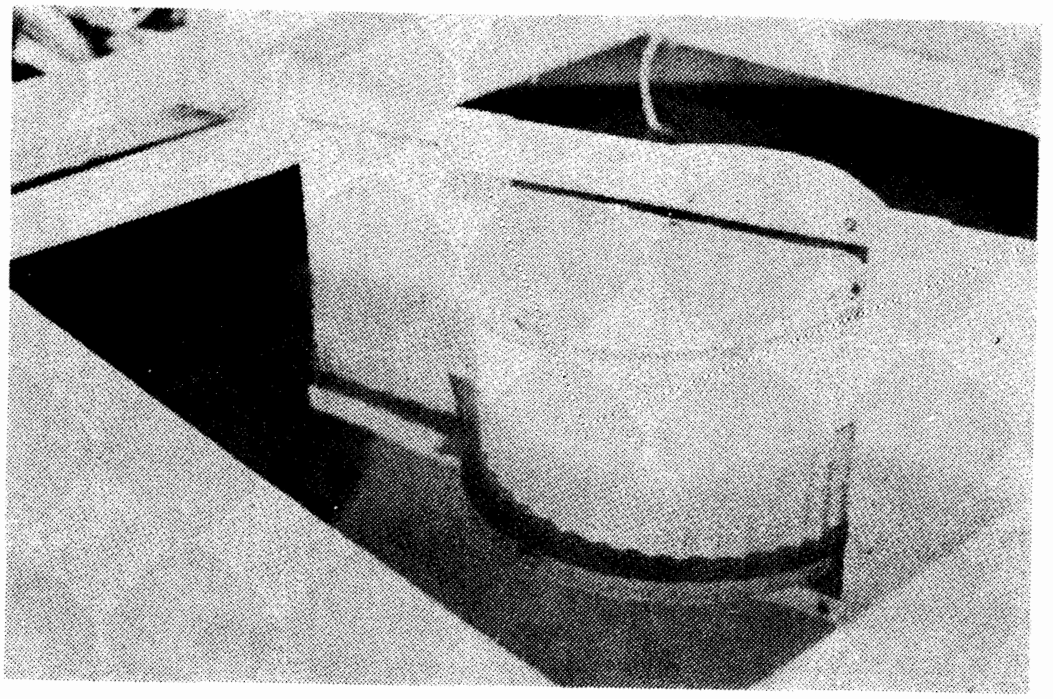


FIGURE 34. NAPA Oscillatoria INOCULATED INTO A SHADED 3 m^2 CIRCULAR POND. CHRONOLOGICALLY THESE EXPERIMENTS PRECEDED THOSE REPORTED IN THE PREVIOUS CHAPTER. A DIFFERENT MIXING SYSTEM WAS USED CONSISTING OF A CENTRALLY PIVOTED HORIZONTALLY ROTATING PADDLEWHEEL.

not grow fast enough. A third inoculum fared better, but only because it started out four times heavier than the second attempt (close to 100 times heavier than the first attempt). Figure 35 shows the results. The pond had to be harvested nearly 100% repeatedly due to the fast growth of unicellular algae. Unfortunately, the growth of Oscillatoria did not keep up with the rate of loss due to harvesting. So, although separation was easily achieved, the only moderate harvestability (~ 65%) and slow growth rate of Napa Oscillatoria precluded selective cultivation on Richmond sewage. At this point, it was decided to test a different Oscillatoria strain from a different oxidation pond and the experiments "Woodland Oscillatoria Inoculation" reported in Section III were started.

It was also deemed necessary to change the inoculation procedure. In order to start with a large and healthy inoculum free of contamination, we grew a strain of Oscillatoria isolated from the Woodland oxidation ponds on Allen and Arnon media in the laboratory. The Woodland ponds were almost exclusively Oscillatoria at the time of isolation. These cultures grew very dense and had very long filaments. Several batches were grown and inoculated into very small (150 liter) outdoor ponds. The algae grew well and remained relatively uncontaminated. Small amounts of raw settled sewage were added periodically. After about one week, the small pond cultures were transferred to the 1000 liter ponds. Here the algae were diluted and exposed to a greater percentage of raw sewage, but still given plenty of synthetic media. The rationale was that Richmond sewage was sufficiently different from other municipal wastes to require growing the inoculum on laboratory media prior to "re-adaptation" to a different sewage. The process was successful. We were able to start our experiments with over 200 mg/l of very healthy cultures of Woodland Oscillatoria (referred to as W.O.) There was contamination, however, from a Chlorella-like green algae (3-4 μm diameter) that was present in small amounts in the original Woodland sample. We refer to this as the Woodland Pond Isolate (WPI). Subsequent laboratory studies (see next subsection) revealed that these algae are particularly fast growing, their minimum doubling time being about 8 hours. Since we avoided harvesting a large portion of any pond after the experience with the Napa algae, by the time our first WO ponds were ready for experimentation, there was already 15-25% general contamination.

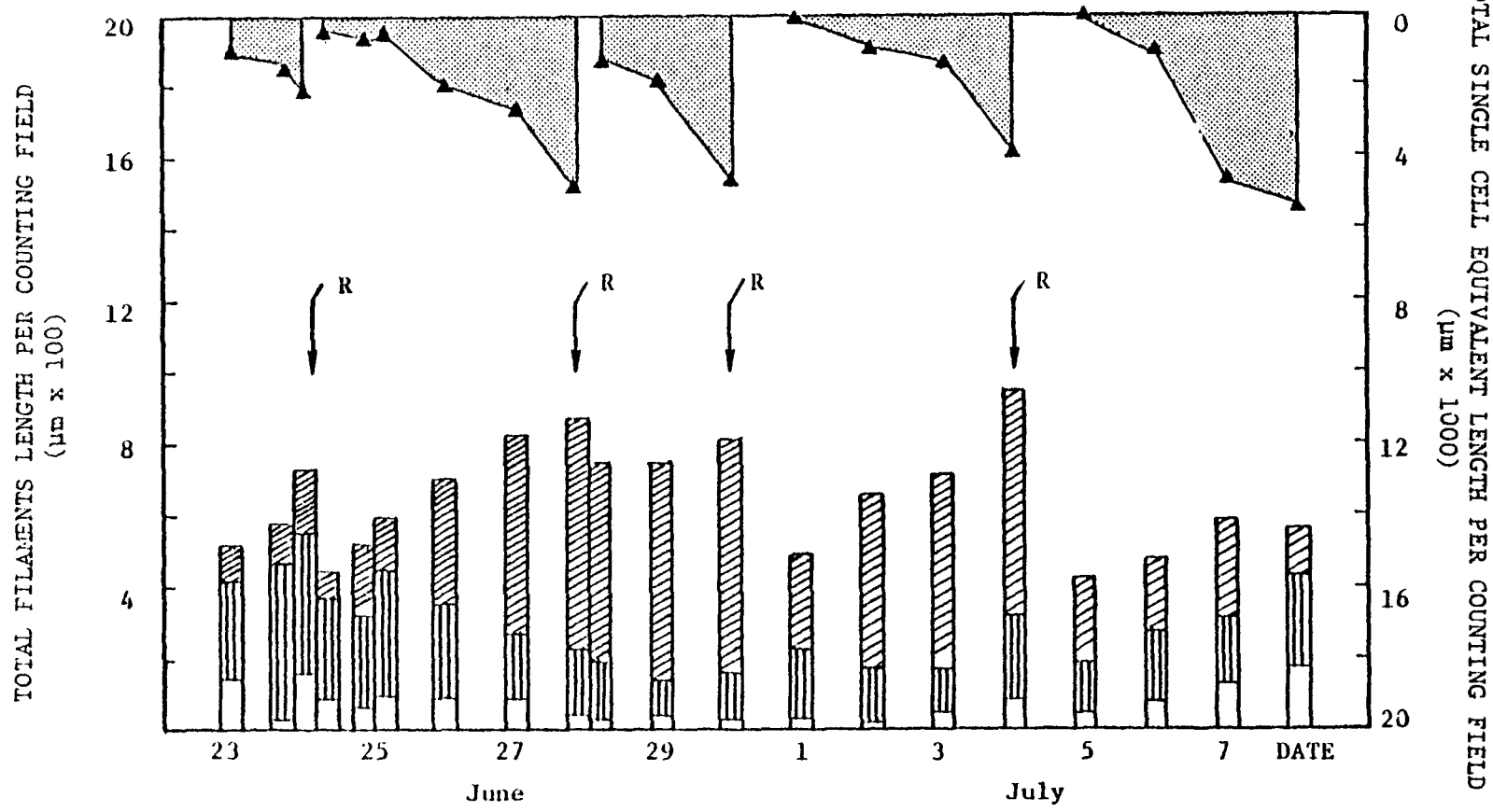


FIGURE 35. MAINTENANCE OF A CULTURE OF NAPA Oscillatoria USING SELECTIVE RECYCLE. BARS INDICATE THE RELATIVE VOLUME CONCENTRATION AND FILAMENT LENGTH DISTRIBUTION OF Oscillatoria (white bar height indicates filaments shorter than 150 µm, vertical striped indicates the filaments between 150 µm and 375 µm, and slashed indicates the filaments longer than 375 µm). SINGLE-CELL VOLUME CONCENTRATIONS WERE CALCULATED ON AN EQUIVALENT LENGTH BASIS AND ARE GRAPHED AS TRIANGLES (shaded area). VERTICAL ARROWS MARKED R INDICATE POINTS AT WHICH THE CULTURE WAS HARVESTED WITH A MICROSTRAINER (using 30-µm opening fabric) AND THE CAPTURED ALGAE (mostly Oscillatoria) RETURNED TO THE POND. EXPERIMENTS WERE CARRIED OUT IN 3.0 m^2 CIRCULAR PONDS MIXED CONTINUOUSLY AT 3 cm sec^{-1}. SETTLED RAW SEWAGE, SUPPLEMENTED WITH 100 ppm $NaHCO_3$, WAS USED AS SUBSTRATE.

Figures A1 through A5 in Appendix A show the pertinent species data for the eight experimental ponds inoculated with WO. (Successive ponds were inoculated from "product" of earlier ponds). The detention times and recycling fractions are different from one pond to another and also within a given experiment due to fluctuations of sewage and climate. Although WO, 1, 2, 3, 6, 9, and 10 started off greater than 75% Oscillatoria and WO 4 and 5 about 50% WO, all ponds except W6 ended up dominated by Micractinium. Detectable proportions (a few percent) of the colonial green algae appeared one day prior to the last bicarbonate additions. The sudden removal (the ponds were on 3 or 4 day detention times) of bicarbonate may well have had deleterious affect on the Oscillatoria if it was "adapted" to the bicarbonate media. This is entirely speculation since the role of bicarbonate has not been satisfactorily elucidated.

The Oscillatoria from Woodland grew on Richmond sewage, but not at a fast enough rate (see Table 9). When in head to head competition with the as harvestable Micractinium, the latter's faster specific growth rate (on sewage) led to its predominance. For example, this competition was followed in two parallel ponds with four-day detention times with one (W9) not recycled and the other recycled (WO 10). The only difference between the ponds was that the recycled pond exhibited a slower decline of Oscillatoria coupled to a slower takeover of Micratinium. Ponds WO1-5 also showed a takeover by Micratinium which usually took 2-3 weeks.

The data thus far do not prove that recycling can be used to maintain a slower growing species in ponds. By the time a satisfactory inoculation method was developed, as well as perfecting the use of microstrainers, Micractinium appeared in the ponds. This precluded doing any controlled experiments with Oscillatoria, since without additional selection methods, Oscillatoria could not be maintained. Thus, we turn to Micractinium as the experimental algae. A controlled experiment was set up using three side-by-side ponds--one was recycled 50%, one was not recycled, and one was recycled in favor of non-harvestable biomass. Productivity and sewage treatment data were obtained from the ponds (see Section III). The species control data showed that Micractinium could outcompete all algae at this time of the year, even when the other algae were selected for mechanically. Table 9 show

TABLE 9

GROWTH RATES OF ALGAE IN RICHMOND PONDS

Exper. No.	Algae[a]	Dates	D[b]	r[c]	μ[d]	τ[e]
WO 1	WO	7/19-7/25	.25	1.0	.08	8.6
WO 1	WO	7/26-7/30	.33	.4	.10	6.7
WO 6	WO	9/5 -9/12	.25	.5	.05	13
WO 8	Unicel.	9/5 -9/12	.25	0	.4	2.0
WO 9	WO	9/28-10/4	.25	0	.13	5.2
WO 9	WO	9/24-10/8	.25	0	.04	17
WO 9	Mic.	9/28-10/4	.25	0	.45	1.6
WO 9	Mic.	9/24-10/8	.25	0	.45	1.6
WO10	WO	9/28-10/4	.25	.67	.08	8.7
WO10	WO	9/24-10/8	.25	.67	.02	30
WO10	Mic.	9/28-10/4	.25	.67	.3	2.4
WO10	Mic.	9/24-10/8	.25	.67	.3	2.6
M 1	Mic.	9/7-9/18	.15	-1[f]	.3	2.3
M 1	Unicel.	9/7-9/18	.15	0[f]	.14	4.8
M 2	Mic.	9/7-9/19	.25	.5	.13	5.1
M C	Mic.	9/7-9/19	.25	0	.26	2.6

[a] WO is an Oscillatoria species isolated from Woodland; Mic. is a Micractinium species appearing spontaneously in the ponds.

This pond was recycled in favor of non-harvestable biomass.

[b] Dilution rate in reciprical days.

[c] r = recycle fraction. The recycle fraction used is equal to the fraction of the total harvested algae recycled to the pond. The assumption here is that all harvestable algae are 100% harvestable. This is true to within 10-15%.

[d] μ is calculated by integrating the equation $\frac{dx^a}{dt}\frac{1}{x^a} = \mu^a - (1 - r^a)D$ assuming μ^a is constant; thus, μ is an average specific growth rate and the calculations are approximate. (See Appendix III).

[e] $\tau = \frac{.69}{\mu}$, the approximate average doubling time.

specific growth rates of Micractinium in these ponds. The ponds showed only minor differences in species composition and harvestability (Fig. 24-29). The anti-recycle pond showed some trend at the end of the experiment towards lower percentage of Micractinium and also lower harvestability.

During the last month of experimentation, the effort was concentrated on eliminating Micractinium from non-recycled ponds. Two pairs of ponds were run consecutively, one pond of each pair was recycled, one wasn't. The first set was run at a four-day detention time, the second at a three-day detention time. Fig. 36 shows the results. At θ = 4.0, Fig. 36a, the only discernable difference is that Micractinium rose faster in the recycle pond. However, at θ = 3.0, Fig. 36b, Micractinium only became predominant in the recycle pond. Both of the results verify that mechanical enrichment supplements specific growth rates. The proportion of Micractinium is shown to decrease in S.C.4 towards the end of the run. In fact, this was the last experiment where Micractinium was found to predominate to any extent. The colonies were breaking up, presumably due to climatic changes. This points out one of the problems with mechanical enrichment of colonial (and also filamentous algae). Uncontrollable and undetermined changes can lead to a morphological change which diminishes harvestability.

LABORATORY EXPERIMENTS

Species control through selective biomass recycling was demonstrated in controlled laboratory chemostats using WPI (the Chlorella-like green algae isolated from the Woodland oxidation ponds) and Spirulina geitheri. The experiments were run in 3.3 liter vessels (30.0 cm liquid height, 12.5 cm outside diameter) stirred magnetically from beneath. Cultures were sparged with a humidified air-carbon dioxide mixture equal to 99.7% air/.3 carbon dioxide. Lighting was provided by two T12F96 UHO "Vita-Lite" fluorescent lamps placed eight inches from one side of the culture vessels. These lamps closely resemble the solar spectrum from the near UV to the near infra-red. The maximum intensity at the outside of the culture vessels was about 2.0×10^2 ergs/cm^2/sec. The pH and temperature were adjusted so that both algal strains had a high maximum specific growth rate. The pH was maintained at 8.5 $\pm$ 1.5 pH units using 25 mM $NaHCO_3$

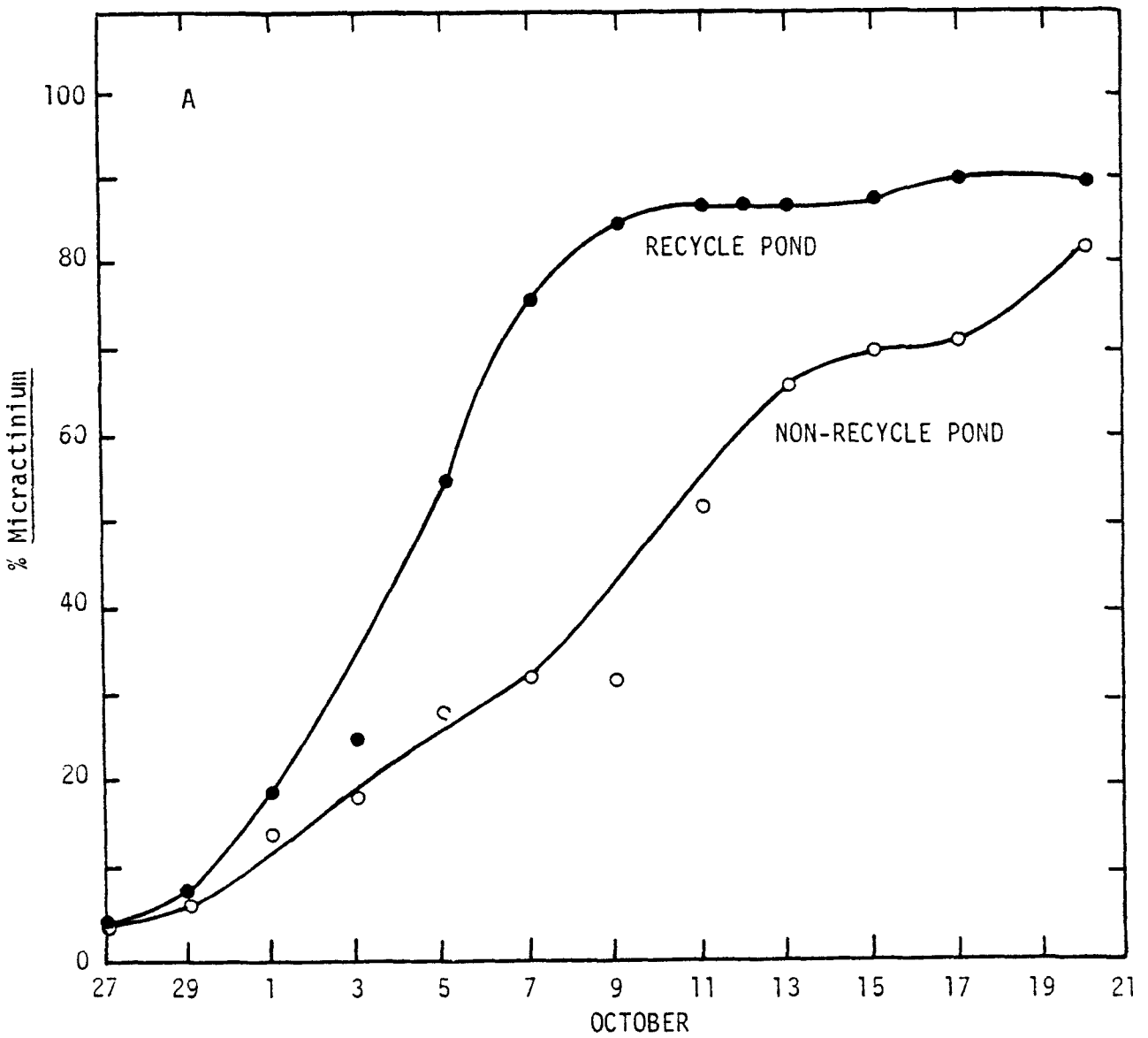


FIGURE 36a. SPECIES CONTROL THROUGH RECYCLING IN PONDS
Detention time = 4 days, Recycle = 50-60%

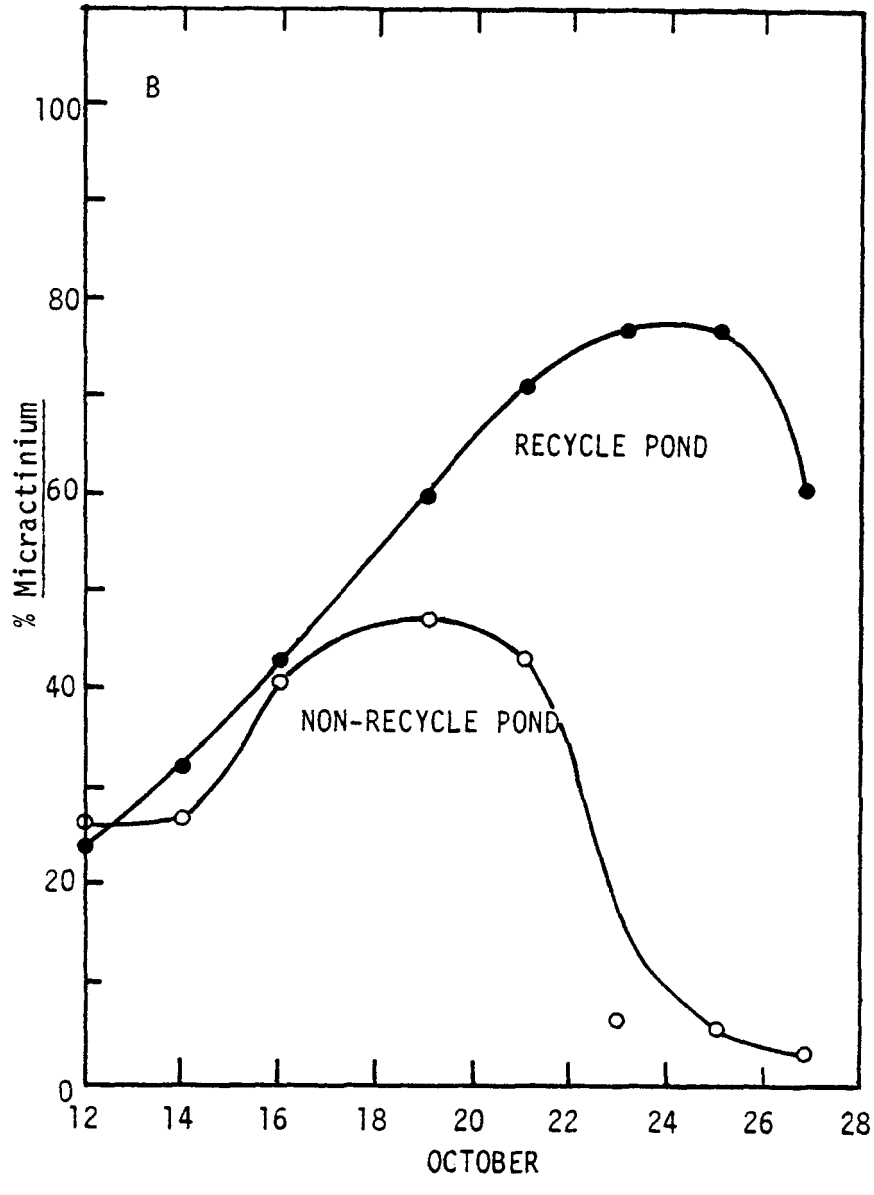


FIGURE 36b. SPECIES CONTROL THROUGH RECYCLING IN PONDS
Detention time = 3 days, Recycle = 50-67%

and the .3 carbon dioxide in the gas phase. The culture temperature was maintained constant by controlling the room temperature at 25.5 ± .5°C. The room has the capability of being sterilized, but was only kept "clean" for these experiments since both algal strains are bacterially contaminated. By using inorganic media, this bacterial contamination was kept to below .5% of the total biomass (microscopic observation).

The selective separation was achieved by straining (screen mesh 26 μm) The Spirulina is greater than 90% harvestable and the WPI is close to 0% harvestable. A fraction of the harvested biomass was recycled.

The following data were taken daily on steady-state cultures: temperature, pH, Klett, dilution rate (and thus detention time), optical density at 750 nm, dry weight, chlorophyl a and b, and protein content. For non-steady-states, we measured temperature, pH, Klett, dilution rate and species composition. This last measurement was done by cell count of the WPI in mixed cultures. The reproducibility of this technique is about ± 10% at best. Table 10 shows the averaged values obtained for the above measurements with the total range of variability in parenthesis. This table also shows the dynamics of species composition for two mixed cultures; one recycled, one non-recycled, run at about the same detention time. The faster growing WPI outcompeted the harvestable Spirulina in the chemostat without recycling. The Spirulina became dominant in the recycled chemostat.

Species composition right after mixing is very accurately determined by mixing known volumes and known densities of each culture. The additivity of density was previously checked. The final predominance of one or the other species was corrobrated by viewing samples under the microscope. The minority algae were extremely difficult to find in each case, indicating less than 1% present. Limitations of the experimental set-up and algal counting precluded more detailed calculations of, for instance, the specific growth rates and the minimum recycling necessary to achieve dominance of the Spirulina. The algal counts were not reliable except as a measure of a general trend over a fairly long period of time. There is no doubt that the cultures became unialgal, but the daily progression based on counts could not be relied upon. In the future, species composition will be determined not only by count but also by phycocyanin content and careful harvestability measurements.

TABLE 10

CHEMOSTAT CULTURES DEMONSTRATING SPECIES CONTROL THROUGH RECYCLING

	WPI	*Spirulina geitleri*	Mixed[c] WPI	Mixed[c] *Spirulina geitleri*
State	Steady	Steady	Non-4 Steady	Non-4 Steady
# of days averaged	5	6	11	11
Apparent[a] Recycling Fraction	0	.26 (11%)	0	.26 (20%)
Temperature	26.8(.5)	27.0(.4)	26.7(.8)	26.9(.8)
pH	8.58(.11)	8.54(.06)	8.57(.20)	8.54(.16)
Klett	215.1(5.6%)	222.0(9%)	211.6(13%)	224.6(15%)
Dilution Rate: hr^{-1}	.0312(8%)	.0335(6%)	.0321(9%)	.0336(9%)
Detention Time: hr.	32.1(8%)	29.8(6%)	31.2(9%)	29.8(9%)
Optical Density-750nm	1.57(19%)	.87(10%)	———	———
Dry Weight mg/l	241.1(13%)	302.8(3%)	———	———
Chlorophyll *a*. µg/ml	9.61(13%)	5.40(16%)	———	———
Chlorophyll *b*[b] µg/ml	3.72(13%)	.23(45%)	———	———
Protein mg/l	88 (46%)	172 (10%)	———	———
% WPI	99	.3	75 → 100	25 → 0

Numbers in parenthesis indicate the total range of variation of the measurements, in percentage of average value (except for temperature and pH which are in °C and pH units respectively). Thus 26.8(5)°C means that the highest and lowest temperature reading differed by .5 °C and that the average of all measurements was 26.8°C.

[a] See text.

[b] The polychromatic chlorophyll estimation used is an empirically derived set of simultaneous equations. It is usual to calculate a small (erroneous) positive or negative value of chlorophyll *b* in blue-green algal extracts using any of these sets of equations.

[c] The individual WPI and *Spirulina* cultures were mixed, yielding one nonrecycled mixed culture (Mixed WPI) and one recycle mixed culture (mixed *Spirulina geitleri*).

[d] The mixed cultures are non-steady in terms of species composition (and thus dry weight, C.D., chlorophyll, protein), but steady with respect to temperature, pH, Klett, and detention time.

Only the blue-green algae contain phycocyanin and its extraction can be made quite quantative. Preliminary results indicate a precision of better than ± 5%.

Another problem involved the chemostat overflow tube. The conventional overflow tube used in these experiments is itself selective. Filamentous organisms are preferentially retained in the vessel (presumably due to water surface tension). Thus, the effluent from a monoculture of WPI is the same density as the culture, but the effluent of a Spirulina culture is significant less dense than the culture itself. This effect has been observed by Dutch workers studying Oscillatoria. The difference in our experiments was 15 35% and was density dependent. For the experiment shown in Table 10, the difference was 18-27%. Thus, the true recycling was .45-.55 whereas the appar recycling fraction was .26. To avoid this problem in future experiments, we h devised a new effluent tube.

The experiments described in this section unequivocally demenstrate the validity of the theory of species control through selective recycle. With the improvements mentioned above, recycling under controlled laboratory conditions provides a long-sought-after methodology for studying species interactions, that is, the proper choice of recycling fraction can result in a steady-state with two species coexisting, as shown in the following section on turbidostats Until now, it has been difficult to achieve steady-state of mixed cultures in laboratory. One of the species inevitably becomes predominant.

TURBIDOSTATIC CULTURES

In Section II on species control theory, the turbidostat was discussed as an alternative way of operating ponds. Development of a turbidostatic syst in the laboratory was undertaken as preliminary to any such set-up on the pon

Initially, turbidostats were loaned to the Algae Research Project by Dr. J.A. Bassham of the LBL Biodynamics Laboratory. After a total modificatio of the sampling system, these optical density regulators successfully held cul ture densities within ± 5%, but they required continual maintanance to keep them operating. Only two of about ten runs were successful.

Table 11 shows data for the two steady-states achieved. The steady-

states were run consecutively with the turbidostat set for the same density. With 70% recycling a pure (>95%) culture of Spirulina was maintained at a density of 179 klett units, a dilution rate of .065 hr^{-1} (μ can be calculated from $\mu = AD$ where A = 1-recycling fraction). This culture was mixed with a unialgal culture of WPI (70% Spirulina, 30% WPI) of the same density, with $D = \mu = .035\ hr^{-1}$. After mixing, the percent of Spirulina recycled was reduced to 30% of the total algal density. This figure was calculated to give the Spirulina a supplemented specific growth rate of .035 hr^{-1}. With this supplemented rate equal to the unsupplemented specific growth rate of the WPI, the two algae should coexist in a steady-state at approximately the same ratio to which they were mixed. This, in fact, occurred, requiring only a one-day transition period for the turbidostat to adjust from $D = .065\ hr^{-1}$ to $D = .035\ hr^{-1}$.

A survey of the available literature produced several designs that were reported to be workable for short periods of time (they were used primarily on bacterial systems) with small deviations. A new design was developed by project and LBL personnel (Figure 37) that was assembled and tested in the laboratory. The results shown in Table 11 indicate extremely good control. However, this design proved unreliable over a period of two weeks. The problem was in the electronic stability since the sampling procedure used--modeled after a procedure described in the literature (52)--overcomes the standard problem of wall growth. It is a flush-and-fill system where the sample tube is flushed out with an air pump for all but the few seconds during which sampling occurs. At this point, a system is being developed that incorporates the precision of regulation of previous designs into a reliable system.

TABLE 11

TURBIDOSTATIC CULTURES

Turbidostat	LBL	LBL	ARP
Culture	*Spirulina geitleri*	*Spirulina geitleri* + WPI	*Spirulina geitleri*
Days	3	3	5
Temperature ^{o}C	24.2(1.6)	23.7(1.6)	24.0(4.0)
pH	8.45(.1)	8.50(.15)	8.60(.1)
Klett	179.0(10)	171.5(4)	173.5(1)
Dry wt. mg/l	305(4)	277(15)	———
Dilution rate hr^{-1}	.065(4)	.035(6)	.055(25)
μ[a]	.020(5)	*Spirulina*:020(7) WPI: .035(7)	.029(30)
Recycle Fraction[b]	.70(4)	.30(4)	.46(4)
% *Spirulina* (by cell count)	>95	69.1(6)	>99
State	Steady	Steady	Constant Density

[a] $\mu \cong$ (1-recycling fraction) D

[b] Due to the larger volumes of liquid flowing through a turbidostat when it is in the dilute cycle, the discrepancy between culture density and effluent density was constant and only 10%. This adjustment for this internal recycling is included in the values reported.

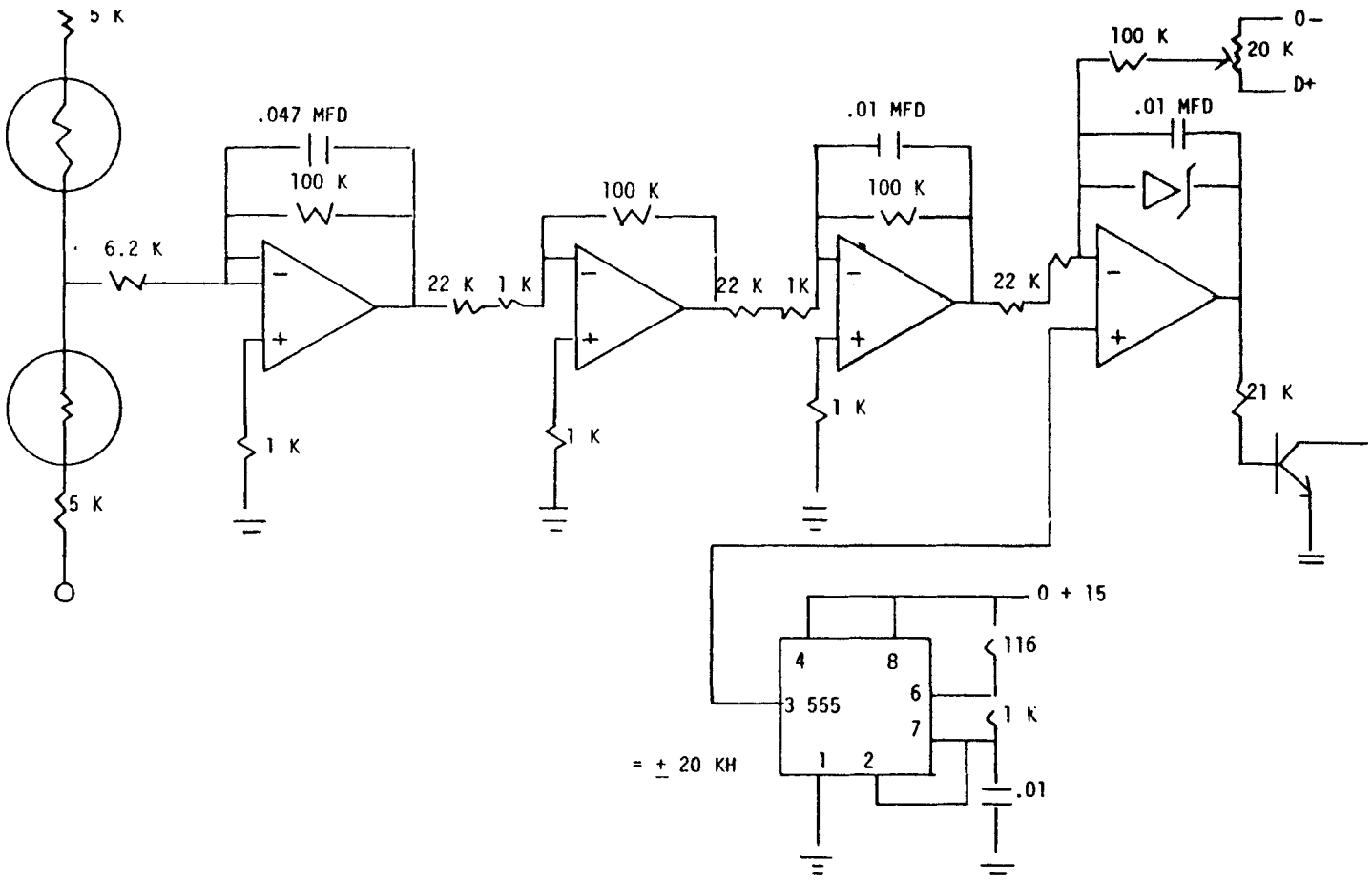


FIGURE 37. DESIGN OF A TURBIDOSTAT

V. CULTIVATION OF Spirulina

INTRODUCTION

One problem revealed by the work reported in Section III and IV is the breakup of Oscillatoria filaments or Micractinium colonies. Methods must be developed to prevent or mitigate the duration of such occurrences. This will require investigation of both the causes and mechanism of breakup. Another approach is to select a morphological shape that lends itself particularly well to microstraining, even if size is relatively small. The large corkscrew spirals of the filamentous blue-green algae Spirulina are particularly amenable to removal by straining processes. The straight filaments of Oscillatoria have a tendency to slip through the openings in the fabric, resulting in loss of significant numbers of filaments not long enou to bridge the fabric openings. Spirulina exhibited much better harvestability as a function of filament length.

Recently, it was shown that Spirulina could be cultivated on secondary effluents fortified with bicarbonate where it reduced significantly the concentrations of nitrates and phosphates (53). Spirulina has been shown to be one of the richest protein sources known (54-56). These algae also show potential in the area of animal waste treatment, especially in conjunction with cattle feedlots and poultry operations where it could be used as a feed supplement (57). Spirulina were shown to be a particularly good substrate for methane fermentations in work recently completed at this laboratory (18). Thus, Spirulina is a prime candidate for cultivation in algal biomass production systems. The absence of sufficient information about the growth requirements of Spirulina led us to study the nutritional needs of these algae in batch laboratory cultures. We also were able to cultivate Spirulina in small outdoor ponds on both primary and secondary sewage.

BATCH CULTURE EXPERIMENTS

Literature Review

Spirulina is a filamentous blue-green alga of the family Oscillatoracea.

Spirulina are usually found in saline waters with low hydrogen ion ncentrations. The best known and most widely distributed species, S. atensis is most common to tropical or subtropical climates but is also und in temperate areas. S. geitieri, the major organisms used in this udy, has been reported in only two sites--the evaporation basins of Sosa xcoco near Mexico City and San Francisco Bay (58-59). The natural brines of Sosa xcoco are composed mainly of Na^+ and K^+ carbonates, bicarbonates and lorides. These salts are concentrated by solar evaporation as they pro- ess through a large (4 km) spiral basin. Spirulina begin to appear when e salts reach 60 g/l and develop very rapidly when salinities of 120 g/l e reached. Densities decline and the population finally dies as evapora- on continues beyond this point. Analyses of the zones most favorable to owth show Na^+/K^+ ratios of 18:1, carbonates/Cl^- ratios of .7/1 and low lfate levels.(60)

Analyses of some natural lakes near Lake Tchad where S. platensis e dominant among the phytoplankton show considerably higher carbonate/ oride ratios (up to 80:1 in the most productive lakes) than the Lake coco waters. Na^+/K^+ ratios ranged between 3:1 and 30:1 and sulfate levels ched values of 1.5 eq/L. Although present in waters ranging in salinity m 8.5 g/l to 260 g/l, S. platensis reach the highest densities in lakes h salinities between 30 and 60 g/l. Spirulina were also found in tem- ary ponds of low salinity which form in the rainy season but the densities e lower with other photosynthetic organisms tending to dominate.(61)

Considerable attention in recent years has been devoted to the influence environmental factors on the productivity of Spirulina, including light ensity and temperature, on growth (62,63). The influence of several com- ents in the media on amino acid composition of Spirulina and the mineral position of S. platensis and S. geitleri was studied extensively (64,65 . Zarrouck (67) in a study of the chemical and physical conditions for imal growth of S. maxima, formulated a media which is used by most in- tigators for the cultivation of these algae. He found S. maxima to be rmophilic and salt tolerant and capable of growing in salinities between nd 56 g/l. Bicarbonate was essential for growth with optimum levels ging between 21 and 42 g/l. Maximum growth was obtained with nitrate

as the nitrogen source, although nitrite, ammonia and urea also supported growth but at much lower rates. The high levels of bicarbonate and sulfate, however, make this media unsuitable for mass cultivation. To evaluate the feasibility of using Spirulina for sewage treatment or biomass production, it was deemed necessary to determine the minimal nutrient requirements and growth characteristics in sewage effluents.

Material and Methods

Two unialgal strains of Spirulina were used--S. geitleri and a Spirulina strain collected from a Texas drainage ditch receiving oxidation pond effluents which we refer to as S. texas. The culture was almost unialgal when obtained and quickly lost its contaminating unicellular green algae with several transfers in Zarrouck's media. They differ in shape from S. geitleri in that they exhibit tigher spirals of larger diameter and have larger cells. One peculiarity is their unusually great propensity to form gas vacuoles. When not agitated or at high cell densities, they rise rapidly, forming a dense mat on the surface.

The experimental cultures were grown in 250 ml Erlenmeyer flasks containing 100 ml of media on a uniformly illuminated (5×10^4 ergs/cm^2/sec) reciprocating shaker table. A 12-hour alternating light-dark regime was used except where noted. Air temperature varied between 79°F in the light period to 65°F in the dark.

Growth was followed on a daily basis by measuring through a side-arm tube the optical density of the cell suspension using a Klett-Summerson colorimeter with a red (#66) filter. Optical density readings were correlated with dry weight by concentration of an exponentially growing culture and preparation of a series of dilutions. Each dilution was filtered on a tared filter and dried to constant weight at 80°C. The conversion factor between dry weight and Klett values was 1.66 µg/ml/Klett unit.

Inoculum was prepared from exponentially growing cells by filtration through a fine mesh nylon screen. The cells were then washed several times with a dilute saline solution to remove most of the media salts. The experimental cultures were usually carried through two consecutive transfers

to eliminate problems of nutrient carryover, storage materials and adaptation. The mean doubling time G in the exponential phase was calculated according to

$$G = \frac{0.301}{k}$$

$$\text{and} \qquad k = \frac{\log_{10}N - \log_{10}N_o}{t}$$

Results

A typical growth curve for S. geitleri grown in batch culture in synthetic ıedia is shown in Figure 38. After a short lag period, growth proceeds :xponentially until Klett values of approximately 200 are reached. Growth :hen increases linearly with maximum cell densities obtained in 14 days. ,ight limitations due to self-shading at high cell densities are a likely ause of the decline of exponential growth. pH values of 10 to 10.5 typically btained at this point may contribute to reduced growth rates. Kosaric et al.(53) ave shown pH 9.5 to be optimum for growth of S. maxima with growth reduced y 50% at pH 10.5. The high pH resulting from algal growth can also affect he solubility and, hence, availability of other nutrients. Iron, as well s trace metals, are only slightly soluble at high pH. At pH 10, a visible recipitate forms in Zarrouck's media which x-ray fluorescence analysis showed o contain calcium and phosphorus in a ratio of 3:2.

Zarrouck's media contains 0.22M $NaHCO_3$ as the major salt. In the batch ılturing system, reduction of $NaHCO_3$ (with equivalent NaCl substitution to aintain ionic strength) had little effect on initial growth rates of S. eitleri. However, cultures with low bicarbonate levels (0 to 0.06M) ex- ibited a rapid pH rise (to 11.5) correlated with cessation of growth. In ıe batch culturing system, the optimum bicarbonate level, in terms of growth ıte and yield, was 0.1M. Bicarbonate can, however, be completely replaced :th chloride when the pH is controlled between 9 and 10 by vigorous aeration. ıder these conditions, growth rates and yields are similar to those observed ı the presence of normal bicarbonate concentrations.

The effect of nutrient levels on the growth of S. geitleri was studied varying the salt concentrations from the levels of Zarrouck's media.

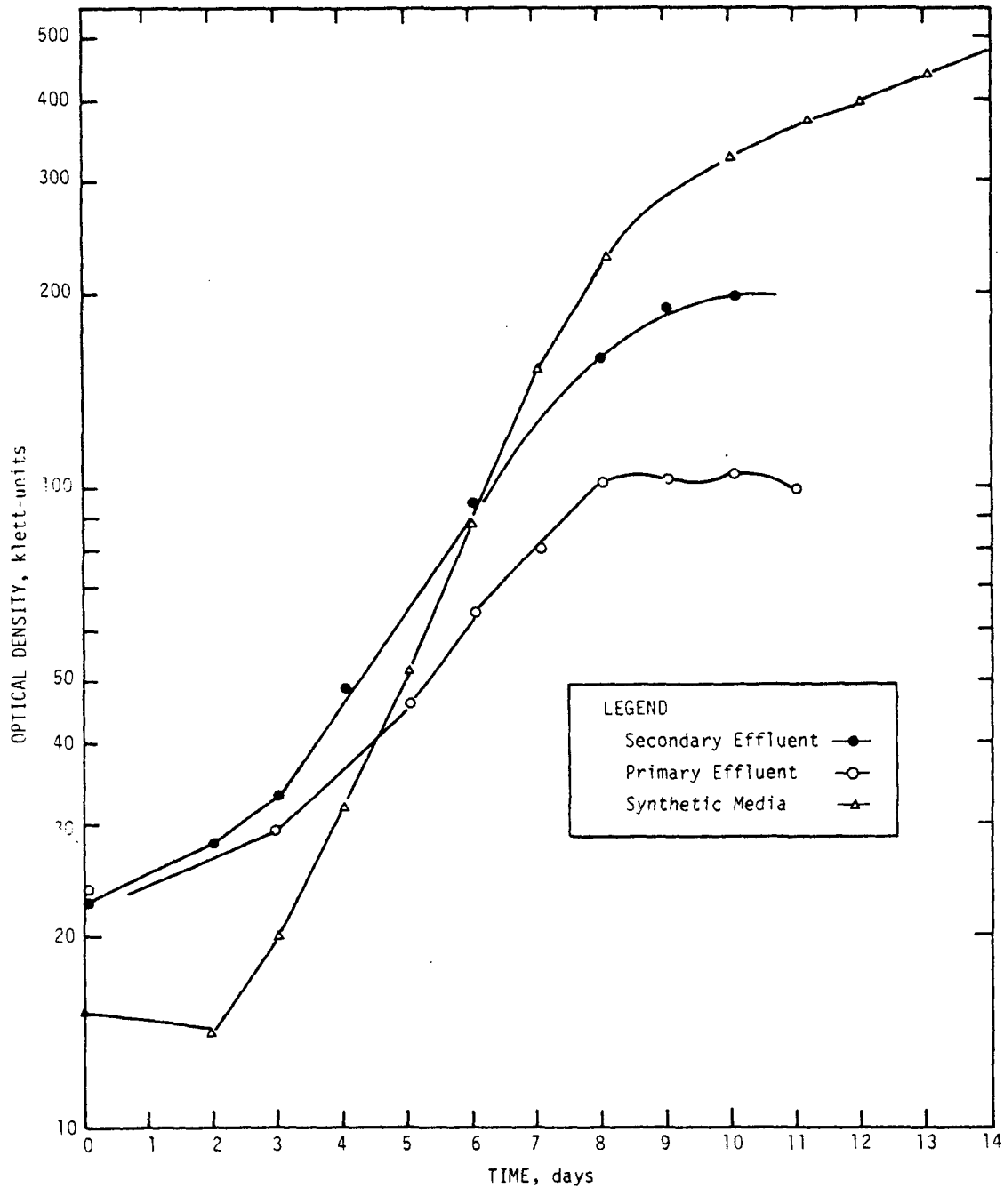


FIGURE 38. GROWTH OF S. geitleri ON SYNTHETIC MEDIA (Table 12 #1) AND SEWAGE EFFLUENTS OBTAINED FROM THE FACULTATIVE POND (10) AND 2° EFFLUENT FROM AN ACTIVATED SLUDGE UNIT. The effluents were fortified with 0.1 M HCO_3^- .

Table 12 describes various modifications of Zarrouck's media and gives the mean doubling time, G, and yield in mg/l (dry weight) after 11 days growth. Although normally found in highly saline waters, S. geiterli were found to be tolerant of a wide range of ionic strength. In Expts. 1 through 4, the salt concentration was reduced ten-fold without varying the macronutrients nitrogen and phosphate or iron, magnesium, and trace methals. Although there was little effect on growth rate, the yields were reduced at lower salt concentrations and this correlated with pH values above 11.5. Using similar culture techniques, Zarrouck observed maximum growth (G = 40 hours) of S. maxima in salinities ranging from 22 to 56 g/l. He reported that growth was reduced by 30% at salinities of 86 g/l and by 35% when salts were lowered to 5 g/l. When bicarbonate concentrations were decreased to 0.022M, the pH increased to almost 12 by the eleventh day of growth. This reflects the buffering capacity of the media due to bicarbonate, the major salt. The levels of the macronutrients, nitrogen, and phosphate could also be reduced from those of Zarrouck's media (Table 12). Reduction of nitrate by 75% had little effect on initial growth rates or final yield. On the other hand, doubling the nitrate level (to 25 mM) did not affect growth rates markedly but the algae appeared more blue in color, suggesting a higher phycocyanin content. A 75% decrease in K_2HPO_4 enhanced the growth rate and increased the yield by 30%. Reducing the iron, trace metal or $MgSO_4$ concentrations by 50% resulted in a slight (25%) reduction in growth rate but made little difference in yield.

The optimum levels of $CaCl_2$ were determined. Deletion of Ca, an essential nutrient in higher plants and many algae, caused the filaments to clump together after a few days of growth and accurate cell density readings could not be obtained. Maximum growth was obtained at 0.4 mM but $CaCl_2$ concentrations between 0.1 and 1 mM had little affect on growth. Spirulina are often found in waters high in sulfates. However, varying SO_4^{2-} to Cl^- ratios (between 30:1 and 1:30) had no affect on growth rates. Na^+/K^+ ratios between 195:1 and 14:1 (the optimum found by Zarrouck) had little influence on growth; however, at a ratio of 1:10 growth and yields were considerably reduced.

Batch culture experiments were also carried out to assess the feasibility of using seawater as a component in Spirulina growth media. Seawater is

TABLE 12

EFFECT OF SALTS AND NUTRIENTS CONCENTRATION ON THE GROWTH OF *Spirulina geitleri* IN BATCH CULTURES

	G (hrs)	Yield (mg/l)
Standard Media*	34.3	689
2 x $PO_4^{=}$ 2 x N	34.9	664
0.5 x $PO_4^{=}$	34.5	846
0.5 x Fe	40.8	747
0.5 x Mg	42.2	689
0.5 x N	39.6	647
2 x salts	40.0	564
0.5 x salts	36.2	474
0.2 x salts	38.8	433

*The Standard Media contained the following salts: 110 mM $NaHCO_3$, 2.85 mM K_2SO_4, 8.5 mM NaCl, 0.18 mM CaO_2, and nutrients: 13.25 mM $NaNO_3$, 3.6 mM K_2HPO_4, 0.036 mM Fe/EDTA and 2 ml of Allen and Armon trace elements.

deficient in several essential nutrients which necessitated supplementation. The addition of iron, nitrate, phosphate and bicarbonate resulted in growth rates approximating those in synthetic media but tended to decline sooner in seawater. Optimal growth was achieved at about 1/10 strength seawater but dilutions from full strength to 1/40 strength were tolerated. Bicarbonate supplementation was required to obtain high yields but, as was the case with synthetic media, this requirement could be eliminated with proper aeration. The requirement for additional phosphate was absolute (Fig. 39). The high calcium and magnesium levels in seawater, however, allowed only low concentrations (100 mg/l) to be used. Higher phosphate concentrations resulted in the immediate formation of a thick white precipitate. Iron supplementation was also necessary to achieve maximum growth. There was no stimulatory effect of additional potassium, sulfate or trace metals.

The growth characteristics of Spirulina on primary and secondary sewage effluents were tested in batch culture. Bicarbonate supplementation was required for satisfactory growth on secondary effluents, but could be eliminated if the cultures were aerated. With the addition of 0.1 M bicarbonate (the optimum concentration in synthetic media), doubling times of 43 hours for S. texas and S. geitleri were observed with a yield of 500 mg/l dry weight after 12 days. In synthetic media, both strains divide every 33 hours with a yield of about 1 g/l (Fig. 38). To determine if the lowered growth response was due to nutrient deficiencies in 2° effluents, the stimulatory effect of additional nutrients was studied. Fe/EDTA, $MgSO_4$, K_2SO_4, $CaCl_2$ and trace metals were added singularly and in combination. Only iron enhanced growth. The addition of nitrate (2.5 mM) when growth began to decline markedly resulted in the recovery of growth. The growth response in 1° effluents varied considerably between different effluent samples and additions of bicarbonate and nitrate exhibited no consistent pattern. The average growth rate, however, was somewhat slower than those observed in secondary effluents (Fig. 38). Furthermore, the length of the exponential growth phase and final yields were considerably decreased. The duration of the light period also influenced the growth of Spirulina. A comparison of growth of S. geitleri under continuous and 12:12 light regime showed that the growth rate in inorganic media decreased by 40% with a dark period while in primary effluents growth rates were decreased by only about 10%.

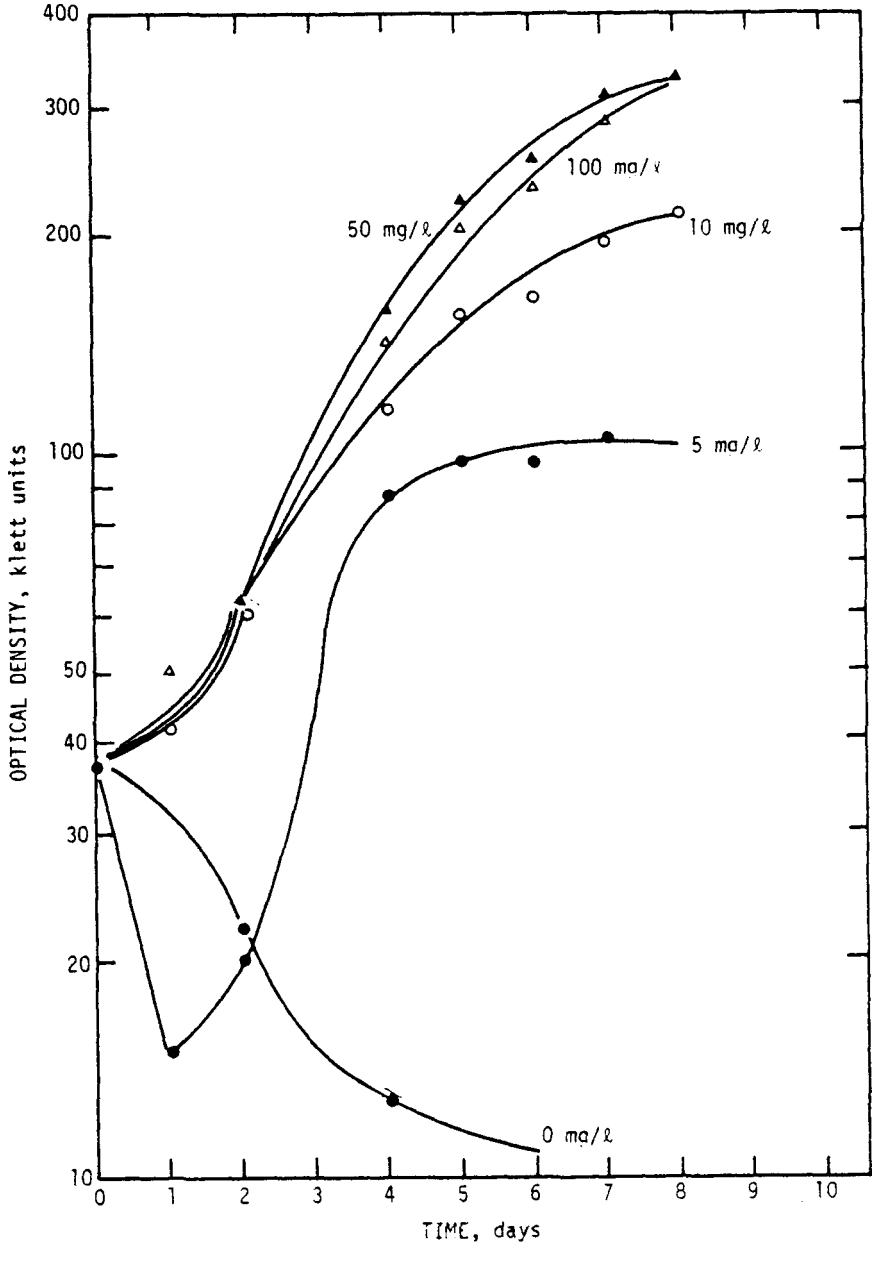


FIGURE 39. THE EFFECTS OF PHOSPHATE CONCENTRATION ON THE GROWTH OF S. geitleri IN 1/2 STRENGTH SEAWATER SUPPLEMENTED WITH $NaNO_3$ (26.5 mM), and $NaHCO_3$ (110 mM)

DISCUSSION

These experiments show that Spirulina are tolerant to wide ranges in composition and levels of mineral nutrients. Of major importance in terms of their use in sewage treatment is the finding that the high levels of bicarbonates and salinity, which characterize their natural habitat, are not essential requirements for the normal growth of S. geitleri. Indeed, growth was enhanced with lower salt levels (Table 12) and bicarbonate had no stimulatory affect if carbon dioxide was supplied at a sufficient rate by vigorous aeration. Zarrouck (67) found maximum growth of S. maxima (which is also described as S. platensis) in salinities between 22 and 56 g/l. Growth was inhibited above and below these levels and arrested at salinities below 5 g/l. Our results indicate that S. geitleri and the Spirulina isolated from a Texas drainage ditch are less sensitive to very low ionic strengths and maximum growth occurs at lower salinities. S. geitleri differs from the better known African species S. platensis in morphological features, but it is not known if physiological differences also exist.

Busson (60), in seeking to establish the taxonomic relationship between the Mexican Spirulina and the S. geitleri once reported in the San Francisco Bay (59), demonstrated growth of S. geitleri in media resembling Pacific Ocean seawater in composition. Our results show that with nitrate, phosphate and iron additions, S. geitleri grow well (at rates comparable to those in synthetic media) in natural seawater. Yields, however, were reduced considerably as compared with artificial media, in batch cultures where the pH soon rose above 10.5. At high pH, nutrient solubility is a particular problem in seawater because of the high Ca^{+2} and Mg^{+2} levels. The affinity of phosphate for these cations allowed for only low concentrations (10-100 mg/l) to be used. The use of low phosphate levels had no effect on growth rates but decreased final yields. Fauchner (68) reduced the calcium and magnesium levels in seawater by pretreatment with high levels of sodium bicarbonate. The supernatant, supplemented with normal amounts of phosphate, nitrate and iron, gave results similar to Zarrouck's media. However, pH control by carbon dioxide enrichment would minimize these solubility problems and eliminate the high cost of bicarbonate additions. This is the preferred approach that should be followed in large-scale algal biomass production in seawater media.

Optimal growth of S. geitleri was obtained at concentrations about 1/10 normal seawater strength (when supplemented with .1M sodium bicarbonate). Although growth rates are reduced at higher salinities, full strength seawater may prove advantageous in large-scale biomass production of this species of algae by inhibiting the growth of faster growing unicellular algae and thus effecting species control. (See also Section IV).

Growth response in primary sewage effluents was inconsistent but generally much lower than in secondary effluents. Nitrate additions, in some cases, stimulated growth in primary effluents which contain nitrogen in reduced form. Zarrouck (67) showed that ammonia and urea supported Spirulina growth but at much lower rates than nitrate. The inconsistency of growth on primary sewage effluents may be due to action of nitrifying bacteria during the course of experiments. Faster growth rates and higher yields were obtained in secondary effluents although the growth response was lower than in synthetic media. That available nitrogen became limiting after a week was suggested by the recovery of growth after nitrate additions. Nguyen et al. (69) also found nitrogen to limit Spirulina growth on secondary effluents.

On the basis of our studies, Spirulina should not be considered a physiologically distinct type of algae; the morphological differences between Spirulina species and other common Oscillatoreacea are merely a matter of cell wall and junction phenomena. Thus, for the primary purpose of species control for harvestability, selection of Spirulina for large-scale biomass production systems should be both desirable and possible. Spirulina and other blue-green algae would have species dictated differences which make them useable for freshwater, brackish and marine systems, as well as primary and secondary effluents.

OUTDOOR POND EXPERIMENTS

Several attempts were made to grow Spirulina in outdoor ponds using sewage-based media. These experiments were moderately successful in that Spirulina was grown and harvested outdoors in Richmond on primary treated sewage with a minimum of nutrient additions (Figure 40).

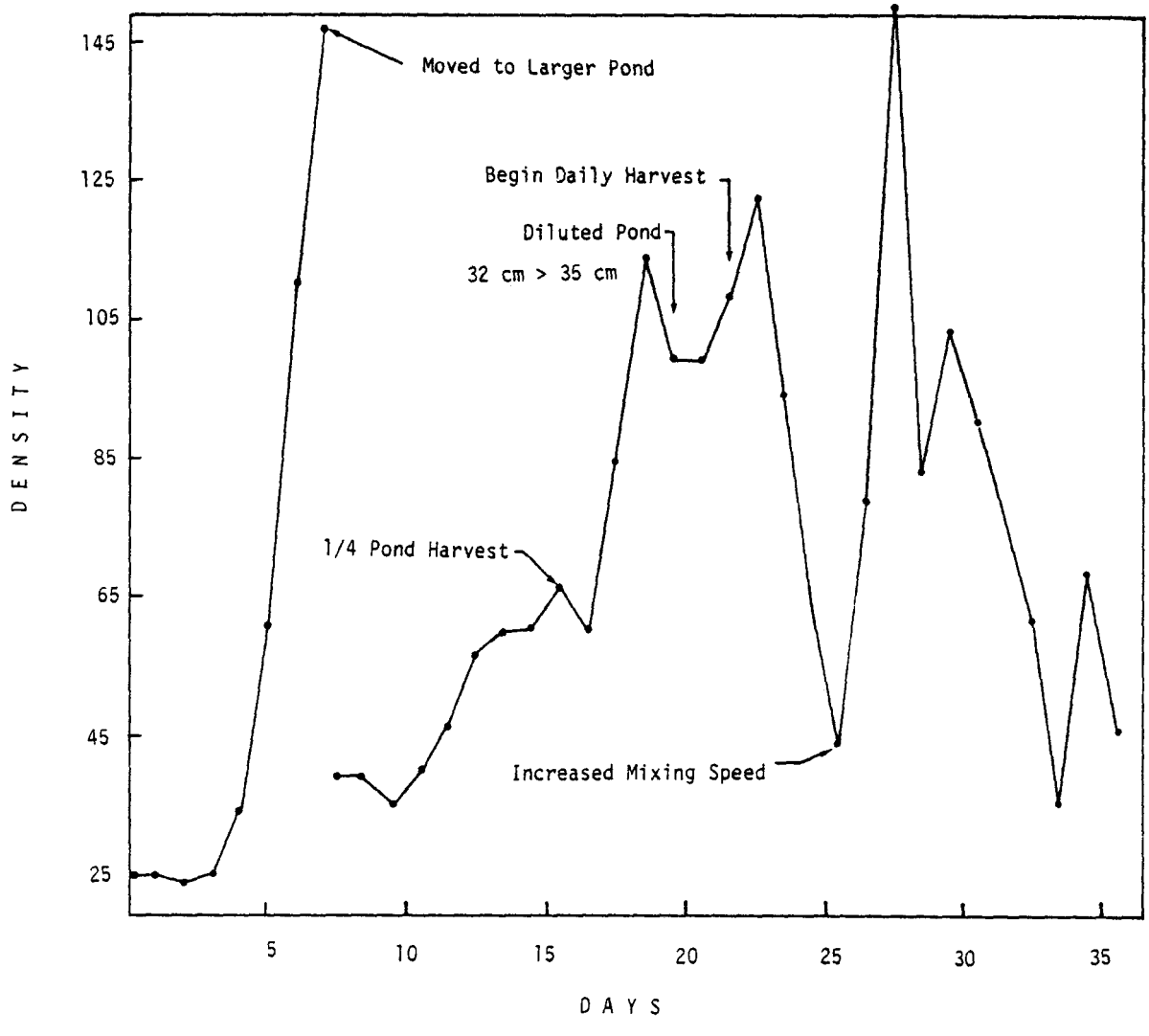


FIGURE 40. OUTDOOR CULTIVATION OF Spirulina

The first inoculum was grown in a small (.5 m^2) plastic box with a volume of 160 liters and a depth of 30 centimeters. This was filled with a medium consisting of settled sewage diluted to half strength with tap water and spiked with .5 g/liter $NaNO_3$ and 2 mg/liter $FeSO_4$ · EDTA. This growth unit was inoculated with about 10-15 mg/liter of laboratory-grown Spirulina geitleri. The temperature was maintained around 22°C. Initial growth was very good with three doublings in the first week. At the end of this time (7 days) this culture had reached about 160 mg/liter and it was used to inoculate a larger pond in which the harvesting experiments were carried out.

This pond was a long, narrow rectangular plywood pond (2.6 m x .7 m) with an operating depth of 35 cm and a volume of 600 liters. This pond was filled with 520 liters of half-strength sewage to which was added 1 g/liter $NaHCO_3$ (640 g), .5 g/liter $NaNO_3$ (320 g), 2 mg/liter Fe · EDTA. The entire 120 liters from the smaller ponds was transferred into this pond as an inoculum. This gave a total of 640 liters at a concentration of about 30 mg/liter. This pond was aerated and heated to a constant 21°C. The pond was also shaded, with the shading being reduced as the density of the culture increased.

This batch culture also grew reasonably well, doubling almost three times in two weeks with an average of 50% shading of the pond. After 13 days, pond operation was changed from batch to a semi-continuous mode and microstraining experiments were begun. One-fourth of the culture was pumped daily out and through a microstrainer. The strained media was wasted, and the harvested algae were initially returned to the pond. Since algal removal was initially almost 100%, the pond was then refilled with settled sewage. The result was a four-day media detention time and an indefinite cell detention time. The intention was to increase pond density by adding sewage nutrients without diluting the culture. Cell density did increase slighly at first, and, after two days, it was decided to return only two-thirds of the daily harvest with one-third remaining as harvested production. At this point, the health of the culture began to degenerate. Up until this point, the culture had been a dark green, homogenous suspension. On the third day of semi-continuous operation, the algae had started to form into

very small clumps. Some larger clumps were also noticed floating at the surface. The harvest schedule was continued, clumping became severe, and considerable settling began to occur, resulting in a precipitous drop in pond density. Algal recycle was increased to 100%, but the four-day detention time was retained. Mixing speed was increased in an attempt to resuspend the algae. The increased mixing velocity suspended both the sludge and the algae from the bottom and temporarily raised the density to a very high level. It soon became obvious, however, that the algae were no longer viable, as the density continued to drop at the same rate as it had before the fast mixing. When nothing remained but grey sewage media and a bottom layer of brackish, putrifying algal sludge, the experiment was terminated.

A small (100 liters) culture was started using the algae that was not recycled in the above experiment. The medium was dilute primary treated sewage. This culture was maintained over a period of several months (August-December) with only occasional (about every ten days), small additions of settled sewage. This culture was mixed with a small submersible pump and was never heated. It reached a high density and showed no signs of clumping until attempts were made to increase the culture volume again. As soon as the dilution rate was increased (roughly from .025 day^{-1} to 0.125 day^{-1}), clumping and settling occurred, followed by rapid die-off of the algae.

In conclusion, it seems likely that Spirulina geitleri can be grown outdoors at Richmond on settled sewage if very long detention times are employed. The harvestability by microstraining is excellent (90-95%) when the algae are healthy. The algae cannot grow on 1° sewage, however, at detention times that are reasonable for a practical system.

It should be possible to decrease the detention time (thereby increasing productivity and treatment capacity). To do so would require the identification of the factors in 1° sewage that are limiting or inhibiting to the growth of Spirulina at short (less than 8 days in summer) detention times. The most likely factors are ammonia toxicity (Spirulina geitleri might prefer nitrate), low pH, inhibitory organics in fresh sewage, and lack of sufficient alkalinity or readily available carbon.

VI. ECONOMIC AND FEASIBILITY ANALYSIS

INTRODUCTION

World production of oil and natural gas is expected to peak by 1990. Increased reliance on alternative fossil fuels, primarily coal or nuclear power, is limited by the economic, environmental, and social costs of these energy sources together with their non-renewable nature. Solar energy is generally most readily adapted to providing individual or local energy requirments. Bioconversion systems based on fuel production from urban or agricultural wastes are also restricted to local markets. Solar thermal, electric, and biological energy production could provide a considerable fraction of local energy demands before the end of the Century. However, the U.S. economy will continue to rely on large-scale, centralized, energy sources. The purposeful cultivation of plant biomass for conversion into fuels has the potential of providing both local and national demands for clean fuels. A multiplicity of plants and cultivation systems are being proposed for bioconversion. It is doubtful that any single approach will provide a general solution. The different requirements for climate, land, water, fertilizer, etc. and products such as electricity, gaseous fuels, etc. of various bioconversion alternatives assures that a diversity of approaches will be more successful than a single concept based on a particular plant.

This analysis considers the feasibility of planktonic microalgal biomass production and conversion to methane and fertilizer with systems increasing in magnitude from about 1 mile^2 to over 100 miles^2. One basic limitation which presently exists in the use of microalgae in bioconversion is the lack of algal type or species specific cultivation technology. Development of this technology is the main effort of our project; the current approaches taken and preliminary results obtained are reported in previous sections. Briefly, we are attempting to cultivate filamentous blue-green or colonial green algae. Such algal types are desirable primarily because they are readily and inexpensively harvested. Microalgal harvesting is presently the key technical and economic limitation in developing bioconversion systems based on microscopic algae. Our present approaches to cultivating filamentous or colonial algae involve the partial recycle of algae harvested by a size-selective harvesting method and the establishment of nitrogen-limiting conditions which favor nitrogen-fixing blue-green algae.

Besides harvesting, other reasons to grow specific microalgal types or species are the requirements for maximum productivity and maintenance of stable anaerobic fermentations. Our basic assumption is that microalgal populations can be controlled through economically permissible adjustments in operating parameters. Therefore, no cost factor is assigned to the species control technology except as represented by the necessary sampling, analysis, and management of the system. This requirement will set a lower size limit to pond systems, estimated at about one square mile. The expected data taking and analysis required for application of predictive mathematical models of algal population dynamics and productivity would involve a sophisticated laboratory and pond management operation. Other factors setting lower limits on algal pond systems are optimal scale for the biomass conversion processes as well as utilization of the products. Since only fuels (methane) and fertilizers are considered as products of the algal biomass in this analysis, their low relative unit market value requires a certain minimum scale for production systems. On the high side, algal biomass production scale is limited by land, water, nutrients, as well as management complexity. Thus, an upper limit below 1,000 mi^2 is considered likely.

It should not be thought that an algal biomass production system would need to be composed of one geometrical unit. Sample analysis and interpretation are the key elements of microalgal biomass production as envisioned by us. Therefore, large-scale systems with a centralized management and scattered non-contiguous ponds would be as acceptable an engineering design (and certainly more applicable to most geographical situations) as a square or other single perimeter system. Limitations must, of course, be faced in the number of subunit ponds that could be economically feasible for any one system. Distance, sampling, operations, product utilization, etc., all would limit the break-up of a large-scale system. Topography, suitable land availability, markets, and resources would tend to lead to an optimal subunit size and number. Obviously, these factors are very dependent on local situations; they cannot be easily subjected to a general analysis and must be evaluated for specific localities.

In addition to fuel and fertilizer production, algal biomass systems are also, simultaneously, usable for wastewater treatment and reclamation, drainage or brackish water disposal and protein or petrochemicals production. The latter will be ignored here since they conflict somewhat with energy production and are presently not yet developed. In Table 13 are summarized various types of microalgal bioconversion systems and their estimated size ranges. These systems range from oxidation ponds to nutrient integrated systems; as they increase in scale, they become economically more dependent on their outputs of methane and fertilizers. (Marine or aquaculture microalgal systems are not considered in this analysis). Each of the systems indicated in Table 13. will be discussed independently. First, however, the common features of planktonic microalgal biomass production ponds will be discussed: high-rate ponds and microalgal harvesting. Harvesting is emphasized since it is the key technical limitation of algal biomass production.

HIGH-RATE PONDS

Intensive microalgal production systems must by necessity utilize a high-rate pond design. Its basic features are shallow depth, large cells with channels, slow mixing, and short detention times. Details are variable; depth can be from a few inches to over one foot; cells and channels can be of varying size and width; mixing must generally be slow enough not to consume a significant fraction of the energy produced; and detention times would be on the order of a few days. The concept of the high-rate pond was formulated almost 20 years ago (70,71) and considerable experience exists with construction and operations of high-rate ponds, both experimental (72-76) and operational in multiacre waste treatment systems (77). The experience with the California oxidation ponds of St. Helena and Modesto is particularly noteworthy. Thus, a reasonable cost analysis can be made of the capital, labor, and energy investments into high-rate pond construction and most operations. (A quantitative cost estimate for a high-rate pond system is given in Table 19).

The design and construction of high-rate ponds is relatively simple. A site of suitable size is chosen with proper topographical, hydrological, meterological, and soil conditions. After operational parameters (depth, detention time, etc.) and their ranges are determined on the basis of rational formulae, the site is surveyed and the pond design layed out. Factors such as

TABLE 13

MICROALGAL BIOMASS PRODUCTION FOR BIOCONVERSION SYSTEMS

Approx. Size Range (mi^2)	System	Location	Water Source	Nutrients C N P	Products
1	Oxidation Ponds	Near Urban Areas	Liquid Wastes	Liquid Wastes	Methane, Fertilizer, O_2
1-10	Advanced Wastewater Treatment	"	"	CO_2, Liquid Wastes,	Methane, Fertilizer, Nutrient Removal
3-30	Complete Wastewater Treatment	"	"	CO_2, Liquid Wastes, N_2	Methane, Fertilizer, Evaporative Wastewater Disposal
5-50	Agricultural Energy Fertilizer	Rural Areas	Drainage irrigation	CO_2, N_2, mineral fertilizer	Methane, N Fertilizer, drainage water disposal
10-100	Nutrient Integration	Waste Lands	Any Locally Available Source	CO_2, Recycled Nutrients	Methane

and wind direction must be considered in orienting the ponds' cells and channel Wind mixing is aimed for; a slight slope could be used to provide current flow and some mixing. Channels must have properly rounded turns and deflectors must be incorporated into the design where necessary to prevent eddying. Grading is a most important requirement; rough finish grading can be accomplish by contractors to within 0.1 foot per 1000 feet. This would limit channel length to about 2000 feet in one dimension. The nature of the soil is important; percolation should be avoided if water loss is a problem or leads to pollution. For this purpose, percolation data must be obtained and a suitable soil sealing method used. Compaction, clay lining, or sealant applications are all methods that are potentially economical. The observation that oxidation ponds are self-sealing (in a few months to a few years) might find application in biomass production systems. After compaction of the soil,the first few batches of algae could be allowed to settle as a sludge layer to clog the soil pores. This would represent a calculable expense in terms of operational start-up time and loss of biomass and fertilizer. Allowable costs for pond sealing processes are at most a few cents per ft^2 which excludes all but the least expensive methods.

Earthwork construction is a well established technology. After estimation of required freeboard (depending on rainfall, wind, and depth), a 6 to 10 ft wide top with sloping (3:1) sides mound is constructed from dirt displaced during grading operations. Preventive measures against pond bottom and earthwork erosion must be included, particularly the use of crushed rock and cement aprons around mixers and weirs. The effect of pond bottom roughness on the power requirements for mixing has been calculated (20). The use of wind power for mixing can be considered.

The choice of mixing systems (air lift, jet pumps, paddlewheels, etc.) need to be evaluated for large-scale systems. Baffle material, weir construction, piping, and other engineering details can be approximately cost estimated. Lifetimes and amortization rates are obviously different for pumps, baffles, pipes, earthwork, etc. The steps of a construction program for high-rate ponds are given in Table 14.

A critical aspect of high-rate ponds is the introduction of required algal nutrients. Of these, carbon dioxide presents the greatest engineering unknown; its double function as carbon source and pH control suggests a

TABLE 14

CONSTRUCTION PROGRAM FOR HIGH-RATE PONDS

(after site selection)

1. Site Survey, Data on Soils, Winds, Slopes, etc.
2. Engineering Design of Pond System
3. Site Preparation, Removal of Obstacles and Grading
4. Earthworks for Cells
5. Sealing of Soil (compacting, etc.)
6. Installation of Baffles
7. Installation of Mixing System
8. Influent-Effluent Systems for Cells (weirs, pumps, etc.)
9. Cross-Connecting Piping Between Cells (for flexible series or parallel operations)
10. Water and Fertilizer(including CO_2) Systems
11. Algal Harvesting Systems
12. Operations and Maintenance Facilities
13. Facilities for Algal Inoculum Preparation
14. Laboratory and Management Building
15. Start-Up Operations (considered part of construction)

requirement for feedback and forward controls. Several methods have been suggested for introduction for carbon dioxide. Most combine the mixing with the carbon dioxide enrichment mechanism. Air lift pumps and Venturi pumps are two good examples; the use of diffusion tubes is not ruled out for large-scale systems. An alternative design is a small dome with an aerator inside, placed next to the mixing system. This has the potential of high efficiency of carbon dioxide transfer into the liquid phase and thereby theoretically diminish power requirements. This needs further experimentation. The source of carbon dioxide for algal growth is an important problem for any large-scale algal biomass production system. This was recognized during the early proposals of algal biomass production for energy conversion which envisioned on-site generation of electricity from the methane produced and return of the flue gases to the ponds (20). However, the generation of electricity from methane is a relatively wasteful process; transportation costs for methane gas are lower than for electricity and the use of methane is overall more efficient for many processes. Thus, alternative carbon dioxide sources must be developed. Use of carbon dioxide from the air is not considered feasible for highly intensive algal biomass production because of the low content of carbon dioxide ir air (0.03%). A more concentrated carbon dioxide source is available in the stack or flue gas of fossil, solid waste, or dry biomass fueled electricity generation plants. Use of this CO_2 source in algal production systems is the principal method being presently considered (43). Advantages of power plant flue gas scrubbing and waste heat disposal can be enumerated and integration of the energy system is also a positive factor.

The CO_2 supply would be limited by a number of considerations, particularly siting and implementation. A large number of natural and anthropogenic carbon dioxide sources exist. Natural sources include fossil carbon dioxide present in a large number of geological formations and available at drilled wells, geothermal sources, etc. Anthropogenic carbon dioxide sources are more widespread and applicable to the requirements of algal biomass production. Stationary sources only can be considered which include power plants, cement or lime factories, many industrial sources, and, of particular interest, some agricultural sources. The transportation costs of carbon dioxide from a major point source to the biomass production system would

be a critical siting consideration. Although little experience exists in long distance carbon dioxide transportation, this can be roughly cost estimated from available data. It is expected that some carbon dioxide would be recycled from the algal biomass digestion process.

In operation of high-rate ponds, the economically feasible parameters that can be regulated, within their limits, are given in Table 15. The critical operational parameter is light. All other parameters must be designed to allow most efficient light utilization compatible with predominance by the desired algal species. No statements can yet be made regarding proper pond operations. Many of the parameters and control methods are interrelated and cannot be varied independently. Some control methods are yet to be demonstrated, e.g. zooplankton, detention time, or oxygen tension control by mixing. The operation of high-rate ponds is facilitated by the hydraulic nature of the system which allows achievement of nearly uniform conditions. With the pond operational parameters listed in Table 15, plus inoculations, algal species control must be achieved. The purpose of research into microalgal bioconversion must be to determine the proper sequence of operations required to encourage and maintain desirable algal types at high-net photosynthetic efficiencies.

MICROALGAE HARVESTING

The scientific basis for the mass culture of microalgae was comprehensively reviewed by Burlew *et al.* (8) 25 years ago. This pioneering work was immediately followed by more extensive laboratory, pilot- and field-scale studies so that by the early 1960's most of the fundamental engineering aspects of algae production had been reported in the literature (72 ,78). Still, no large-scale (>100 acre) systems now exist for the purpose of producing algal biomass, primarily because of the lack of a cost-effective harvesting method for the microscopic pond algae. Research in this field has failed to develop a suitable method: centrifugation is too capital and energy-intensive (79), chemical coagulation processes require large quantities of lime or alum and result in a contaminated product (80,81), intermittent sand filtration (82) or in-pond sedimentation (83) do not allow practical algae recovery. Some proposed algal-removal systems offer optimistically high performance and low-cost estimates, but are not without inherent limitations. For example, high-

TABLE 15

ECONOMICALLY FEASIBLE POND OPERATIONS

PARAMETER	CONTROL METHOD(S)	NORMAL LIMITS
1 Algal Concentration	Harvesting, dilution, recycle	100-300 mg/l
2 Depth	Dilution, harvesting	20-50 cm
3 Hydraulic Detention Time	Dilution	1.5-8 days
4 Phytoplankton Detention Time	Biomass Recycle, dilution	1.5-10 days
5 Zooplankton Detention Time	Harvesting-recycling w/DSM screen	1.5-30 days
6 Hydraulic Loading	Dilution	2-20 cm/day
7 pH	CO_2 addition	7.5-9.5
8 Nutrient Additions	Waste flows, CO_2, N_2, etc.	Should not normally be limiting for desirable algae
9 O_2	Mixing (CO_2)	0-25 mg/l
10 Light	Parameters 1-4 Mixing	Absorb 99-99.9 of incident penetrating light

gradient magnetic separation processes involve highly sophisticated equipment installations and, in addition, require the addition of chemicals (coagulants, flux) to the influent algal culture (84); non-fouling membranes for ultrafiltration promise to increase operational life and flux rates, but their use involves highly mechanized ultrafiltration systems requiring extra pumping (minimum filtration pressure is 20 psi) (85); and continuous-flow, dark, deep-basin settling of algae remains to be demonstrated over a long time period or on a significant scale. Thus, microalgal harvesting remains an unsolved problem.

Analysis of Cost Effectiveness

The key parameters which determine the effectiveness of algal-harvesting methods are separation efficiency, concentration factors, product quality, and process dependability. The importance assigned to each parameter depends largely on the application; in waste treatment systems, separation efficiency and process dependability are the most significant; whereas product quality and concentration factors are of more importance in biomass production. Obviously, algae-recovery processes must have a minimum efficiency; about 80% appears to be the lower limit where waste treatment is the primary objective with a somewhat lower efficiency (65-75%) being tolerable in biomass production systems, especially in multistage ponds where the effluent from one pond feeds another. Similarly, a lower degree of dependability on a day-to-day basis can be tolerated as long as it remains reasonably high on a seasonal basis. The concentration and quality of the recovered algae are especially important because it must serve as methane fermentation substrate; for this reason, the algal dry solids concentration must equal or exceed about 2% of wet weight and should not contain any chemicals or inert matter which would upset or slow the fermentations or which make disposal of residues difficult.

Energy requirements are a function of the required direct electrical and fuel inputs plus indirect energy requirements (e.g. extraction, processing, and transportation of chemicals). As an example of the significance of indirect inputs to total energy consumption, Hagan (86) has estimated that the energy required to produce and transport the lime used in the coagulation/

filtration of activated sludge effluent is actually greater than the direct energy inputs.

The most poorly established facet of algal-removal processes is their cost; only now are full-scale algal-removal systems being applied to oxidation ponds (87,88); several years will be required before the total cost of operating these systems can be reliably calculated. Until then, cost estimates must be extracted from the literature. Because of inflation, lack of long-term operational experience,and uncertain scale-up factors such estimates can be viewed with only a low degree of confidence.

Comparison of Presently Available Harvesting Methods (Nos. 1-8, Table 16)

The historical impetus to research on algal-removal methods has been for improvement of oxidation pond effluents in order to comply with Federal and State discharge standards. Thus, highly efficient, dependable methods have received the greatest attention although these methods are also the most expensive. Relatively inexpensive processes such as microstraining and filtration, which rate poorly according to waste treatment criteria, have been, to a large extent, neglected.

Table 16 reviews the performance, energy requirements, and cost of available or proposed algal-removal methods. Only a few of these methods produc an algal product readily utilizable for bioconversion. The first, centrifugation, rates well in all performance parameters but is prohibitively expensive; and the second, microstraining, is low in cost but poor in separation efficiency and dependability. Direct filtration of algae, which produces an algal product of fair quality, suffers from rapid clogging and the propensity of smaller algae (e.g. Chlorella) to completely pass through the sand beds. Intermittent sand filters achieve better removals through maintenance of a biologically active layer at the filters' surface; however, allowable loading rates are small and the recoverable algae (by removing the top layer of the filter) are partially degraded and contaminated with sand.

The most popular methods involve coagulation of algae prior to sedimentation, air flotation, or filtration. The two important coagulants are aluminum sulfate (alum) and lime. These chemicals are required in large

TABLE 16

COMPARISON OF PRESENTLY AVAILABLE MICROALGAL HARVESTING PROCESS

NO.	PROCESS	REMOVAL EFFI-CIENCY	CONCENTRA-TION FACTOR	ESTIMATED COST 1976 $/MG	RELATIVE ENERGY REQUIREMENTS	RELIABILITY	QUALITY FOR BIOCONVERSION
1	Centrifugation	80	40	500	Very High	Good	Good
2	Coagulation-flocculation-sedimentation	85	50	400	High	Good	Poor
3	Coagulation-flotation	90	85	450	High	Good	Poor
4	Coagulation-Clarification-filtration	95	-	600	Moderate	Very Good	Poor
5	Direct filtration without coagulants	40	-	200	Moderate	Poor	Fair
6	Intermittent discharge ponds (with chemical)	90	-	150	Very Low	Not Established	None
7	Intermittent sand filtration	80	-	250	Low	Fair	Poor
8	Microstraining	50	35	50	Low	Poor	Good
9	High Gradient Magnetic Separation	95	Unknown	600	Very high	Very Good	Poor
10	Ultrafiltration using non-fouling membranes	95	50	350	High	Very Good	Good
11	Dark, deep sedimentation	80	Unknown	<50	Low	Poor	Fair

Removal efficiencies and concentration factors are based on dry algae concentrations. Concentration factors are ratios of product algal density to culture density. The estimated cost includes capital recovery, operation and maintenance. Relative energy requirements are ranked considering both direct (electricity) and indirect energy (e.g. extraction, processing and transportation costs for chemicals) inputs. The principle criterion used to evaluate reliability is the degree of fluctuation in process removal efficiency and concentration performance with algal, water, and operational characteristics. Quality refers to the degree of contamination or degradation of the algae removed. Costs are estimated for 10 mgd (million gallons day^{-1}) capacity. (From Ref.22).

quantities to be effective; about 1 mg alum is required for every mg algae (89) and about double that dosage for lime. Before either feeding or fermentation of the recovered algae is possible, the chemical coagulants must be at least partially removed. Shelef (15) was able to remove only about 50% of the alum originally added using an acid (H_2SO_4, pH 3.5) washing step, the remainder being tied up in inert form by phosphates. The acid-alum solution is then reused for coagulation. Lime is more difficult to recover in a reusable form; lime can be recovered from water softening sludge (primarily Mg(OH) and $CaCO_3$) by recalcination (90), but lime sludges, high in organic content, probably are inapplicable to this process. The costs included in Table 16 for coagulation processes do not include the cost of the extraction of chemical coagulants from the algal biomass.

Interest in reusing the chemically flocculated algae has spurred research on the application of synthetic organic polymers (cationic, anionic and non-ionic) to algal coagulation. These polymers would be compatible with methane fermentation, thus not requiring extraction. Results have not been encouraging, however; polymers are ineffective when used alone, although some reduction in the required dose of lime or alum is possible when polymers are used in conjunction with these coagulants (80).

Comparison of Proposed Harvesting Methods (Nos. 9-11 Table 16)

Recognition of the crucial nature of the harvesting step to algal biomass production has restimulated interest in this field and several new algal-removal processes have been proposed. Because of their still largely undefined performance and cost characteristics, it is unfair to subject such process to the cost effectiveness analysis presented in Table 16 . However, due to the critical nature of harvesting to bioconversion, a preliminary analysis has been made for the three more important methods: high gradient magnetic separation (HGMS), ultrafiltration using nonfouling membranes, and dark, deep sedimentation. Table 16 shows that 1) much of the information needed to comprehensively evaluate the alternative methods is missing (the application of HGMS and ultrafiltration to algal removal has been mostly limited to small-scale experiments not processing typical pond algae); 2) one method, HGMS, is not applicable because of its high costs and poor product quality; 3) ultrafiltration and dark, deep sedimentation might become marginally cost-effective.

Since little "hard" data exist on the performance of these processes, a brief discussion of their function, advantages, and limitations can serve to underscore the conclusions drawn from Table 16. HGMS is a new but rapidly developing technology which enables the separation of only weakly magnetic particles from water; these particles include biological colloids (e.g. viruses, bacteria), dissolved nutrients (e.g. phosphate) and algae (84). However, pretreatment of the water to be processed with magnetic seeding material (1000 ppm Fe_3O_4) and a flocculating agent (aluminum sulfate, no dose reported) is required. Even if most of the magnetite can be recovered (this is doubtful because it will be bound in a magnetite-alum-algae matrix) the chemical costs will not be inconsiderable. It should be noted that if enough alum is added to attain flocculation (approximately one mg alum per mg algae) then the magnetite and magnet could be dispensed with and the algae separated by sedimentration. The quality of the algal product would be severely decreased by HGMS because of the chemical additions.

Membrane ultrafiltration depends upon a pressure driving force and a membrane that is permeable to some components of a solution or mixture and impermeable to others. The upper molecular weight limit for ultrafiltration is usually defined as 500,000, above that size, separation occurs by conventional microporous filtration (85). In the case of microalgae, ultrafiltration is better termed simply as pressure filtration, especially for oxidation pond algae such as Scenedesmus, Micractinium, and Euglena, which are considerably larger and heavier than the bacteria, viruses, paint pigments or other low molecular weight colloids which have been concentrated using ultrafiltration in typical applications. Ultrafiltration requires moderate pressure (20-100 psi) to achieve economical flux rates. Besides pumping costs and membrane flux rates, the key cost factors are membrane life and replacement cost. Another additional cost factor is pretreatment; "large" particulate matter ("large" depends on the construction of filtering cartridges; Gregor (91) has indicated that particulates as small as 15 μm diameter will clog the cartridge windings) will need to be removed by microstraiing. This could be a significant expense as the finest straining fabrics now available for large-scale microstrainers have 23 μm openings; finer fabrics severely decrease flux rates. Even if 23 μm openings are acceptable, there is still a problem with pretreatment; at times when colonial algae predominate, there would be no algae left to filter after microstraining. In ultrafiltration

two main factors affect flux rates achievable in continuous flow applications concentration, polarization, and fouling. Retention of particles and solutes creates a layer that must back-diffuse into the bulk fluid, this polarization becomes increasingly severe with the increasing size of the impermeable phase. High operating temperatures, high flow rates parallel to the membranes, or both are necessary to reduce the polarization effect. Irreversible loss of flux capacity is referred to as fouling, generally due either to the entrapment of matter within the matrix of the membrane or biological growth (slime layer) developing on the membrane surface. Newly developed sulfonic acid nonfouling membranes might result in longer operational life; this savings must be balanced against any increased fabrication costs for the membranes.

In-pond algal sedimentation without chemical additions is now practiced successfully by Woodland (92); however, their process requires isolation of the settling pond for two to three weeks and they do not recover any of the settled biomass. Recent experience at this laboratory (93) with continuous flow-dark sedimentation indicates that this process is reasonably efficient, but still too slow to be practical for use in biomass production systems. Experiments proposed to improve this system are the use of deep (20 ft) basins in which algae are introduced near the bottom with a low upflow velocity. Draw-off of the settled algae could be done several times a day. No performance data is available for this process, although, in shallower, dark sedimentation experiments, as high as 90% separation efficiency was achieved. The only significant cost would be for excavation to about two-thirds the required depth; the rest of the depth could be provided by using the excavated earth as dike material.

Role of Species Control in Microalgal-Harvesting

Each of the harvesting methods reviewed in this section are to varying degrees affected by the algal species composition. The important differences between algae in this context are physical dimensions and shape, motility, sur electrical charge, and specific gravity. Table 17 identifies the algal cell characteristics which affect algal removal processes.

TABLE 17

IMPORTANCE OF ALGAL CELL CHARACTERISTICS TO

PERFORMANCE OF ALGAL-REMOVAL PROCESSES

Rank	Process	Score	Sensitivity to Algal Cell Characteristics			Estimated Cost 1976 $/mg
			Size and Shape	Surface Elect. Charge	Specific Gravity	
1	Microstraining	4/4	CS	IS	IS	50
2	Dark, deep sedimentation	2/4	S	IS	S	< 50
3	Intermittent sand Filtration	2/2	S	IS	IS	
4	Direct Media Filtration	1.3/4	S	MS	MS	200
5	In-Pond Sedimentation with Chemical Adds.	1/3	MS	MS	MS	150
6	Flotation Following Coagulation	1/3	MS	MS	MS	---
7	Centrifugation	1/2	MS	IS	MS	500
8	Sedimentation Following Coagulation-floccula.	1/2	MS	IS	MS	--
9	Alum Coagulation	1/1	IS	MS	IS	--
10	Lime Coagulation	1/1	MS	IS	IS	--
11	HGMS with Alum + Magnetic Adds.	1/2	IS	MS	IS	600
12	Ultrafiltration	1/1	MS	IS	IS	350

Legend - CS - critically sensitive weighting factor 4
S - sensitive 2
MS - moderately sensitive 1
IS - insensitive 0

The score was calculated by dividing the sum of weighting factors by the number of non-insensitive tallies for each process; for example, "microstraining" has a sum of 4, from one tally which yields a score of 4/4. Ranking was decided by considering first the sum divided by no. tallies, then the sum alone. Estimated costs based on a 10 mgd capacity.

Three of the straining or filtering processes listed--intermittent sand filtration, microstraiing, and ultrafiltration--are influenced predominately by the physical size and, to a lesser extent, shape of the algal cells. Microstraining is the most sensitive; cells smaller than 23 μm in all dimensions cannot be retained unless there are enough larger cells present to form a matt on the fabric. A biologically-active matt is formed on the surface of intermittent sand filters, allowing smaller cells to be retained. Very small cells (such as Chlorella) or flexible-bodied cells (Euglena or other flagellates), however, can still pass through. Membranes used in ultrafiltration are the least sensitive to cell size.

Cellular specific gravity is important where sedimentation or flotation is employed. High specific gravities are beneficial to centrifugation, all sedimentation processes, and direct media filtration (sedimentation is one of the more important particle removal mechanisms operating in depth filtration). Conversely, a low specific gravity is needed where flotation is employed. Algal cells grown in waste ponds typically exhibit a bimodal distribution of specific gravities (12). This fact was confirmed in a study of alum coagulation/flotation where Ramani found that roughly 50% of the algal cells floated in the separation cell and the rest settled. Ives (94) first related to surface electrical charge of algae to the performance of cationic charge neutralization; at the pH range of ponds, algal cells carry a net negative surface charge. Before the cells can aggregate, this charge must be neutralized, a task accomplished in alum coagulation by the aluminum ions. Reduction of the net surface charge of algae prior to coagulation can reduce the coagulant dose required. This could be achieved through selection of species which exhibit favorable characteristics. (It is clear that the surface properties of algae are species specific--blue-green algae possess bacterial type cell walls and under natural conditions blue-greens exhibit reversible clumping, whereas greens are rarely observed to naturally aggregate) Alum coagulation, which depends largely on charge-neutralization to promote cellular aggregation, is necessarily affected by the surface electrical charge, whereas,lime coagulation, which is more dependent on hydroxide precipitate formation and enmeshment of particulates in the flox matrix, is not generally affected. Similarly, in-pond sedimentation and HGMS, when they

incorporate alum or polymeric flocculation, are influenced by the surface charge. Media filtration depends to a minor degree on electrostatic attraction between particulates and the media.

As shown in Table 17, algal-removal processes fall roughly into three categories: those insensitive to algal species (coagulation, HGMS, and ultrafiltration), those which are highly sensitive (microstraining and dark, deep sedimentation), and the remaining processes which are moderately affected. Interestingly enough, the estimated costs roughly increase with the decreasing sensitivity of performance to algal cell characteristics. Two strategies exist for algal removal: the first is to use processes which can effectively remove any species; such processes tend to be relatively high in cost; the second is to selectively cultivate algae which are harvestable by inexpensive means. The latter strategy is the principal topic of our research. Since microstraining is favored by us as the microalgal harvesting method of choice, it deserves further analysis.

Cost Analysis of Microstraining

The monetary costs and energy requirements for microstrainers are well established. However, unit cost figures for microstraining quoted in the literature ($/mg) are generally calculated for systems treating river or lake waters. These waters have lower algal concentrations and do not possess the fouling or corrosive properties of sewage. Therefore, costs for systems handling these waters are likely to be lower than the costs of similar systems involved in removing algae from production pond effluents. In Table 18 are given a breakdown of costs associated with microstraining flows of 10 mgd and 100 mgd. Some of the criteria used to develop the table are discussed here; the others are given in the legend to the table.

The largest microstrainer unit manufactured by the Crane Company is 10 ft in diameter and 10 ft wide. Assuming a maximum fabric loading of 250 gal/ ft^2/hr (submerged area), eight 10 ft x 10 ft units would be required to process 10 mgd. For the flow rate of 100 mgd, it is assumed that a larger unit (15 ft diameter, 20 ft wide) could be fabricated; 27 units of the larger size would be necessary. Cost for the 10 ft x 10 ft unit was quoted as $66,500 (1967 dollars). No allowances were taken for discounts on orders of several units at a time, although it is standard business practice to give such discounts. Erection cost for the 10 ft x 10 ft unit was estimated at $5,250

(50% above the quoted 1967 cost) and $6,500 for the 15 ft x 20 ft unit. Total installation costs for the 10 mgd and 100 mgd microstraining systems (including automatic regulation equipment and concrete basins) are $912,000 and $5,110,000 respectively. Annual capital costs for the two systems, assuming 6% interest and a 10-year useful life, are $128,000 and $715,000 respectively.

Operational and maintenance costs given by Diaper in 1969 (95) were $4 per mg. Recent large increases in manpower and energy costs make this value unrealistic even when increased by 50%. The costs per mg were calculated as $15 and $9 for the 10 mgd and 100 mgd systems, respectively. These costs include energy requirements (using Diaper's value of 12.5 HP required for the 10 ft x 10 ft unit, an estimated 15 HP required for the 15 ft x 20 ft unit, and an energy cost of 1.5¢ per kw-hr), maintenance, and manpower (operators and supervisory personnel).

Capital, operational, and maintenance costs for microstraining total $183,000 yearly for the 10 mgd system and $1,050,000 yearly for the 100 mgd system, or $50 and $29 per million gallons, respectively. Of these amounts, approximately 70% is made up of capital cost. The capital-intensive nature of microstraining, combined with its relatively low absolute costs, suggest that the costs of microstraining could be reduced considerably by advances in fabrication methods and use of more economical materials. Additionally, it is important to note that energy requirements (see Table 18) amount to 172 kw-hr and 69 kw-hr; this would be $2.58 and $1.04 per mg respectively or about 5% of capital, operational, and maintenance costs. Although microstraining co: are expected to decrease by half in bioconversion applications, costs per ton biomass (roughly equal to $/mg) would still be too high in systems producing methane alone. However, it might be possible to develop much cheaper large-scale microstrainers applicable to such purposes.

OXIDATION PONDS

The concept of algal biomass production for conversion to methane originated from the development of algal waste treatment systems (17). The cultivation of algae on sewage has the potential of providing significant quantities of algal biomass whose quality makes it unsuitable for most higher uses (food, feed) without expensive processing. Methane fermentation, being an accepted sanitary engineering disposal method for unwanted sludges, was the obvious choice for handling algal biomass produced during waste treatment. The main objective of current sewage and liquid waste treatment practices is the oxidation of the organic matter in the wastes. Oxidation ponds provide the necessary dissolved oxygen through algal photosynthesis.

TABLE 18

ESTIMATED CAPITAL AND OPERATIONAL COSTS OF MICROALGAE-HARVESTING WITH MICROSTRAINERS

CAPITAL COST	10 mgd CAPACITY	100 mgd CAPACITY
Microstrainer Units		
10 ft x 10 ft (@ $99.750)	$798,000	
15 ft x 20 ft (@ $170,000)		$4,590,000
Automatic Regulation		
Equipment (@ $3,000)	24,000	
(@ $3,750)		101,250
Erection (@ $5,250)	42,000	
(@ $6,500)		175,500
Concrete Basins (@ $6,000)	48,000	
(@ $9,000)		243,000
TOTAL	$912,000	$5,109,750
ANNUAL CAPITAL COST (@ 6% 10 yrs)	127,680	715,365
OPERATIONAL & MAINTENACE COST		
HP Requirements	$ 9,417	$ 37,843
Maintenance	5,500	23,000
Manpower (@ 4 units/cap)	40,000	270,000
TOTAL O & M COST	54,917	330,843
TOTAL CAPITAL + O & M	$182,597	$1,046,208

LEGEND TO TABLE 18

Unit Costs for Microstrainer Units

1967	10' x 10' Unit	$ 66,500 (Diaper, 1967) (Ref. 95)
1977		99,750 (Estimated)
1977	15' x 20' Unit	$170,000 (Estimated)

Fabric Type: Glenfield Mark 0 23 μm openings
Number of Units Required:
Fabric Loading = 250 gal/ft^2/hr or 5,750 gal/ft^2/day @ 23 hr/day
Required Straining Area
10 mgd 1740 ft^2
100 mgd 17400 ft^2
Available Straining Area
10' x 10' unit: 314 ft^2 less 25% not submerged
5% external fittings, seams
220 ft^2 available
15' x 20' unit: 942 ft^2 less 25% not submerged
5% fittings, seams
660 ft^2 available
Number of 10' x 10' Units Needed for 10 mgd: 7.9 → 8
Number of 15' x 20' Units Needed for 100 mgd: 26.4 → 27

Erection Cost

1967	10' x 10' Unit	$ 3,500 (Diaper 1967)
1977		5,250 (Estimated)
1977	15' x 20' Unit	6,500 (Estimated)

Concrete Basin Construction

1977	10' x 10' Unit	6,000 (Estimated)
1977	15' x 20' Unit	9,000 (Estimated)

ENERGY REQUIREMENTS FOR MICROSTRAINING

		KW-hr / day	K BTU / day
10 mgd: 8	10' x 10' units		
	drive	688	6992
	wash	1,032	10,400
	Total	17,20	17,392
	$/yr @ 1.5¢ KW-hr	$9,417	
	$/mg	$ 2.58	
100 mgd 27	15' x 20' units		
	drive	2781	28,350
	wash	4131	43,200
	Total	6912	71,500
	$/yr @ 1.5¢ KW-hr	$37,843	
	$/mg	$ 1.04	

The amount of algae that can be produced depends on the nutrient content of sewage; carbon is the normal limiting nutrient. A reasonable current estimate is that per liter of typical municipal sewage, about 250 mg of algae/l can be grown on available carbon (Table 3). Per capita sewage flows (about 200/l/day) are not substantial enough to produce significant quantities of methane from algae harvested from oxidation ponds. (If algae are grown to the growth potential of carbon in sewage, only about 80 lbs of algae/person/year could be produced representing about 400,000 BTU of methane). In many localities waste flows are of different chemical constitutions, and particularly where agricultural or food processing wastes are available, significantly higher, per capita, outputs might be achievable. The methane produced from the primary sludge would also contribute to the energy outputs of oxidation pond systems.

Although the potential energy outputs from oxidation ponds might be considered negligible by some, they could represent a significant municipal resource of value to local industries or residential users. About 0.5% of per-capita methane consumption could not be satisfied by such systems using the C in municipal sewage alone, with additional waste flows they would increase in magnitude. In the future, natural gas is expected to make up a smaller share of U.S. energy consumption. If algae are grown to the N and P growth potential of sewage, methane outputs from oxidation pond systems could become a very significant and crucial local energy resource.

Waste treatment systems will have impact on energy consumption beyond just substitute natural gas production. The energy value of the fertilizer content of residual algal sludge could be a significant output from such systems (wherever transportation does not limit its utilization). However, the most important energy aspect of oxidation ponds is their energy conservation when compared to present conventional sewage treatment. The generation of dissolved oxygen requires electrical energy to run the pumps (or, more recently, the cryogenic equipment of oxygen purification). A 100 MGD activated sludge plant requires about 70,000 kw-hr/day to oxidize the organic material (86). This represents an energy consumption approaching the net energy production expected from the algal biomass harvested from the oxidation ponds. Thus, impact on local energy sources would be doubled if, in addition to SNG outputs, energy conservation and fertilizer outputs are also considered.

The economics of oxidation pond systems are dictated by present technol and EPA wastewater treatment standards and regulations. Additional considerations are the relative land and construction costs: at equal total costs, conventional energy intensive waste treatment will normally be favored over th land extensive oxidation pond system. The reasons are complex, involving the interests of governmental agencies, construction companies, labor unions, municipalities, consulting engineers, land owners, and so on. EPA regulations are important factors; with presently accepted microalgal harvesting technolog it is difficult to meet effluent standards at costs much below conventional methods. Recently, the high national costs of waste treatment programs led EPA to significantly relax standards for small-scale oxidation pond systems.

ADVANCED WASTEWATER TREATMENT

After oxidation of organic materials, the effluents from conventional waste treatment systems still contain sufficient inorganic nutrients to suppor the growth of extensive algal blooms in receiving waters. This eutrophicatior role of liquid waste treatment plants is being solved by advanced wastewater treatment systems designed to lower N and/or P levels in plant effluents. The enormous costs of these systems assures their installation in only a few selected sites, those able to most rapidly garner EPA construction funds. This might become a mixed blessing; the rapidly increasing operating costs of advanced wastewater treatment systems must be borne solely by the local governments.

A recent GAO report to Congress (96) calls for more data collection before any additional appropriations for construction of advanced waste treatment facilities. The projected federal expenditures exceed those devoted to e research and development. The additional energy requirements of a 100 mgd advanced wastewater facility, above those required for activated sludge, are between 140,000 kw-hr/day for alum treatment followed by nitrification/denitrification to over 1,000,000 kw-hrs/day for coagulation/filtration-lime recalcination-activated carbon adsorption and Zeolite ion exchange (86). (The latter method approaches complete wastewater treatment discussed in the next section).

Oxidation ponds can be expanded to meet advanced wastewater treatment

standards and nutrient removal by growing algae to the N and P growth potential of the waste (Table 3). This requires the introduction, in most cases, of carbon dioxide into the ponds. As indicated in the table, up to ten times as much algae can be grown on the phosphorus present in typical sewage as on the available carbon. This, however, exceeds the requirements of waste treatment. The phenomenon of luxury uptake assures complete P (and N) removals well before it becomes a limiting nutrient. About a three to four-fold expansion of oxidation ponds would suffice for advanced wastewater treatment. Since this also corresponds to the additional costs of conventional systems capable of effecting nutrient removals, the economic advantage enjoyed by oxidation ponds over conventional systems in secondary waste treatment will carry over into advanced (tertiary) waste treatment. Operation of advanced waste treatment ponds will require a successive harvesting of algal crops from the same wastes, or a dilution of the incoming nutrient with pond effluents already depleted of nutrients. The distinction is important in pond operations, the first alternative requires a series of ponds, each with a particular algal species or type; while the second could involve a single algal type (e.g. nitrogen-fixing blue-green algae).

The development of advanced waste treatment with algal pond systems could result in a significant effect on local, regional, and even national energy consumption. It could be expected that for many sites up to 10% of local methane requirements could be satisfied; fertilizer and energy conservation aspects of such systems would also be significant. The application of such systems will be favored by the high costs of conventional systems. Thus, even if biomass productivities are half those presently expected and algal harvesting and digestion costs higher than CH_4 outputs, these systems would still be cost effective. The significant energy outputs possible in large urban areas, together with their strong economic potential, make these systems the prime candidates for development of large-scale microalgal bioconversion systems.

COMPLETE WASTE TREATMENT SYSTEMS

Advanced wastewater treatment has for its objectives zero discharge of pollutants. At present, this is possible with pond systems designed to com-

pletely evaporate the waste stream. This is accomplished by large-scale ponding systems of large enough surface area to effectuate complete holding and eventual evaporation in the ponds. Since large areas are required, since algal cultivation helps significantly in evaporation, and since algae can be cultivated in very high salinities, the combination of algal biomass production systems with terminal evaporation systems for waste treatment is of obvious appeal in the design of large-scale systems. The economics of such systems would be aided by the nearly astronomical costs of alternative disposal methods for wastewater too highly contaminated with salts to allow ground water recharge or stream disposal. The complete evaporation of wastewater would also be roughly equivalent to an exhaustive utilization of nutrients.

Because algae, particularly blue-greens, are capable of growing at high salinities, they should not be a problem to produce suitable algal biomass in all but the final evaporation ponds. Indeed, when salinities exceed those of seawater, certain algae such as Dunaliella could be selectively cultivated for glycerol and other petrochemicals (97). The increase in salt concentration as a function of time or in successive ponds would affect algal cultivation technology, pond designs, harvesting, etc. Build-up of toxic materials is a possibility that would need to be considered. Micronutrient availability is not a primary problem; they are present in high concentrations in wastes and could be supplemented at relatively low costs.

The size of systems required for complete waste treatment can be easily approximated by multiplying the local yearly evaporation rates by a factor (presently unknown) representing increased evaporation due to algal heat absorption and pond operations. If local evaporation is 4 feet, somewhat more water would be evaporated yearly from an algal pond. This allows calculation, from daily waste flows, the acreage capita^{-1} required for complete waste treatment. Assuming 100 gal capita^{-1} day^{-1}, this corresponds to about 0.1 acre foot year^{-1} capita^{-1}, or 60 people per acre of evaporation ponds. A system of 25 square miles would thus serve a 100 MGD sewage flow representing about one million people.

Thus, these types of systems would only achieve very large sizes near urban areas where municipal, industrial, and agricultural waste streams are

available. A number of such urban areas exist, including the San Francisco Bay Area, Los Angeles, Houston, Atlanta, etc. The limitations of waste transportation distances and land availability would dictate a scattered set of pond locations grouped on the fringes of the urban areas and managed by the local water utility (with, possibly, the bioconversion and methane marketing being handled by a local energy utility). Land costs and availability might be limiting factors which must be solved through land use planning, zoning, and acquisition through eminent domain. Ponds could serve as open space or green belts.

The waste treatment credits available to such systems, above those already received for nutrient removal, might be substantial in particular local cases where salt buildup is a problem. In other cases, complete waste treatment systems would need to be designed instead of nutrient removal pond systems because of high local evaporation. Using the evaporation value of 6 feet/year, it can be easily calculated that at 20 tons dry algae/year/acre and 5,000 BTU's methane/lb algae, about 100 BTU's are produced per gal of wastewater disposed of evaporatively. This is a key parameter for algal bioconversion.

AGRICULTURAL FERTILIZER-ENERGY SYSTEMS

The average value of U.S. agricultural land is about $500/acre and the gross output per acre per year is below $200/year. Algal bioconversion systems with expected capital investments of several thousand dollars per acre and gross expected outputs of $500/year/acre and above could easily absorb these land costs and compete with agriculture on the basis of outputs. In this sense, algal biomass production would be a higher land use than agriculture. However, the realities of implementation and our dependence on food could not allow significant competetion and encroachment by bioconversion systems on the land, water, and fertilizer required by agriculture. Bioconversion systems must be devised which utilize resources unsuited for food production and/or which can complement the agricultural system. It has been calculated that one acre of algal biomass production ponds producing 20 tons/acre/year of dry volatile solids biomass would (at 10% N content) provide the fertilizer needs of 10 to 30 acres of intensive non-leguminas agricultural crops (23). The 8 million tons of N fertilizer consumed annually in the United States could thus be supplied by 4 million acres or about 6,000 mi^2 of pond systems. Since the highest intensity agriculture is usually locally concentrated, and since many

large-scale irrigation schemes exist in the southern U.S. regions where the localities most climatically favored exist, the concept of producing algal biomass in conjunction with agriculture is appealing. In such a scheme, land and water availability are not limiting and are of relatively low cost. Marginal lands can be utilized for the ponds and perhaps reclaimed in the long run. Even if land is taken from agricultural use, it would only be about 5% of local acreage and it would be producing all local N fertilizer needs. The siting requirements for a large-scale algal biomass production system for production of methane and nitrogenous fertilizer would be where intensive irrigated agriculture is practiced.

Although preferably the plant fertilizer is derived from urban liquid wastes, thereby conserving non-renewable resources, the reality of the present U.S. situation, in which agricultural and urban areas are separated by long distances, suggests the potential of "once through flow systems" for algal biomass production geared to agricultural requirements. The individual ponds and cells could be scattered in between an agricultural locality. The phosphate fertilizer used would be the same presently being used in crop production. The water is the same utilized in irrigation; salinity increases would be mino since it would be a simple once pass-through system (at 5% of local acreage, evaporative water consumption would be proportionally even less). Carbon dioxide would be piped in from the most available source. (This might become a problem; the use of the algal pond systems and irrigation waters for power plant cooling could be considered.) The cost of pumping flue gases (10% carbon dioxide) would probably be too high for longer than one-hundred miles. In liquid waste treatment systems, their location near urban centers would increase proximity to suitable carbon dioxide sources.

The algae cultivated in such systems must be the nitrogen-fixing, filamentous, heterocystous blue-green type. These are selected when nitrogen is a limiting nutrient. Cultivation technologies for blue-green algae still have not been developed; however, no outstanding problems appear obvious. Provision of micronutrients (including Fe, Mn, Mg, etc.) and trace elements could be provided inexpensively by small local waste flows or mineral fertilizers.

The utilization of the sludge residue after conversion of the algal biomass would allow direct low-cost land application or addition to the irrigation water. Algal sludge storage facilities would have to be incorporated into the bioconversion process designs to allow utilization of the fertilizer when required by agriculture. The value of the algal fertilizer would be given by their N content, which costs at present about $150 per ton and may be assumed to considerably rise in price as natural gas prices increase. Thus, the N value of the algal biomass would be comparable to that represented by the BTU content of the methane obtained by its digestion. This methane output of microalgal bioconversion systems is expected to amount to 250 MBTU's/acre/year, on the basis of a predicted productivity of 20 tons/acre/year and a methane yield of 5 ft^3/lb of algae (20).

Another possibility for such algal biomass production systems combining CH_4 with agricultural fertilizer production is the use of and disposal of drainage waters. Large amounts of nitrogenous fertilizer are lost in agricultural drainage areas; the disposal of drainage water is a severe problem because of its salinity and nitrate content. Through phosphate fertilizer and evaporative ponds, it might be possible to simultaneously dispose of this water, recover lost nitrogen, and produce large amounts of algal biomass. Such projects have already been studied in the San Joaquin Valley in California (74) where great potential for applying such technology exists. The results of that study are informative: phosphorus, carbon dioxide, and iron were required to support algal growth on tile drainage water (average composition 6,000 ppm TDS and 20 ppm nitrogen as nitrate). The use of algal ponds to reclaim agricultural runoff and to dispose of salt-laden drainage waters is an attractive possibility.

NUTRIENT INTEGRATED SYSTEMS

As discussed above, algal biomass production is not limited by water quality but by quantity of evaporative losses. Thus, a value of 100 BTU's/gal water consumed,which has been estimated above,compares favorably with alternative biomass production systems, hydroelectric power, and even coal gasifications or nuclear power (whose process or cooling water requirements are

high). Large fertilizer and land requirements, however, present limiting facto that prevent the application of algal-bioconversion systems except for favorabl urban or agricultural sites (as described above). To be able to consider the very large tracts of suitable, flat, non-productive land presently available in the sunniest regions of the U.S., the problem of nutrient supply must be considered preeminent.

This problem was recognized long ago and a set of experiments was undertaken to determine whether digested microalgal biomass could be utilized for the further cultivation of algae (16). Microalgal digestion results in 50 to 70% destruction of volatile matter (17,18), the digestion residues containing the original N and phosphorus. These were utilized to grow additional algal biomass (16). A small-scale laboratory culture and digestion unit were operated in a closed loop fashion for nearly one year with good results. Although these data suggest the feasibility of such operations, it does not provide sufficient basis for an engineering analysis. The return (recycling) of the digester sludge residues would be most economically accomplished by their direct return to the ponds, since there is no need for separate sludge ae tion. Indeed, the surplus oxygen in the ponds would be a readily available source, whose depletion would help productivity. Introduction of the digestion residues into ponds is an engineering and systems operation problem readily subjected to analysis. The requirements of nutrient control and turbidity avoidance can readily be met by distribution (spatially and in time) of the algal residues. The design of a nutrient integration system must incorporate such a nutrient recycling system.

In nutrient integration (recycling) systems, the problem of make-up nutrients has to be considered. It is possible that a few percent of the nutrients (or specific nutrients) are lost each cycle. Ammonia could, for example, be lost due to the high pH of ponds. However, it could be resynthesized rather easily through nitrogen fixation. Phosphates and some minor nutrients could precipitate into the bottom sludge layer and remain there (although pond maintenance operations may be devised to make them available for algal growth). Discharge or losses from the digestion system might not be unavoidable. These factors would have to be minimized by proper pond operations. The lowering of the pH by pond carbonation would prevent most losses. Since blue-green algae are not necessary in such ponds (because nitrogen

fixation would not be required), lowering of the pH to neutrality would not have serious effects on algal species control. Nutrient makeup could be provided by minor, locally available waste streams. The start-up of such systems requiring "stocking" of the system with sufficient nutrients to grow and digest the first crop cycle (and "saturate" the bottom of the pond) would be a significant cost factor. Use of mineral fertilizers and nitrogen-fixing algae might be required during the first weeks of operation. Another alternative is to permit the system to "grow" over a period of time.

The same considerations apply to nutrient integrated systems as to the concepts described above. Terminal evaporation systems resulting in complete consumption of water requires algal species succession to the halophylic species. The high-rate pond designs and operations must allow algal species control and low-cost algal harvesting. Nutrient integrated systems would not be economically supported by the waste treatment or fertilizer production aspects of the previous systems. By being virtually independent of waste flows or algal residue disposals, these systems could be sited in an outlying compact pond layout. This would allow better design of pond connections required for flexible operations.

If this concept can be demonstrated to be capable of delivering algal biomass for a cost of about 1¢/lb volatile solids, it could become a major source of methane for the U.S. If a net energy production of fuels of 10^{11} BTU/s mi^2/year is assumed which uses about 3000 acre-feet/acre/year water, 1% of present U.S. natural gas requirements could be produced in about 2,000 mi^2 of pond systems utilizing 10 million acre-feet of water. This corresponds approximately to an equivalent volume as presently shipped in California on the California Aquaduct. Since much or most of this water could be wastewater, no longer suitable for agriculture, or other purposes, it appears that this water usage would not be excessive for the purposes required.

Although water use has been minimized in these concepts, it is still substantial in large-scale systems. Its value would depend primarily on cost of water transportation from an available surplus or "spent" water source to the algal biomass production site. An additional water charge which must be considered is the added cost of the final brine evaporation ponds in which algal production would be impossible (or of only low efficiency). Furtheremore, any

large-scale bioconversion engineering design must include the use of accessory storage and distribution systems capable of equalizing water consumption over the year. Besides costs these accessory water systems would entail inevitable losses which would raise overall water consumptions.

Although nutrient integrated systems are the least economically viable (having no other reason for their existence but their methane outputs), they are the subject of considerable interest since they potentially represent the large-scale centralized systems of biofuel production required to meet demands by interstate gas pipelines. Being dependent mainly on water and carbon dioxide (both of which can be from waste sources) for major external inputs their potential usage would be extensive if their technical and economic feasibility could be achieved. Since technical feasibility is not yet demonstrated, economic costs cannot be assessed. However, it is possible to list the specific inputs required for nutrient integrated microalgal biomass production in high-rate ponds. Obviously, a cost estimate must be based in large part on favorable assumptions (e.g. topography, mixing speed requirements etc.) and thus represents a minimum cost analysis. Some aspects of a nutrient integrated system remain to be agreed upon or designed (e.g. mixing and carbonation systems). In some cases, a "likely" or "allowable" value must be arbitrarily assigned. Nevertheless, such an exercise, detailed in Table 19, can be instructive.

The legend to Table 19 details the assumptions made; the total allocated costs for the system are about $1,200/HA/yr or about $450 less than expected revenues from the methane produced. Thus, $450 must cover all management, data collection, profits, contingencies, etc. involved in operating the system. This is considered a major uncertainty; it is presently not feasible to indicate operations and manpower effort required. For large-scale systems, however, it is likely that they could be minimized. It is important to point out that the analysis is based on some unproven concepts (carbonation, methane production by covered ponds, etc.) still to be developed. The number of locations meeting the indicated requirements might also be limited. It is however, not apparent why, in theory, such systems could not be developed. Table 19 also contains a net energy analysis. The data indicate a potentially very favorable net energy output by such systems with only 10% of the energy

TABLE 19

COST AND ENERGY INPUT ESTIMATES FOR NUTRIENT INTEGRATED SYSTEMS

COMPONENT		COST 1976 $/HA/yr	ENERGY INPUT 10^3 Kcal/HA/yr
Growth Ponds	Earthworks	100	70
	Installation	225	20
	Paddlewheels	30	3
	Mixing Power	7	700
Water Supply	Water Costs	180	10,000
	Installation	60	40
	Pumping power	13	1,300
Nutrients	N, P, salts, minor elements	0	--
	CO_2 (transport, injection)	70	1,000
Harvesting	Settling ponds	100	400
	Microstrainers	200	2,000
Anaerobic Digestion	Ponds, Installation	200	30
	Operations	15	700
Management & Operations		???*	?
	TOTALS	1,200	16,263
Expected Gross Methane Outputs**		1,650	165,000
	Net Outputs	450	~150,000

*Major uncertainty

**Assuming $10/M Kcal, 5.5 Kcal/gm algae, 50 metric tons ash-free dry weight algal biomass, 1 HA/yr, and 60% conversion into methane

TABLE 19 LEGEND

COSTS: All capital costs amortized at 6%, 20 year-life (except microstrainers a 10-year life). Growth ponds are designed as 20 hectare cells with earthworks 1 m high and 2 m across at the top with 3:1 sloping walls, channels about 25 m wide, with leveling and earthwork charges of about $\$1/m^3$ and \$3/m for baffles. Water supply was calculated at $\$30/10^6$ liters, which is a realistic cost for non-subsidized irrigation water. Nutrients were assumed to have no net cost. Recycling of nutrients is considered part of harvesting-digestion operations and make-up nutrients are provided by local waste flows. Water and carbon dioxide supply installations were assigned values based on rough estimates which considered necessary pumping and minimum piping costs. The harvesting system consists of a primary settling pond followed for each 200 hectares by a 15' x 20' microstrainer unit (940 ft^2 straining area) at \$170,000 which functions continuously. Anaerobic ponds and installations were approximated from anaerobic pond construction costs with a submerged or floating cover at $\$2/ft^2$ and minimun piping and pumping. Operation costs were based on no heating (except solar) and minimum mixing (by recirculation).

ENERGY INPUTS were calculated in part using data from Alich and Inman (3). Direct energy inputs (fuel) for earthwork were calculated for three stages of construction: bulk earth moving with a 15-m^3 capacity "scraper," levee shaping a D9 bulldozer or equivalent, and grading to $\pm$ 3-cm with a blade. These fuel re quirements were amortized over the 20-yr assumed pond life. Fuel use was assume 85% efficient and a heat value of 139,000 BTU's/gallon diesel fuel used. The energy needed to process the steel used in the heavy machinery was calculated, using a value of 19,000 kw-hr/ton steel and a thermal efficiency of 0.33. Weigl for the scraper, bulldozer and blade were pegged at 25, 15, and 10 tons, respectively. The fabrication energy input was adjusted for the relative portion (the machinerys' useful life spent constructing each hectare of growth pond and amortized over the 20-yr life span. Energy inputs needed for installations and fabrication of paddlewheels consisted of the amortized fuel requirements of equ ment and vehicles needed; the indirect inputs for raw materials were considered insignificant. Mixing energy requirements were calculated assuming 25-hectare cells with L:W ratios of 4:1. A constant slow mix of 5 cm/sec interrupted by short durations (2 hours or less/day) of fast (50 cm/sec) mixing was found to r quire at most 270 kw-hr/HA/yr (\$7/HA/yr @ 2.5¢/kw-hr) or 700 x 10^6 cal/HA/yr at thermal efficiency of 33%. For the water supply, a final lift of 8 meters was assumed to be required and Hagan's data (86) of 1.3 kw-hr/acre-ft/ft used to ar at a pumping energy requirement of 540 kw-hr/HA/yr (\$15/HA/yr) or 1400 x 10^6 cal/HA/yr. Installation energy requirements consisted of the energy cost for t pump and pump housing raw materials and the construction of the housing.

HARVESTING COSTS: Microstrainers at \$170,000 per 15' x 20' unit (total 940 ft^2 straining area) were assumed to be available. It was estimated that one such u would be needed for each 200 hectares of growth ponds to function as a secondar construction step following sedimentation. The amortized cost was calculated u a useful life expectation of ten years and an interest rate of 6%. The unit wa assumed to function 23 hrs/day. Energy requirements for harvesting assumed a t of 14 HP (drive motor and backwash pump) was estimated to be required for each which gave a requirement of 700 kw-hr/HA/yr (\$18/HA/yr) or 1,800 x 10^6 cal/HA/y which, in addition to the capital cost of ca \$86/HA/yr, gave a total cost for microstraining of \$200/HA/yr. Assuming a weight of 4 tons steel per unit, the indirect energy input was calculated to be ca 70 kw-hr/HA/yr or 200 x 10^6 cal/H

generated being consumed in operations. This is even more dramatic than the favorable cost estimate. If it can be substantiated by future analysis, it would represent a major argument in favor of bioconversion with microalgae. Nutrient integrated microalgal bioconversion systems could provide the large-scale centralized solar energy systems required to provide SNG for the interstate pipeline on which the climatically less-favored regions of the U.S. depend.

CONCLUSIONS

The development of the various microalgal bioconversion concepts proposed above will require a common research effort since the basic problems are similar. Each has specific limitations and advantages over the others which will favor or restrict it in particular locations. It is expected that large-scale systems (above 10 mi^2) would combine aspects of two or more of the proposed concepts. The time scale or cost of the research required for practical applications cannot yet be stated with confidence. Past progress is not a good measure of future expectations; lack of interest in and support of biological solar energy conversion has restricted research and practical applications of microalgal systems to relatively small-scale oxidation ponds for liquid waste treatment. Their upgrading and expansion to combine waste treatment-bioconversion systems and the general adoption of such systems, might be accomplished within the effluent quality (10 mg/liter BOD + 10 mg/liter suspended solids) and time (1985) limits set forth by PL 92-500. Besides a major research and development effort, this would also require major shifts in engineering practice and federal construction grant policies. At present, however, outlook is for a major retreat from PL 92-500 time tables in face of rapidly mounting expenditures. Microalgal systems have the potential of greatly reducing costs and energy requirements for wastewater treatment while simultaneously producing a net energy output. The economics of wastewater treatment would allow sophisticated management and operations (e.g. microstraining) which could not be justified by their energy outputs alone. Large-scale agricultural fertilizer energy or nutrient integrated systems would, in principle, be easier to operate since they are not subjected to the variability exhibited by waste flows or to stringent treatment standards. However, their much stricter economic limitations make it likely that they would require a longer process of experimentation and demonstration. Aggregate microalgal bioconversion systems could have a significant impact on national energy supplies before the end of the century. Quantitatively, this could easily be

1% of U.S. energy usage if waste treatment and fertilizer outputs are counted along with SNG derived for biomass. The limits of microalgal bioconversion are less easily predicted; however, considering water and land requirements, they certainly are below 10% of present U.S. fuel consumption. In combination with other bioconversion systems and solar energy technologies, microalgal bioconversion can be an alternative to fossil or nuclear energy.

VII. FUTURE DEVELOPMENTS

This report covers only the initial laboratory studies and the first set of ourdoors experiments performed with the 3 m^2 pond systems. A new set of experiments was started in January 1977 using the 12 m^2 ponds. The first nine month of operations of these ponds will be reported on in the next report*which will also contain the new information on continuous cultures. The initial attempts to cultivate nitrogen-fixing blue-green algae are the subject of a separate report in preparation** is the continued development of the economic and engineering analysis of microalgae bioconversion which were carried out during 1977.***

In Oct. 1977 a new research plan was initiated dealing in part with cultivation of algae on chemical fertilizers and digester effluents. The aim will be the development of truly large scale systems not limited by waste flows or urban land costs. From the experimental point of view such project should be more readily able to develop the necessary species control techniques since it does not involve the unknown and changeable nature of the sewage. This severely lowers the information content of the present data and is a major limitation in the development of sewage based bioconversion systems. A shift in harvesting methods from microstraining to settling and flotation will also be instituted, since these are potentially much cheaper.

We forsee that microalgae bioconversion technology will be ready within about two years for larger pilot and demonstration projects. We predict that large scale systems will be widley utilized in the United States before the end of the century.

* An Integrated System for Solar Energy Conversion Using Sewage-Grown Microalgae, Benemann et al. 1978, Final Report, San. Engr. Res. Lab., Univ. of Calif., Berk.,

*Fertilizer Production with Nitrogen-fixing Heterocystous Blue-green Algae Benemann et al. 1978, Final Report, San. Engr. Res. Lab., Univ. of Calif. Berk.

*Preliminary Design of the Algae Pond Subsystem of the Photosynthesis Energy Factory, 1977, Final Report, Benemann et al. San. Engr. Res. Lab., Univ. of Calif., Berk.

APPENDICES

A. ALGAL VOLUME CONCENTRATIONS

B. SAMPLE DATA SHEETS

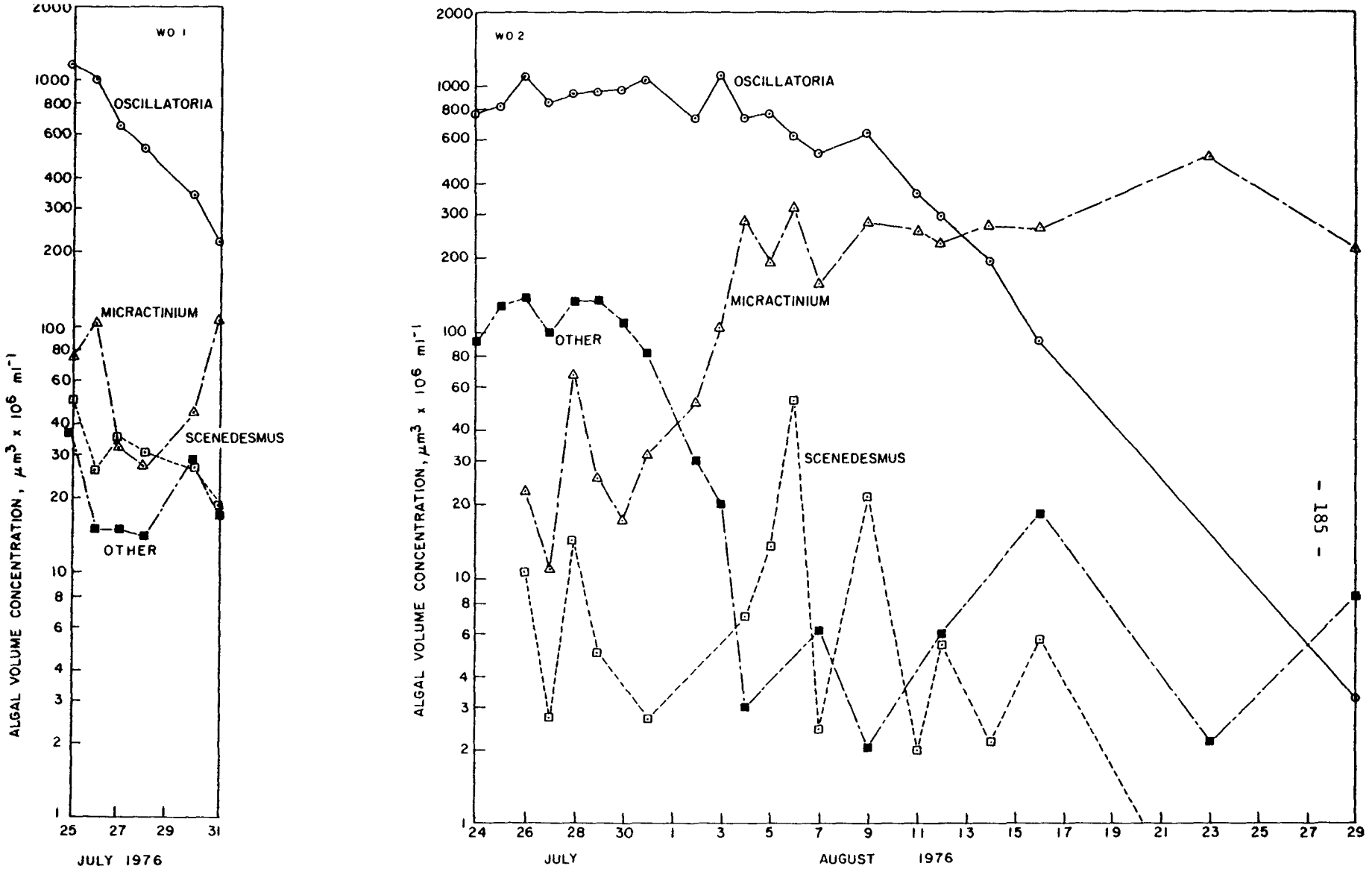


FIGURE A-1. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS WO1 AND WO2.

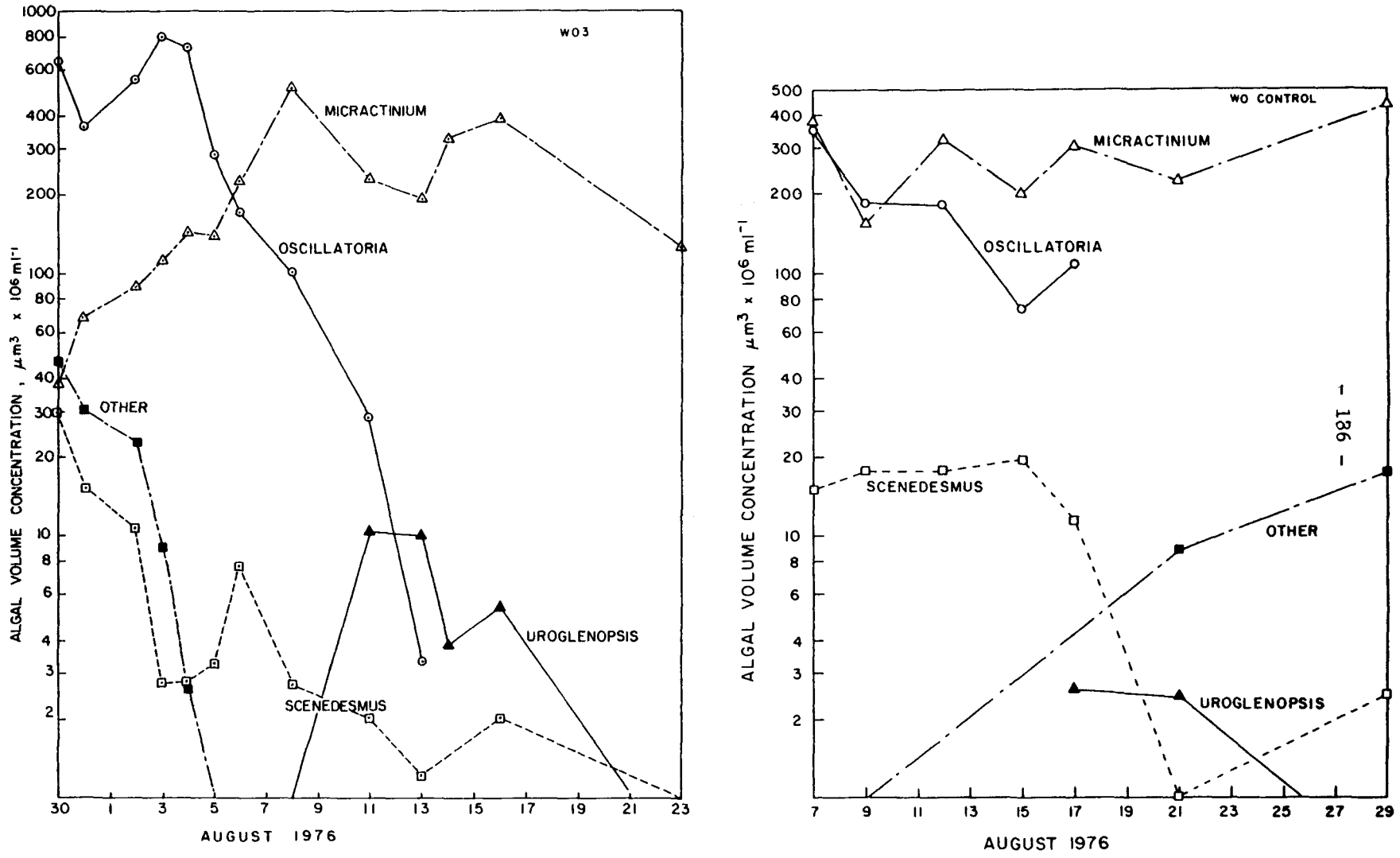


FIGURE A-2. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS WO 3 AND WO CONTROL (W04)

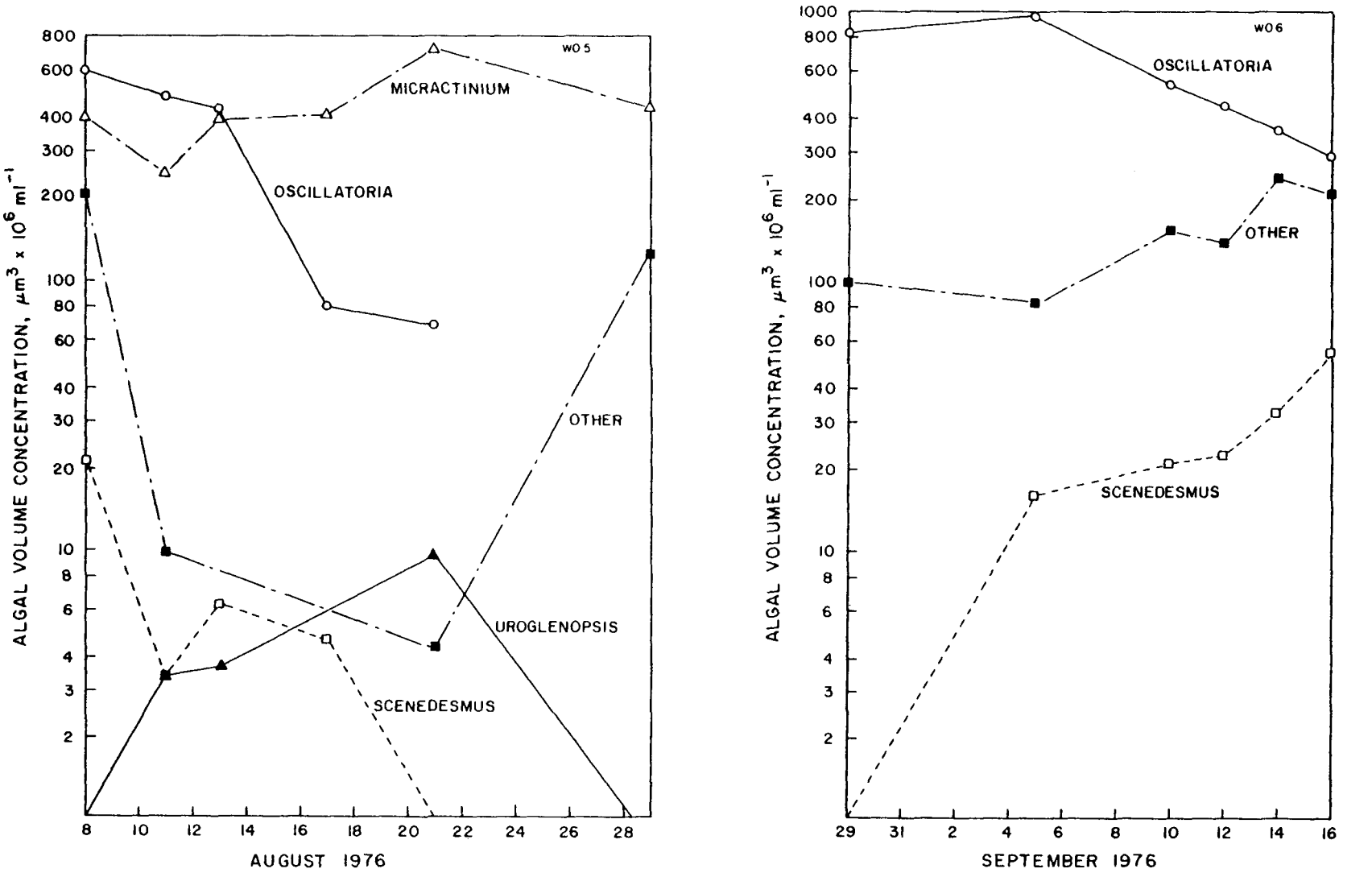


FIGURE A-3. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS WO 5 and WO 6

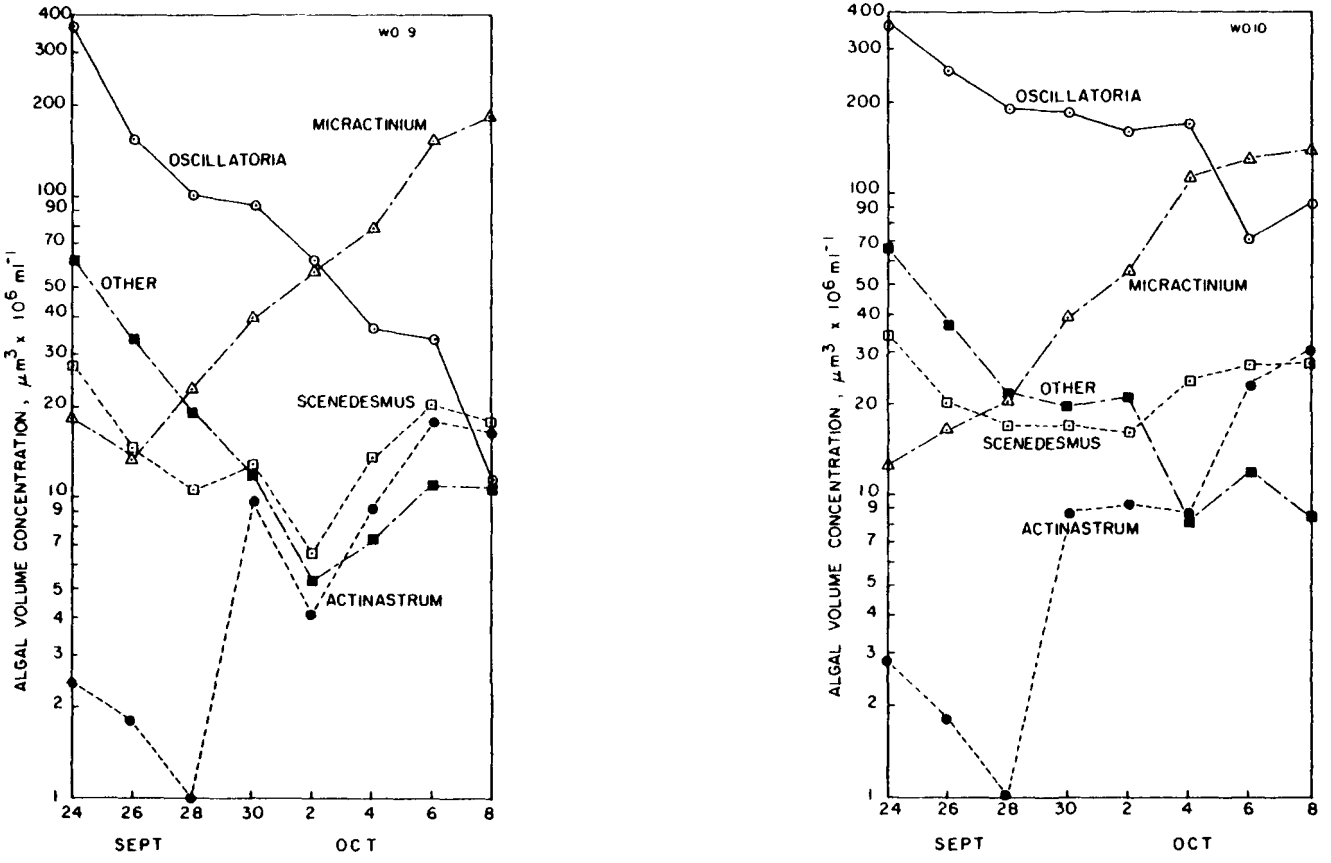


FIGURE A-4. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS WO 9 AND WO 10

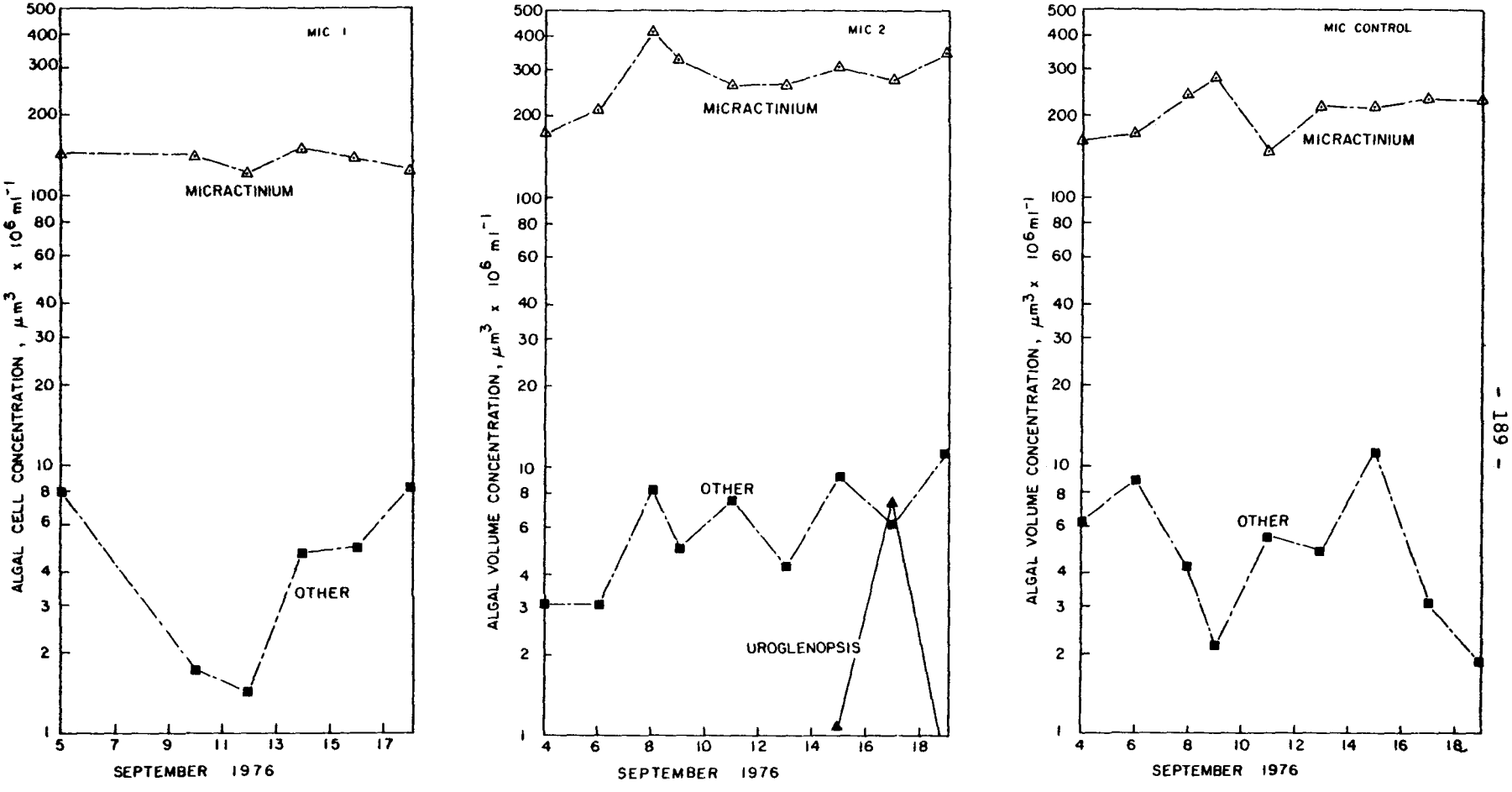


FIGURE A-5. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS MIC 1, MIC 2, AND MIC CONTROL

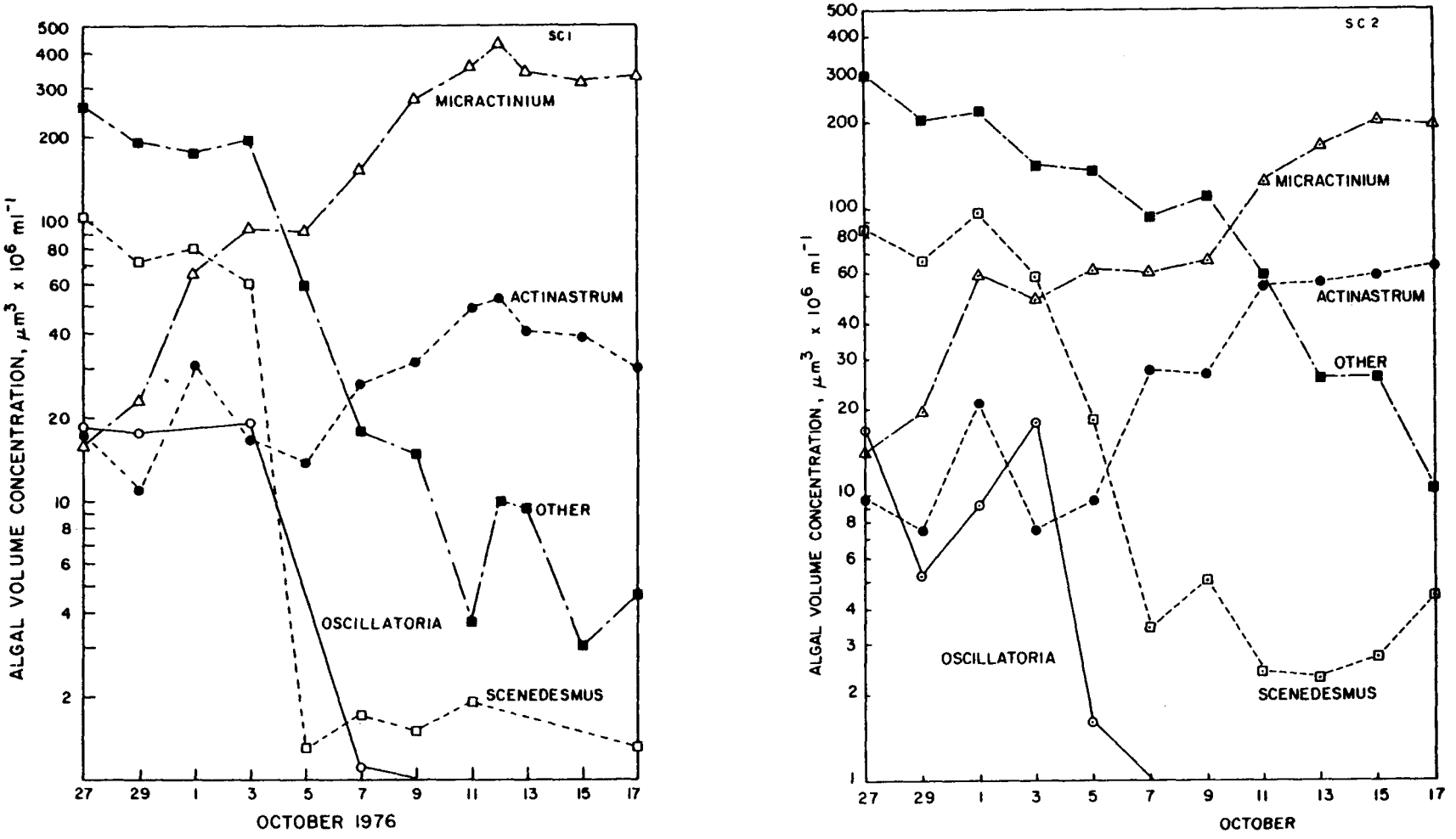


FIGURE A-6. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS SC1 AND SC2

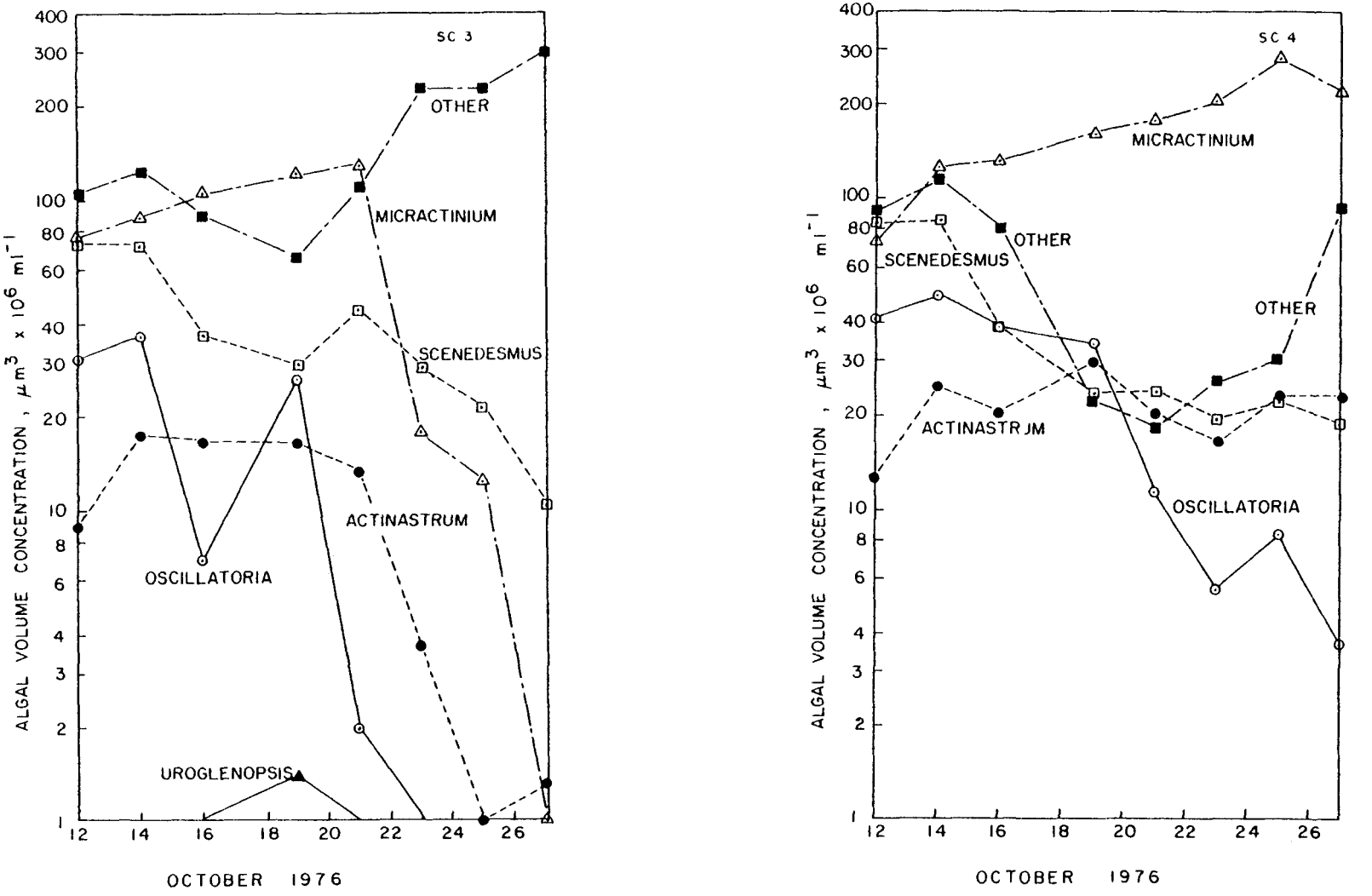


FIGURE A-7. ALGAL VOLUME CONCENTRATIONS FOR EXPERIMENTS SC3 AND SC4

POND DAILY OBSERVATIONS

DATE 8/23/76 DAY Monday

TIME 0900 am / pm WIND none (appreciable) INSOLATION Clear, sunny, no clouds AIR TEMP 19.5 °C NOTES

EXP.	POND I.D.	TEMP. °C	*	pH	KLETT	COVER, %	DEPTH, cm	COLOR		COMMENTS
W.O. 3	mini pond 1	18	A	8.8	78	10	17.5 *	DK olive grn		* Pond depth down from normal level ⇒ will result in lower Klett tuesday due to increased sewage dilution effect.
			B	8.75	79					
W.O. 2	mini pond 2	18	A	9.3	(2x61=)122	10		DK olive green		
			B	9.2	(2x64=)128					
W.O. 5	mini pond 3	17.5	A	9.9	(2x81=)162	10		olive green		
			B	9.75	(2x85=)170					
W.O. Control	mini pond 4	18	A	9.5	(2x40)=80	10		olive green	60	(better Klotts)
			B	9.5	(2x35)=70				58	
Spirulina g. 3	µ pond 2	25	A	8.53	(2x62=)124	40		blue green		filaments are in small clumps
			B	8.5	(2x61=)122					

GENERAL COMMENTS A & B samples are sample taken to evaluate reproducibility of sampling technique which is: large 1000 ml beaker = immersed in pond immediately behind paddlewheel. Beaker of pond water taken out, stirred, and sub-sampled.

	EXP.		W.O. #6	MIC #1	MIC #2	MIC CONTROL	Sp. g. #3	W.O. 7		
	POND I.D.		mP.1	mP.2	mP.3	mP.4	μP-2	μpond 3		
	HARVEST	% Pond	25	30	25	25	—	—		
		Volume, liters	250	300	250	250	—	—		
	RECYCLE,	%	50	-0-	50	-0-				
MICRO-STRAINER	FABRIC	Type	Nylon	Nylon	Nylon	Nylon				
		Openings, μm	26	26	26	26				
	UNIT	Drum #	2	5	4	3				
		Frame #	4	3	2	1				
	ROTATION SPEED	Minarik	30	45 *	30	100				
		RPM								
FLOW	TIME	START	1000	1000	1000	1000				
		STOP	1300	1225	1145	1140				
	PUMP	Minarik, %	70	23 *	27	28				
	AVG FLOW	Liters min^{-1}	1.39	2.07	2.38	2.50				
CONCEN-TRATE	TOTAL,	Liters	16	25	14	16				
	RECYCLED,	Liters	8	-0-	7	-0-				
	EXCESS TO		μpond 3	HRP	HRP	HRP				
REFILL	LIQUOR		SS	150 ℓ effl. +	SS	SS	—	S.S.		
	ADDITIVES			150 ℓ sewage	NONE	NONE		1gm/ℓ HCO_3^- + 1gm/ℓ NO_3^-	+ 7ℓ of osc. from m.p. 1	
	POND DEPTH	Initial	25	25	25	25				
		Final								
PERFORMANCE	KLETT	Pond Comp	—	—	—	—				
		Effluent	30	11	8	5				
		Concentrate	88x25 = 2200	11x49 = 490	50x20 = 1000	10x68 = 680				
	REMOVAL,	%								
	DURATION,	Days								
	PRODUCTIVITY,	Klett-liters / pond-day	35.2	12.25	14.0	10.28				
			17.6	12.25	7.0	10.28				

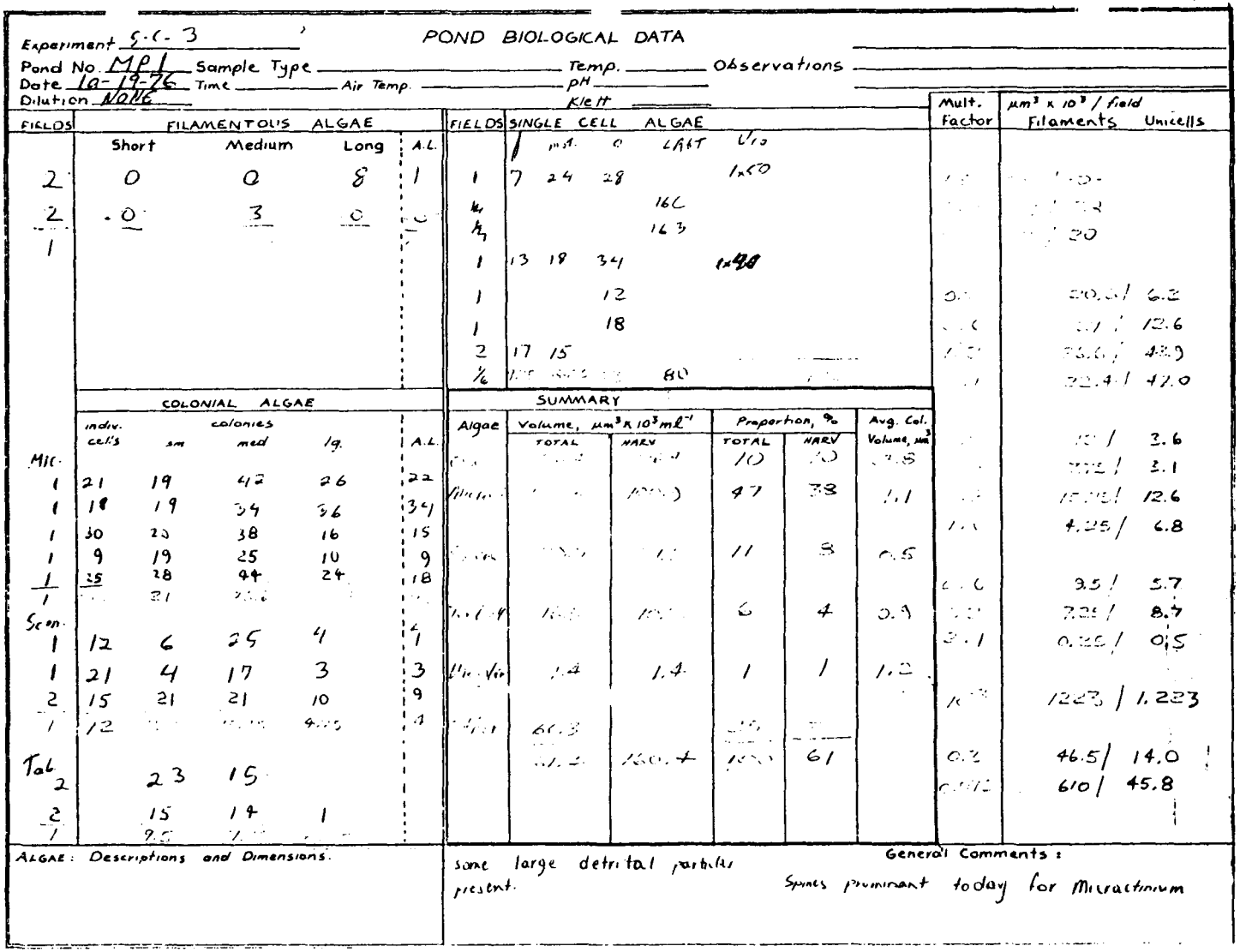
COMMENTS

Stop = 10:25

(* New Jabsco pump (plastic) + ⅓ Hp motor)

Vol μpond 3 @ 30 cm depth = 30 x 80 x 61 cm = 146.4 ℓ
add 146.5 gm HCO_3^-, 146.5 gm NO_3^-.

FIGURE B-2. "POND HARVESTING DATA" WORKSHEET



POND BIOLOGICAL DATA

Experiment E-C-3
Pond No. MP1 Sample Type ____ Temp. ____ Observations ____
Date 10-19-76 Time ____ Air Temp. ____ pH ____
Dilution None Kleit ____

FILAMENTOUS ALGAE

FIELDS	Short	Medium	Long	A.L.
2	0	0	8	1
2	0	3	0	1
1				

COLONIAL ALGAE

	indiv. cells	colonies sm	colonies med	colonies lg.	A.L.
Mic.					
1	21	19	42	26	22
1	18	19	34	36	34
1	30	20	38	16	15
1	9	19	25	10	9
1	35	28	44	24	18
1		31			
Scen.					
1	12	6	25	4	4
1	21	4	17	3	3
2	15	21	21	10	9
1	12				
Tab. 2		23	15		
2		15	14	1	
1					

SINGLE CELL ALGAE

FIELDS					
1	7	24	28		1x50
½				160	
½				163	
1	13	18	34		1x98
1			12		
1			18		
2	17	15			
⅙				80	

SUMMARY

Algae	Volume, µm³ x 10³ mL⁻¹ TOTAL	Volume, µm³ x 10³ mL⁻¹ HARV	Proportion, % TOTAL	Proportion, % HARV	Avg. Col. Volume, µm³
			10	10	
			47	38	1.1
			11	8	0.5
			6	4	0.9
	1.4	1.4	1	1	1.2
		160.4		61	

Mult. Factor	µm³ x 10³ / field Filaments	Unicells
0.1	20.4 /	6.2
		12.6
		48.9
	22.4 /	47.0
		3.6
		3.1
		12.6
	4.25 /	6.8
	3.5 /	5.7
	7.35 /	8.7
0.1	0.35 /	0.5
	1223 /	1.223
0.2	46.5 /	14.0
	610 /	45.8

ALGAE: Descriptions and Dimensions.

some large detrital particles present.

General Comments:
Spines prominent today for Micractinium

FIGURE B-3. "POND BIOLOGICAL DATA" WORKSHEET USED IN COMPUTING ALGAL VOLUME CONCENTRATIONS

POND PRODUCTION DATA SHEET

Calculated by D.E.
Checked by BLK

POND I.D. MP-1
POND AREA 3.0 m^2
EXPERIMENT S.C. #3

DATE			10/12	10/13	10/14	10/15	10/16	10/17	10/18	10/19	10/20	10/21
HARVEST		%	33	33	33	33	33	33		33	33	33
		liters	330	330	330	330	330	330		330	330	330
RECYCLE,	CONC., %	Nominal	0	0	0	0			NONE			
		Adjusted	0	0	0	0						
	EFFL.	%	0	0	0	0						
		liters	0	0	0	0						
CONCENTRATE, liters		Total	32	16.5*	24	22	21	24		24	15	16
		Recycled	0	0	0	0						
		Net	32	16.5	24	22	21	24		24	15	16
KLETT BASIS		Conc.	190	370	200	150	150	160		200	150	130
		Pond	53	53	66	53	51	58	61	58	50	57
		Effl.	46	40	48	47	43	43		50	53	55
		Removal, %	13.2	24.5	27.3	11.3	15.6	17.24		13.8	51	3.5
NET PRODUCTION, Klett-liters / m^2-day x 10^3		Total	7.[illegible]	6435	6[illegible]	[illegible]10	5780	6[illegible]	3550 ?	3550	[illegible]	6745
		Harv.	[illegible]027	2035	1[illegible]	[illegible]10	1[illegible]	130[illegible]	800 ?	800	75[illegible]	6[illegible]
DRY WEIGHT BASIS, mg/l		Conc		858							334	
		Pond		124							[illegible].7	
		Effl.		[illegible]5							[illegible]	
		Removal, %		42.5							22.8	
NET PRODUCTION, grams / m^2-day		Total		12.[illegible]4							14.34	
		Harv		4.71[illegible]							1.[illegible]7	
ASH-FREE DRY WEIGHT BASIS, mg/l		Conc.		74.[illegible]							250[illegible]	
		Pond		25.[illegible]							12.2	
		Effl.		63.6							[illegible]	
		Removal, %		[illegible]							13.1	
NET PRODUCTION grams / m^2-day		Total		[illegible].[illegible]							12.43	
		Harv		4.37[illegible]							1.75	
CHLOROPHYLL a BASIS, mg/l		Conc		[illegible]3							[illegible].[illegible]	
		Pond		[illegible].[illegible]							2.2	
		Effl.		[illegible].[illegible]							[illegible].[illegible]	
		Removal, %		57.2							12.[illegible]	
NET PRODUCTION mg Chl a / m^2-day		Total		202.7							253.1	
		Harv		[illegible]6.[illegible]							40.75	
COMMENTS flow l/min			1.94	2.75*	3.47?	3.30	3.14	3.[illegible]	[illegible] 3.33		5.55	5.55

FIGURE B-4. "POND PRODUCTION DATA SHEET" USED TO CALCULATE ALGAL-REMOVAL EFFICIENCIES AND PRODUCTION VALUES

EXPERIMENT NO. Mic #1 NET HARVESTABLE
POND NO. MP-2 PRODUCTION

DATE 1976	KLETT-LITERS / m^2-day	gm dry wt. / m^2-day	gm. ash-free dry wt. / m^2-day	mg Chl a / m^2-day	
9-3	3200 *	13.3 *	12.7 *	201 *	first two harvests dropped
9-4	3480 *				
9-5	4400				
6	4083				
7	2667	7.84	7.04	153	
8	2383	7.10	6.72	177	
9	4853	13.0	12.2	328	
9-10	4160	10.4	9.53	273	
11	3109				
12	4206				
13	3520	4.64	3.84	214	
14	4390 *	15.6 *	14.2 *	181 *	mixed too fast
9-15	4373	13.1	12.1	268	
16	3787	12.0	10.9	232	
17	3080	11.4	10.4	216	
18	3413				
19	3563				
	$\bar{P}$ = 3685	$\bar{P}$ = 9.93	$\bar{P}$ = 9.09	$\bar{P}$ = 233	

FIGURE B-5. "NET HARVESTABLE PRODUCTION" WORKSHEET USED IN AVERAGING DAILY VALUES FOR HARVESTABLE PRODUCTION

EXPERIMENT NO. MC #1 NET TOTAL PRODUCTION

POND NO. MP-2

DATE 1976	KLETT-LITERS m^2-day	gm dry wt. m^2-day	gm ash-free dry wt. m^2-day	mg Chl. a m^2-day	
9-3	4350 *	17.7 *	14.1 *	212 *	first two harvests dropped
4	4230 *				
9-5	5000				
6	4633				
7	3617	9.20	8.34	169	pond densities not available for this day
8	3183	8.10	7.72	197	
9	5903	14.4	13.4	347	
9-10	4760	11.5	10.5	295	
11	3759				
12	4681				
13	4270	5.69	4.86	227	
14	4940 *	16.7 *	15.0 *	192 *	mixed too fast
9-15	5023	13.9	12.8	279	
16	4187	13.0	11.9	241	
17	3730	12.7	11.6	229	
18	4033				
19	4153				
	$\bar{P}$: 4352	$\bar{P}$ = 11.06	$\bar{P}$ = 10.14	$\bar{P}$ = 248	
	$\Delta P = \frac{(42.8-52)1000}{3(14)}$	$\Delta P = \frac{(.149-.141)1000}{3(9)}$	$\Delta P = \frac{(.141-.130)1000}{3(9)}$	$\Delta P = \frac{(3.43-3.22)1000}{3(9)}$	
	= -219	= +0.30	= +0.41	= +7.8	
	$\bar{P}_G$ = 4133	$\bar{P}_G$ = 11.36	$\bar{P}_G$ = 10.55	$\bar{P}_G$ = 256	

FIGURE B-6. "NET TOTAL PRODUCTION" WORKSHEET USED IN AVERAGING DAILY VALUES FOR TOTAL PRODUCTION AND IN CALCULATING GROSS PRODUCTION VALUES

EXPERIMENT Mic #2

MP-3

		DATE										
		9-3	9-7	9-8	9-9	9-10	9-13	9-14	9-15	9-16	9-17	
TSS	Settled Sewage	103.29	119	71.86	84	65.1	[illegible]	75.38	102.17	64.17	110.0	93.7
VSS	Settled Sewage	88.29	99.71	61.43	60.71	55.5	79.29	60.77	87.17	53.67	87.67	78.2
BOD5	Settled Sewage											
	Effluent											
	Removal, %											
												Avg.
COD	Settled Sewage	379.4		365.8		284	324		398		365	347
	Effluent	229.2*		186.9		232	49*		[illegible]		112	160
	Removal, %	39.6		48.1		18.3	[illegible]		72.4		69.3	53.9
	Pond			42.9		47			56.7			
												Avg.
$NO_3^- + NO_2^-$-N	Settled Sewage					10.9						—
	Effluent										4.1	—
												Avg.
NH_3-N	Settled Sewage		25.30			24.7		[illegible]		[illegible]	[illegible]	23.0
	Effluent		2.15		1.75	[illegible]		2.28*		[illegible]	[illegible]	1.75
	Removal, %		91.5			78.7		86.5		94.2	85.3	92.4
												Avg.
ORTHO-P	Settled Sewage		6.9		6.0			7.27		7.0		7.44
	Effluent		7.2		[illegible]			8.65*		[illegible]		5.21
	Removal %				37			25.5		48.3		30.0
												Avg.
TOTAL-P	Settled Sewage		13.2		6.21			10.26		12.4		[illegible]
	Effluent		[illegible]		[illegible]			[illegible]*		[illegible]		7.18
	Removal, %		34.5		.15			28.1		[illegible]		34.9
	Pond				12.0			[illegible]		[illegible]		
												Avg.
Cl, Cl + Br	Pond	2.95		7.45	[illegible]	7.23		[illegible]	[illegible]	[illegible]	[illegible]	
	Effluent	0.27	0.54	2.66	0.18	0.36	[illegible]	0.13	[illegible]	0.14	[illegible]	
	Removal, %	90.8*		91.1	89.1	[illegible]		[illegible]*	[illegible]	[illegible]	[illegible]	95.0
	Concentrate	75.14	74.50	[illegible]	[illegible]	[illegible]	106.10	[illegible]	[illegible]	[illegible]	[illegible]	

FIGURE B-7. WORKSHEET USED TO SUMMARIZE AND AVERAGE NUTRIENT CONCENTRATIONS IN INFLUENT AND EFFLUENT STREAMS OF EXPERIMENTAL PONDS

REFERENCES

1. Kilgore, J.D., L. J. Shannon, and P. Gorman. "Environmental Studies on the St. Louis Union Electric Refuse Firing Demonstration" in Clean Fuels from Biomass, Sewage, Urban Refuse, Agricultural Wastes, Institute of Gas Technology, pg. 413, 1976.

2. Jewell, W. T., H. R. Davis, W. W. Gunkel, D. J. Lathwell, J. H. Martin, Jr., J. R. McCarty, G. R. Morris, D. R. Price, and D. W. Williams. Bioconversion of Agricultural Wastes for Pollution Control and Energy Conservation, ERDA-Natl. Tech. Inf. Service, TID-27164, 1976.

3. Alich, J. A. and R. E. Inmann Effective Utilization of Solar Energy to Produce Clean Fuel, Stanford Research Institute, Menlo Park, California, 1974.

4. Ryther, J. H. in Proc. Capturing the Sun through Bioconversion, Washington Center for Metropolitan Studies, Washington, D.C., March 10, 1976.

5. Round, F. E. The Biology of the Algae, Edward Arnold Ltd., London, 1965.

6. Fogg, G. E. Algal Cultures and Phytoplankton Ecology, Univ. Wisconsin Press Madison, Wisconsin, 1965.

7. Stewart, W.D.P. Algal Physiology and Biochemistry, Univ. of Calif. Press, Berkeley, 1974.

8. Burlew, J. S. (Ed.). Algal Culture from Laboratory to Pilot Plant, Carnegie Institute of Washington, Publication 600, Washington, D.C., 1953.

9. Durand-Chastel, H. and G. Clement. Proc. 9th Int. Cong. Nutrition, Mex., 1972, 3:85-90 Karger, Basel 1975

10. Oswald, W. J. and H. B. Gotaas "Photosynthesis in Sewage Treatment," Trans. Am. Soc. Civil Engrs., 122, 73-105, 1957.

11. Gloyna, E. F., J. Melina, Jr., and E. M. Davis (Eds.). Ponds as a Wastewater Treatment Alternative, Center for Research in Water Resources, Univ. of Texas, Austin, 1976.

12. Golueke, C. G., W. J. Oswald, and H. B. Gotaas. Recovery of Algae from Waste Stabilization Ponds, Univ. of Calif., Berk. SERL Issue No. 7, IER Series 44, 1957.

13. Oswald, W. J. and R. Ramani. "The Fate of Algae in Receiving Waters," In Ponds as a Waste Treatment Alternative, E. F. Gloyna, J. Melina Jr., and E. M. Davis (eds.), Center for Research in Water Resources, Univ. of Texas, Austin, p. 111-122, 1976.

14. Soeder, C. J. "Primary Production of Biomass in Freshwater with Respect to Microbial Energy Conversion, In Proc. Seminar on Microbial Energy Conversion, Gottingen, p. 59-68, 1976.

15. Shelef, G., R. Moraine, A. Meydan, and E. Sandbank. "Combined Algal Production-Wastewater Treatment Systems," In Proc. Seminar Microbial Energy Conversion, Goettingen, Germany, p. 286, 1976.

16. Golueke, C. G., and W. J. Oswald. "Biological Conversion of Light Energy to the Chemical Energy of Methane," Applied Microbiology, 7:219, 1959.

17. Golueke, C. G., W. J. Oswald, and H. B. Gotaas. "Anaerobic Digestion of Algae," Applied Microbiology, 5:1 47, 1957.

18. Uziel, M., W. J. Oswald, and C. G. Golueke. Solar Energy Fixation and Conversion with Algal-Bacterial Systems, Final Report to National Science Foundation, San. Eng. Res. Lab., College of Eng., and School of Pub. Hlth., Univ. of Calif., Berkeley, April 1975.

19. Oswald, W. J. "Gas Production from Microalgae," Proc. Symposium "Clean Fuels from Biomass, Sewage, Urban Refese, and Agricultural Wastes, Institute of Gas Technology, Orlando, Florida, p. 311-324, 1976.

20. Oswald, W. J. and C. G. Golueke. "Biological Transformation of Solar Energy," Adv. Appl. Microbiol., 2:223-262, 1960.

21. Oswald, W. J. and J. R. Benemann. "A Critical Analysis of Bioconversion with Microalgae," Biological Solar Energy Conversion, pgs. 379-396, 1977.

22. Benemann, J. R., B. L. Koopman, D. C. Baker, J. C. Weissman, and W. J. Oswald. "A Systems Analysis of Bioconversion with Microalgae," Clean Fuels from Biomass and Wastes, Institute of Gas Technology, Chicago, Ill., pp. 101-126, Jan. 25-28, 1977.

23. Oswald, W. J. and J. R. Benemann. "Fertilizer from Algal Biomass," Proc. RANN II Symposium, Natl. Science Foundation, Washington, D.C., 1976.

24. Goldman, J. C. and J. H. Ryther. "Temperature-Influenced Species Competition in Mass Cultures of Marine Phytoplankton," Biotechnology and Bioengineering, 1976.

25. Golueke, C. G. and W. J. Oswald. "Harvesting and Processing Sewage-Grown Planktonic Algae," J. Water Poll. Cont. Fed., 37, 471-497, 1965.

26. Parker, D. S. in Ponds as a Wastewater Treatment Alternative, E. F. Gloyna, F. F. Malina, Jr., and E. M. Davis (Eds.), Center for Research in Water Resources, College of Engineering, Univ. of Texas, Austin, Texas, p. 401-416, 1976.

27. Oswald, W. J. Ibid, p. 257-272, 1976.

28. Uziel, M., W. J. Oswald, and C. G. Golueke. "Integrated Algal-Bacterial Systems for Fixation and Conversion of Solar Energy," Proc. Ann. Meeting Am. Assn. Adv. Science, 1975.

29. Herbert, D., R. Elsworth, and R. C. Telling. "The Continuous Culture of Bacteria: A Theoretical and Experimental Study," J. Gen. Microbiol., 14, 601-622, 1956.

30. Herbert, D. "A Theoretical Analysis of Continuous Culture Systems," In Continuous Culture of Microorganisms, Monograph No. 12, Soc. Chem. Ind., London, p. 21-53, 1961.

31. Canale, R. P. "Predator-Prey Relationships in a Model for the Activated Process," Biotech. Bioeng., 11, 887-907, 1969.

32. Ghosh, S. and F. G. Pohland. "Population Dynamics in Continuous Cultures of Heterogeneous Microbial Populations," Dev. Ind. Microbiol., 12, 295-311, 1971.

33. Grenney, W. J., D. A. Bella, and C. Herbert. "A Theoretical Approach to Interspecific Competition in Phytoplankton Communities," Am. Naturalist, 107, p. 405-425, 1973.

34. Ramanathan, M. and A. F. Gaudy. "Steady-State Model for Activated Sludge with Constant Recycle Sludge Concentration," Biotech. Bioeng., 13, 125-145, 1971.

35. Reilly, P. J. "Stability of Commensalistic Systems," Biotech. Bioeng., 16, 1373-1392, 1974.

36. Stewart, F. M. and B. R. Levin. "Partitioning of Resources and the Outcome of Interspecific Competition: A Model and Some General Considerations," Am. Naturalist, 107, 171-198, 1973.

37. Titman, D. "Ecological Competition Between Algae: Experiments Confirmation of Resource-based Competition Theory," Science, 192, 463-465, 1976.

38. Levins, R. Evolution in Changing Environments, Princeton University Press, Princeton, New Jersey, p. 55, 1968.

9. Lotka, A. J. "The Growth of Mixed Populations: Two Species Competing for a Common Food Supply," J. Wash. Acad. Sci., 22, 461-469, 1932.

0. Volterra, V. and U. D'Ancona, "Les Associations Biologiques au Point de Vue Mathematique," Hermannet Cie, Paris, 1935.

1. Koga, S. and A. E. Humphrey. "Study of the Dynamic Behavior of the Chemostat System," Biotech. Bioeng., 9, 375-386, 1967.

2. Ramanathan, M. and A. F. Gandy, Jr. "Effect of High Substrate Concentration and Cell Feedback on Kinetic Behavior of Heterogenous Populations in Completely Mixed Systems," Biotech. Bioeng., 11, 207-237, 1969.

3. Benemann, J. R., B. L. Koopman, J. C. Weissman, and W. J. Oswald. "Biomass Production and Waste Recycling with Blue-Green Algae," Microbial Energy Conversion, Schlegel, H.G. and Barnea, J. eds., (Erich Goltz, K.G., Gottingen, Germany) pp. 413-426, Oct. 4-8, 1976.

4. Benemann, J. R., J. C. Weissman, B. L. Koopman, and W. J. Oswald. "Energy Production with Microalgae," Nature, 268:19-23, 1977.

5. Weissman, J. C., J. R. Benemann, B. L. Koopman, and W. J. Oswald. "Algal Species Control through Biomass Recycle," 1977, in preparation.

6. Standard Methods for the Examination of Water and Wastewater, 13th ed. Amer. Pub. Hlth. Assn., Washington, D.C., 1976.

47. Holden, M. "Chlorophylls," In Chemistry and Biochemistry of Plant Pigment Goodwin, I.W.(ed.), Acad. Press, New York and London, pp. 461-488, 1965.

48. Solarzano, L. "Determination of Ammonia in Natural Waters by the Phenochypochlorite Method," Limnol. and Oceanogr., 14, 799-801, 1969.

49. Henriksen, A. "An Automated Method for Determining Nitrate and Nitrite in Fresh and Saline Waters, Analyst, 90, 83-88, 1965.

50. Gotterman, H. L. Methods for Chemical Analysis of Fresh Waters, Blackwel 1969.

51. Allen, M. B. and D. I. Arnon. "Studies on Nitrogen-Fixing Blue-Green Alga I. Growth and Nitrogen Fixation by Anabaena cyclindrica, Lemm. Plant Phys 30, 366-372, 1955.

52. Sorgenloos, P., E. Van Outrye, G. Persoone, and A. Cottori-Reyanaerts. "N Type of Turbidostat with Intermittent Determination of Cell Density Outsi the Culture Vessel," Appl. Envir. Microbiol., 31, 327-331, 1976.

53. Kosaric, N., H. T. Nguyen, and Bergobnou. "Growth of Spirulina maxima Algae in Effluents from Secondary Wastewater Treatment Plants," Biotech and Bioeng., 26, 881-896, 1974.

54. Clement, G., C. Giddey, and R. Menzi. "Amino Acid Composition and Nutriti Value of the Alga, Spirulina maxima," Journ. of Sci., Food, and Agric., 18, 11, 497-501, 1967.

55. Baron, C. "Alques Alimentaries: Comparison Biochimique de Spirulina platensis et de S. maxima," IX. Int. Congr. Nutr., Mexico, 1972.

56. Leonard, J. and P. Compere. "Spirulina platenis (Gom.) Geitl., Algue Bleu de Grande Valeur Alimentaire par sa Richesse en Proteines," Bull. Jard. B Nat. Belg., 37, 1, suppl. 23 p., 1967.

57. Grisanti, N. and W. J. Oswald. "Protein from Algae", A.I.Ch.E. National Meeting, Kansas City, Missouri, 1976.

58. Ortega, M. "Study of the Edible Algae of the Valley of Mexico," Botanica Marina, 15, 162-6, 1972.

59. Garner, N. L. "Arthrospira maxima," Univ. Calif. Publs. Bot., 6,377-9, 1917.

60. Busson, F. "Spirulina platensis (Gom) Geitler et Spirulina geitlei , J. de Toni, Cyanophycees Alimentaires, Sevvice dr Sante, Parc du Pharo, Mars 1971.

51. Iltis, A. "Phytoplancton des eaux natronees due Kanem (Tchad). I. les lacs permanents a Spirulines." Cah. O.R.S.T.O.M., ser. Hydrobiol. III, 29-44; II 119-125, IIII 3-19, IV 53-59, 1969.

52. Pinevich, V.V., Verzilin, N.N., and Mikhailova, A.A. Fiziol. Rastenii 17: 1037, 1970.

53. Al'bitskaya, N.O. et al. "Physiological and biochemical pecularities of a culture of Spirulina platensis." Microbiologiya 43(4): 649-53, 1973.

54. Wouters, J. "Influence due milieu mineral sur le rendement pondural et le spectra des acides amines de Spirulina platensis (Gom.) Geitler," Ann. Physiol. veg. Univ. Bruxelles 14(4): 85-105, 1969.

55. Pinta, M. et F. Busson. "Note preliminaire sur la composition en elements mineraux et oligo-elements de Spirulina platensis (Gom.) Geitler et de Spirulina maxima (Setch. et Gardn.)"Geitler. Med. Trop. 29: 617-619, 1969.

56. Laquerbe, B., F. Busson, and Maigrot M. "Sur la composition en elements mineraux de deux Cyanophycees, Spirulina platensis (Gom.) Geitler et Sp. geitleri",J. de Toni, C.R. Acad. Sc. Paris 270: 2130-2132, 1970.

57. Zarrouck G. "Contribution a l'etude d'une Cyanophycee. Influence de dovers facteurs physiques et chimiques sur lar croissaner et la photosynthese de Spirulina maxima (Satch. et Gardner) Geitler." These Doctorat, Nr. A.O. 1064, Paris, 1966.

68. Fauchner, O. Etude de l'eau de Mar et de l'utilisation de l'uree comme source d'Azote pour la culture de Spirulina maxima. Univ. de Sherbrooke, 1972.

69. Nguyen, H.T., N. Kosaric, and M.A. Bergougnou. "Some nutritional characteristics of Spirulina maxima algae grown on effluents from biological treatment plant." Can. Inst. Food. Sci. Technol. J. 7(2): 114-116, 1974.

70. Oswald, W.J. "High-Rate Pond in Waste Disposal." Developments in Industrial Microbiology 4, (1963).

71. Oswald, W.J.; Golueke, C.G.; Cooper, R.C.; Meyers, J.H.; Hintz, H.; and Gee, H.K. Nutritional and Disease Transmitting Potential of Sewage-Grown Algae. First Progress Report, Sanitary Engineering Research Laboratory, University of California, SERL Report No. 64-6, Oct., 1964..

72. Oswald, W.J. "Light Conversion Efficiency of Algae Grown in Sewage." Journal of the Sanitary Engineering Division, July, 1960.

73. Shelef, G.; Schwarz, M.; Scheckter, H. "Prediction of Photosynthetic Biomas Production in Accelerated Algal-bacterial Wastewater Treatment Systems." Advances in Water Pollution Research. S.H. Jenkins (ed.). Pergamon Press, Oxford, pp. 181-190, 1973.

74. California Department of Water Resources. "Bioengineering Aspects of Agricultural Drainage, San Joaquin Valley, California." Report No. 174-10, 1971.

75. McGarry, M.G. and Tongkasame, C. "Water Reclamation and Algae Harvesting." Journal Water Poll. Control Federation 43:824-835, 1971.

76. Dodd, J.C. and Dougas, P.P. "High Rate Ponds for Algae Harvesting From Wastewater." Unpublished, 1976.

77. Oswald, W.J.; Meron, Aaron; and Zabat, M.D. "Designing Waste Ponds to Meet Water Quality Criteria." In, R.E. McKinney (ed.). Proceedings of the Second International Symposium for Waste Treatment Lagoons. Kansas City, Missouri. Basin Engineering Health Council and Federal Water Quality Administration, p. 186-194, 1970.

78. Oswald, W.J.; Gotaas, H.B.; Golueke, C.G.; and Kellen, W.R. "Algae in Waste Treatment." Sew. and Indus. Wastes 29:437-457, 1957.

79. Golueke, C.G. and Oswald, W.J. "Harvesting and Processing Sewage-grown Planktonic Algae." Journal Water Poll. Cont. Fed. 37(4), p. 471-498, 1965.

80. McGarry, M.G. "Algal Flocculation with Aluminum Sulfate and Polyelectrolytes." Journal Water Poll. Cont. Fed. 42(5), R191-R201, 1970.

81. Folkman, Yair and Wachs, A.M. "Removal of Algae from Stabilization Pond Effluents by Lime Treatment." Water Research 7(3), 419-435, 1973.

82. Middlebrooks, E.J. and Marshall, G.R. "Stabilization Pond Upgrading with Intermittent Sand Filters." In E.J. Middlebrooks et al (eds.). Upgrading Wastewater Stabilization Ponds to Meet New Discharge Requirements. Logon, Utah: Utah Water Research Laboratory, PRWG159-1, p. 47-69, 1974.

83. Graham, H.J. and Hunsinger, R.B. "Phosphorus Removal in Seasonal Retention Lagoons by Batch Chemical Precipitation." Unpublished paper, Ontario Ministry of the Environment, Research Branch; 135 Street, Clair West; Toronto, Ontario, Canada.

84. DeLatour, Christopher. "Magnetic Separation in Water Pollution Control." Paper presented at the 1973 Intermag Conference. Washington, D.C., April 24-27.

85. Cruver, J.E. "Membrane Processes," Chapter 7. In Physiochemical Processes for Water Quality Control. W.J. Weber, (ed.). New York: Wiley-Interscience.

86. Hagan, R.M. "Energy Requirements for Wastewater Treatment, Part 2." Water and Sewage Works: 52 , December, 1976.

87. Stone, R.W. ; Parker, D.S. ; and Cotteral, J.A. "Upgrading Lagoon Effluent for Best Practicable Treatment." Journ. Water Poll. Control Federation 47(8), p. 2019-2042 , 1975.

88. Lynch, G. Napa, California Sanitation District. Personal Communication, 1976.

89. Ramani, A.R. "Factors Influencing Separation of Algal Cells from Pond Effluents by Chemical Flocculation and Dissolved-Air Flotation." Ph.D. Dissertation, University of California, Berkeley, 1974.

90. Black, A.P.; Wertz, C.F.; and Henry, C.R. "Recalcining Softening Sludge at Miami, Florida." Water Works Eng., 104:214 March, 1951.

91. Gregor, H.P. Personal Communication , 1976.

92. Hiatt, A.L. "PL 92-500: Phase Isolation Helps Oxidation Pond Meet EPA 1977 Discharge Requirements." Water and Sewage Works: 42 , December, 1975 .

93. Oswald, W.J. and Ramani, Roy. "Evaluation of a New Method for Algae Separation." Final Report submitted to Regents of the University of California , June 30, 1974.

94. Ives, K.J. "The Significance of Surface Electric Charge on Algae in Water Purification." J. Biochem. and Microbiol. Techn. and Engr. I: 37, 1959.

95. Diaper, E.W.J. "Tertiary Treatment by Microstraining." Water and Sewage Works: 202 , June, 1969.

96. Comptroller General of the United States, Environmental Protection Agency. "Better Data Collection and Planning Is Needed to Justify Advanced Waste Treatment Construction." Report to the Congress. CED-77-12, December 21, 1976.

97. Avron, M. Personal Communication, 1977.

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