BIOLOGICAL FLOCCULATION OF MICROALGAE

By

HO KANG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1987

TO MY WIFE
KINAM LEE KANG

ACKNOWLEDGEMENTS

Principal funding for this research was provided by the Florida Water Resources Research Center. Additional funds were contributed by the University of Florida, the GRI/IFAS Methane from Biomass Project and the University of Florida Engineering and Industrial Experiment Station.

The author wishes to express his sincere gratitude to Dr. Ben Koopman for his introduction to this most interesting field of study and for his expert guidance, encouragement and enthusiastic support throughout the course of this research. Thanks are also extended to Dr. Edward F. Lincoln, who was always available for discussion and advice, to Dr. John Zeltek, Jr., who provided expert guidance in the wastewater treatment aspects, and to Dr. Roger Nordstedt and Dr. Dinesh O. Shah for their contributions to his education.

The author also acknowledges the patience, understanding and loving support of his parents, his wife, Kinam, and his children, Eyn and Grace, who all contributed in no small way to the successful completion of this undertaking. Special thanks are expressed to his brother-in-law, Kicheon Lee, for his financial support.

The author also wishes to express his appreciation to Dr. Chang-won Kim, Dr. Robert Ryczack, Dr. Sang-ill Lee, Joe Angley, Dr. Jonathan Earle, Ms. Veronica Campbell and

Chan-won Lee for their assistance with laboratory analyses and relevant suggestions during this research. Special thanks are extended to Jong-pil Yoon and Yong-sun Choi for their support in lending their computer and printer.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

BIOLOGICAL FLOCCULATION OF MICROALGAE

By

HO KANG

May 1987

Chairman: Ben Koopman
Co-chairman: Edward P. Lincoln
Major Department: Environmental Engineering Sciences

The effect of environmental, biological and operational variables on biological flocculation of microalgae grown on anaerobically treated swine waste was investigated in laboratory and field scale systems. Environmental variables considered were season, rainfall, temperature and solar irradiance. The biological variable considered was algal species composition. Operational variables included flow mixing, waste loading, waste pretreatment, algal biofloc and bacterial seeding, pH reduction and carbonate supplementation. Parameters measured consisted of algae removal, settled volume, dehydrogenase activity, optical density, dissolved oxygen concentration, chlorophyl a and pH.

The results indicated that the most important factor affecting bioflocculation was continuous flow mixing. Algal removal and settleable matter production were found to be proportional to flow mixing velocity. Continuous mixing inhibited photosynthesis of Synechocystis sp. This species

exhibited the greatest bioflocculation tendency. Waste loading and sludge seeding had moderate effects in the laboratory and slight, a positive effect in the field. Acidification stimulated biological flocculation in laboratory cultures but inhibited it at the field scale. Carbonate supplementation and photosynthetic bacteria seeding had no effect on bioflocculation. Dehydrogenase activity was higher in cultures that were mixed or fast mixed, waste loaded or heavily waste loaded, and algal biofloc or activated sludge seeded.

CHAPTER 1
INTRODUCTION

1.1 Problem Definition

Animal wastes have historically been disposed of by spreading on land. Manure was considered to be a soil conditioner and fertilizer because it supplied organic matter and nutrients. Manure usage has declined markedly in recent years, however. This is partly because chemical fertilizers have become more cost effective. An additional factor is the increasing popularity of confinement operations. These units generate large volumes of wastewater which are expensive to transport to often distant crop lands. Treatment systems for livestock wastes are therefore assuming increased importance.

Techniques applied for municipal wastewater management are not appropriate for livestock wastes, which tend to be more concentrated. These wastes can have chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values in excess of 100 times greater than those of municipal wastewaters. Utilization of microalgae grown in waste ponds is potentially cost effective because this process combines wastewater treatment and feed production. Waste organics are stabilized by bacteria using photosynthetically produced oxygen, thus obviating the need for mechanical oxygenation. Waste nutrients are converted to the form of

algal biomass which can then be removed to leave a treated effluent.

It is essential that algal cells be harvested or removed from the liquid phase of waste pond effluent. Unfortunately, waste-grown algal cells, because of their small size (generally less than 20 microns in any dimension), dispersed nature (cell suspensions are stable, showing little tendency to agglutinate), and low specific gravity, are difficult to harvest. They generally cannot be settled in a reasonable length of time, nor can they be removed effectively by filtration through sand or other media (Oswald, 1965). The most common method of harvesting currently applied is flocculation with inorganic compounds such as aluminum sulfate and lime or organic polyelectrolytes. The necessity to add chemicals adds considerably to the process cost and degrades the quality of the biomass recovered, however. Flocculation with exocellular polymers (biological flocculation) is a promising means of eliminating or reducing chemical requirements.

1.2 Research Objectives

The goal of this research was to determine how various environmental, biological and operational factors affect algal bioflocculation. Environmental variables considered were season, rainfall, temperature and solar irradiance. The biological variable considered was algal species composition. Operational variables included flow mixing,

waste loading, waste pretreatment, algal bioflocs and bacterial seeding, pH reduction and carbonate supplementation. Parameters measured consisted of algae removal, settled volume, dehydrogenase activity, optical density, dissolved oxygen concentration, chlorophyll a and pH.

CHAPTER 2
LITERATURE REVIEW

Flocculation with organic polyelectrolytes results from bridging between extended polymer lengths and vacant sites of adjacent microorganisms to form aggregates of cellular material (Fig. 2-1). In biological flocculation, the polymers are produced by microorganisms instead of being added.

2.1 Nature and Production of Extracellular Biopolymers

Extracellular biopolymers of microorganisms originate from cell lysis, biological excretion, and extracellular synthesis. Accumulation of extracellular biopolymer is closely related to the growth phase of microorganisms as shown in Fig. 2-2. The results of several investigators (Lewin, 1956; Moore and Tisher, 1964; Sakka et al., 1981) indicate that the quantity of extracellular polysaccharides of unicellular algae increases during the late logarithmic phase of growth and reaches a peak at the early stationary phase. Maximum polysaccharide production has been observed to occur under carbon, nitrogen, sulfur or phosphorus limiting conditions (Pavoni et al., 1972; Saviour and Kristiansen, 1983; Salenitro et al., 1983; Dugid and Wilkinson, 1953; Rudd et al., 1984). It was suggested that a higher C:N ratio could enhance the polymer production of

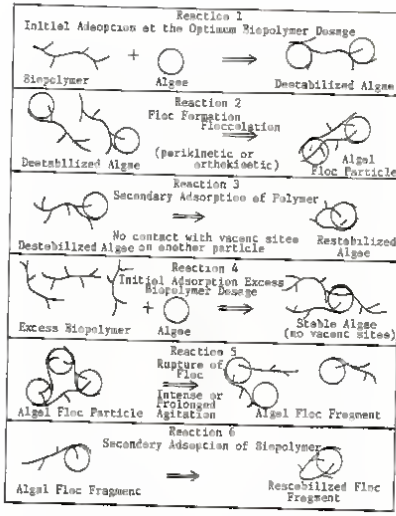

Figure 2-1 Schmatic representation of the bridging model for the destabilization of algal cells by polymers. Adapted from O'Melia (1972).

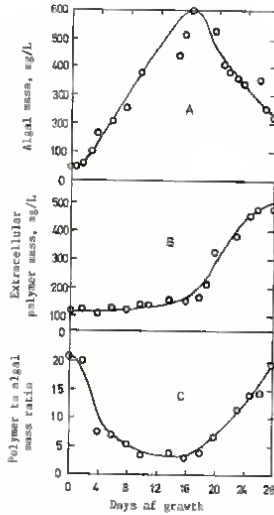

Figure 2-2 Relationship between algal mass and extracellular polymer production (A,B) and their mass ratio (C). Adapted from Pavoni et al. (1972).

capsular rather than soluble materials.

2.2 Algal Bioflocculation Experience in Laboratory Scale Systems

Pavoni et al. (1971) observed that algal flocculation directly coincided with algal polymer accumulation. They found that floc formation was restricted to the declining growth phase and the zeta potential of algal cells remained negative throughout all growth phases.

Algal bioflocculation was successfully achieved in activated algae systems developed by McKinney and his co-workers (Wahbeh, 1965; Sherwood, 1966; McGriff, 1970; Humenik and Hanna, 1971; Regen, 1972). Activated algae is a modification of the basic activated sludge process in which algal sludge is recycled between growth reactors and a gravity sedimentation basin. Growth reactors were separated into a light chamber and a dark chamber to produce a controlled short term light-dark cycle. Typical flow diagrams and experimental conditions are illustrated in Figure 2-3 and Table 2-1, respectively.

In laboratory-scale systems, well flocculated algal sludges were obtained on inorganic and synthetic organic media as well as sewage (Wahbeh, 1965; Humenik and Hanna, 1971; Regen, 1972). McGriff (1970), using 18 connected channels in the light chamber, achieved 97% BOD, 92% nitrogen and 74% phosphorus removals. The laboratory research of McGriff (1970) and Regan (1972) established that (1) a workable light-dark relationship consisted of 3.3 minutes in the light and 2.4 minutes in the dark;

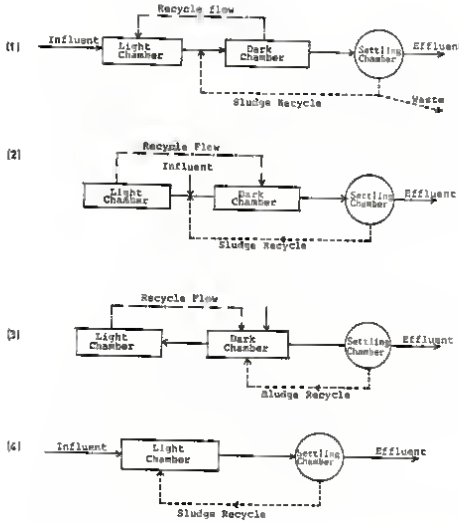

Figure 2-3 Flow diagrams for activated algae syatems used by (1) Wahbeh (1965) and McGriff (1970), (2) Sharwood (1966), (3) Humenik and Hanna (1971) and (4) Regan (1972).

Table 2-1 Summary of experimental conditions of activated algae systems

Year	Investigator	Lab / Field	Reactor Surface Area (Cm^2)	Capacity (L)	Volume of Settling Chamber (L)	Raw Sewage Feed rate (mL/hr)	Fluid Velocity in Light Chamber (Ft/Sec)	Light Intensity (ft-Candles)
1965	Wahbeh	L	775	2	1.5	400	0.79	—
1966	Sherwood	F	111,000	6,750	1,388	112 - 450	2	1,200
1969	Wanna & Humenik	L	*	2	2.7	330	—	1.200
1970	McGriff	L	6,458	15-21.5	8	1,580	0.36-0.48	300-400
1972	Regan	L**	6,960	29	12	41-82	—	200-600

* Annulus type chamber
** Results of phase 3 only

(2) a detention time of 10 hours and a MLSS range of 1,000 to 1,400 g/m^3 were workable operating parameters; (3) at the light-depth ratio of 48 ft-candles/cm, satisfactory operation resulted when systems were loaded at a carbon input rate of 1,150 g/m^3-d; (4) nutrient removal for activated algae operated at 1.0-1.5 kg BOD/day/kg MLVSS was accounted for by microbial synthesis.

Despite this successful experience, it proved impossible to develop a flocculent algal culture in an outdoor pilot plant fed sewage or synthetic media (Sherwood, 1966; McGriff, 1970). It was postulated that the failure resulted from insufficient pH rise due to difficulties in balancing the quantities of algae and bacterial biomass.

Bogan et al. (1960) utilized a similar activated algae system in which algal sludge was recycled between a separator and a growth unit, with species of Chlorella and Scenedesmus predominant. A well-flocculated culture was observed even when the effluent pH fell below 9 (the lower limit for phosphate precipitation).

Sokil and John (1981) reported that the optimum ratio of algae to bacteria for flocculation to take place was 60:40 (w/w). The optimum algal bacterial biomass concentration fell within a range of 1,400-1,600 g/m^3 with a detention time of 6-8 hours. Contrary to the results of McKinney et al. (1971), they mentioned that complicated light-dark cycles were not necessary to develop flocculating

algal-bacterial systems.

2.3 Algal Bioflocculation Experience in Field Scale Systems

Oswald et al. (1978) reported that Scenedesmus grown in continuously mixed (at 5 cm/sec or 30 cm/sec) high-rate ponds treating domestic wastewater in Manila, the Philippines, settled quickly when transfered to quiescent conditions. The experimental regimen included operation at three different pond depths (15 cm, 30 cm, and 45 cm), three different hydraulic loading rates (5, 7.5, and 10 cm/day), and three different feast-mix durations. The growth media was very dilute, coarsely screened sewage with an average BOD of only 42 g/m^3 and ammonia concentration of 8 g/m^3. The average algal solids removal of 77.6% was observed within detention times of 0.25-1.4 days.

A facultative pond system treating domestic sewage from the city of Woodland, California, was the first mass culture system to utilize bioflocculation for algae removal (Hiatt et al., 1977). Koopman et al. (1978, 1981) studied this method, which they termed "Pond Isolation." In this method, effluents from facultative ponds were isolated in a batch operated secondary pond. Most of the algal cells in the secondary pond disappeared from suspension within 2 to 4 weeks. In-pond sedimentation of flocculent algal cultures produced by pond isolation techniques resulted in algal removals consistently exceeding 80%. In work conducted at Richmond, California, Eisenberg et al. (1981) discovered that continuous flow mixing with paddle wheels promoted

dominance of bioflocculating Micractinium sp. in high-rate ponds treating domestic sewage. Mixing power requirements were low (approximately 15 kWh/ha-d) because flow velocities of only 10-15 cm/sec were needed. The algae removal efficiency obtained by settling flocculent cultures averaged 76-80%.

Algal bioflocculation in a mass culture system treating swine waste was first demonstrated by Lincoln and Koopman (1982) at Gainesville, Florida. In this system, continuous paddle wheel mixing at a velocity of 20 cm/sec promoted dominance of bioflocculating Micractinium sp. with the loading of raw swine waste. Settled volumes increased to over 100 mL/L, with a final average of 71 mL/L. It was found that heavy (pulse) loading with raw swine waste enhanced floc formation and that this medium was associated with the best growth of Micractinium.

The flocs produced in high-rate pond bioflocculation at Richmond, California, were composed of algal cells, primarily Micractinium sp., incorporated into matrices of filamentous microorganisms. In their comparative studies, Koopman et al. (1981) indicated a fundamental similarity of structure in both activated sludge and algal-bacterial flocs associated with filamentous microorganisms. Flocs produced in the high-rate pond at Gainesville, Florida, consisted of clusters of Micractinium cells enmeshed in a bacterial matrix composed largely of the purple sulfur bacterium, Thiopedia rosea.

CHAPTER 3

MATERIALS AND METHODS

3.1 Summary of Experiments

A total of 11 laboratory experiments and 14 field experiments were conducted between November 1983 and February 1986. Tables 3-1 and 3-2 provide summaries of the laboratory and field experiments, respectively.

3.2 Laborstory Experiments

3.2.1 Growth Media

Three kinds of waste media were utilized in the laboratory experiments: alum flocculeted anaerobic lagoon effluent, centrifuged anaerobic lagoon effluent and mature high-rate pond culture medium. Descriptions of the anaerobic lagoon and high-rate pond are given in section 3.3.1.

Alum flocculated anaerobic lagoon effluent was used in experiment 3. It was prepared by flocculating anaerobic lagoon effluent with aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$) to remove Thiopedia rossea and other suspended solids. Dosage was 1.2 g alum/g TSS (Freedman et al., 1983). The jar test procedure consisted of 60 sec of rapid mixing at 132 rev/min ($G = 200\ s^{-1}$), 1 hour of slow mixing at 12 rev/min ($G = 5\ s^{-1}$) and 4 hours of quiescent settling. Values of mean velocity gradient (G) were calculated from stirred speed (in rev/min) using the calibration of Cornwell and

Table 3-1. Summary of laboratory experiments

Exp. no.	Dates	Experimental variable	Algal genera dominant initially
1	1/3-1/20(84)	carbon addition	Chlorella
2	1/9-1/26(85)	pH	Chlorella
3	1/17-2/9(85)	pH	Chlorella
4	5/15-5/30(85)	waste loading	Chlorella/Monodus
5	6/10-6/24(85)	mixing	Chlorella/Monodus
6	7/5-7/19(85)	mixing	Chlorella/Monodus
7	10/1-10/10(85)	pH	Synechocystis
8	10/22-11/1(85)	pH	Synechocystis
9	11/1-11/11(85)	waste loading	Synechocystis
10	12/3-12/19(85)	activated sludge seeding	Chlorella/Monodus
11	12/2-12/15(85)	waste pretreatment	Chlorella/Monodus

Table 3-2. Summary of field experiments

Exp. no.	Dates	Experimental variables	Algal genera dominent initially
1	Nov-Dec 83	mixing	Synechocystis
2	Jan-Feb 84	mixing	Synechocystis
3	Mar-Apr 84	CO_3^{2-} addition	Chlorella/Monodus
4	May 84	mixing	Synecho./Chlorella
5	Jun-Jul 84	waste loading	Synechocystis
6	Jul-Sep 84	waste loading rate	Synechocystis
7	Sep-Oct 84	biofloc (algae) seeding	Synecho./Chlorella
8	Nov 84	pH	Chlorella/Monodus
9	Dec 84	purple sulfur bacteria seeding	Chlorella/Monodus
10	Jan-Feb 85	waste type	Chlorella/Monodus
11	Mar-Apr 85	waste loading	Chlorella/Monodus
12	May-Jun 85	mixing velocity	Chlorella/Monodus
13	Oct-Nov 85	mixing velocity	Synechocystis
14	Dec 85	activated sludge seeding	Chlorella/Monodus

Bishop (1983). Supernatant was carefully decanted from individual jars at the end of the settling period and combined in one large beaker. After inoculation, it was dispensed into individual flasks. Centrifuged anaerobic lagoon effluent was prepared by centrifuging anaerobic lagoon affluent at 2,500 x g for 10 min with a centrifuge (RC2-B, Ivan Sorvall, Inc. Norwalk, Conn.). Typical characteristics of alum flocculated and centrifuged anaerobic lagoon effluents are given in Table 3-3.

When alum flocculated or centrifuged anaerobic lagoon effluent was used as the growth medium, inoculation with algae was required. The algal inoculum was obtained from the high-rate pond. Inoculum was concentrated by centrifugation at 2,000-2,500 x g for 10 min. The volume of inoculum added ranged from 20-25 mL/L of culture medium.

At times, the culture medium obtained from the high-rate pond contained significant zooplankton populations (e.g., Brachionus rubens, Diaphenosoma Brachyurum). In experiments 7, 8, and 10, zooplankton in the laboratory cultures were controlled by addition of NH_4Cl. The target free ammonia concentration was reported as 20 g/m^3 (Lincoln et al., 1983), a value sufficient to kill virtually all zooplankton without adversely affecting the algal population. In order to reach this concentration, 153 mg NH_4Cl (40 mg as N) was added per litre of medium, then the pH of the cultures was raised to 9.3 with 10 N NaOH. At this

Table 3-3 Typical characteristics of alum-flocculated and centrifuged anaerobic lagoon effluents

Sources*	Alum flocculated anaerobic lagoon effluent	Centrifuged anaerobic lagoon effluent
TSS	65	84
VSS	60	72
BOD	8	15
COD	86	143
TKN	53	82
TP	15	26

*All values in g/m^3

pH, approximately one-half of the total ammonia present exists in the unionized form (NH_3). The pH was held at this level for 4-6 hours, then readjusted to 8.0 with 10 N H_2SO_4

3.2.2 General Experimental Plan

In all experiments, except exp. 5 and 6, cultures were maintained on a variable speed shaker table (Model 3590, Lab-Line Instruments Inc., Melrose Park, IL) which was operated at 140 oscillations/min. Illumination was provided a bank of five 122 cm long, 40 W, cool white flourescent bulbs spaced on 12 cm centers and suspended 58.8 cm above the top of the shaker table. The light intensity at the surface of the shaker table was measured as 7.80 W/m^2, using a pyranometer/lightmeter (FY1617-7903, LI-Cor., Inc., Lincoln, Nebraska/LI-185, Lambda Instruments Corp., Lincoln, Nebraska).

Cultures were grown in 500-mL Erlenmayer flasks, each containing 300 mL medium volume. Flasks were capped with porous plastic plugs to allow air exchange. Trials were conducted in triplicate.

In experiments 5 and 6, a standard jar test apparatus (Phipps and Bird, Richmond, VA) was used to provide various mixing intensities. Paddle-stirrers were 2-bladed, each blade having a radius of 3.8 cm and length of 2.5 cm. The jars were made of acrylic plastic and had dimensions of 11.5 x 11.5 x 20.0 cm. The liquid volume used was 1.0 L. Jars were covered with a sheet of paper to prevent dust and debris from falling into the cultures. Illumination was

provided by a 30 W fluorescent light located 5 cm from the lateral face of the jars. Light intensity measured at the lateral surface of the jars was 11.0 W/m^2. Trials were conducted in duplicate. Unless otherwise noted, cultures were adjusted to pH 8.0. on a daily basis.

3.2.3 Experimental Procedures

3.2.3.1 Effect of pH--Experiments 2, 3, 7 and 8

The effect of pH was investigated in 4 experiments. Cultures were initially dominated by Chlorella in exp. 2 and 3 and Synechocystis in exp. 7 and 8.

In exp. 2, three different pH levels (5.5, 7.5 and 9.5) were tested. Initial chl a concentration was 10 g/m^3.

In exp. 3, trials at three different pH levels (5.5, 6.5 and 7.5) were repeated based on the results of exp. 2. The pH was not adjusted in the control. The culture medium was a mixture of 2 parts high-rate pond medium and 1 part alum flocculated anaerobic lagoon effluent. Initial chl a concentration was 3.8 g/m^3.

In exp. 7, culture medium obtained from high-rate pond was adjusted to pH levels of 5.5, 6.5, 7.5 and 8.5. Initial chl a concentration was 3.5 g/m^3.

In exp. 8, trials at 3 different pH levels (5.5, 6.5 a.d 7.5) were repeated based on the results of exp. 7. The pH was not adjusted in the control. Initial chl a concentration was 4.1 g/m^3.

3.2.3.2 Effect of Mixing--Exp. 5 and 6

The effect of mixing on bioflocculation was evaluated in exp. 5 and 6. Using the jar test apparatus, mixing speeds of 20 rev/min, 40 rev/min and 80 rev/min were applied to culture media. The control was not mixed. Algal genera were 50% Chlorella and 50% Monodus on a biovolume basis in both experiments. Initial chl a concentrations in exp. 5 and 6 were 13.0 g/m^3 and 8.8 g/m^3, respectively.

3.2.3.3 Effect of Waste Loading Rate--Exp. 4 and 9

The effct of waste loading rate on bioflocculation was investigated in exp. 4 and 9. Protocol in these experiments were identical, except that dominant algal genera were Chlorella and Monodus in exp. 4 and Synechocystis in exp. 9. Cultures were loaded with fixed bed reactor effluent at rates of 2%, 4%, and 8% of culture volume per week. The control was not loaded. Initial chl a concentrations in exp. 4 and 9 were 6.2 g/m^3 and 4.1 g/m^3, respectively.

3.2.3.4 Effect of Waste Pretreatment--Exp. 11

The effect of waste pretreatment on bioflocculation was tested in this experiment. Settled waste supernatant and fixed bed reactor effluent obtained from the field scale syatem were sparged with nitrogen gas to strip out hydrogen sulfide. Typical characteristics of settled waste supernatant and fixed bed reactor effluent are given in Table 3-4. Waste loading rate was 8% of the culture volume (24 mL) per week. Algal genera were 50% Chlorella and 50%

Table 3-4 Typical characteristics of settled waste supernatant and fixed bed reactor effluents

Source*	Settled waste supernatant	Fixed bed reactor effluant
TS	7,465	6,100
VS	5,066	3,989
COD	12,601	8,253
TKN	1,225	1,279
NH_3-N	931	1,064

*All values in g/m^3

Monodus on a biovolume basis. Initial chl a concentration was 4.9 g/m^3.

3.2.3.5 Effect of Activated Sludge Seeding--Exp. 10

This experiment employed activated sludge as a source of bacterial seed. The amount of activated sludge added was 5%, 10%, and 20% of the algae dry weight. Algal genera were 50% Chlorella and 50% Monodus on a biovolume basis. Initial chl a concentration was 4.9 g/m^3.

3.2.3.6 Effect of Carbon Addition--Exp. 1

Three levels of sodium carbonate (50 g/m^3, 100 g/m^3, and 1,000 g/m^3 expressed as carbon) were added to the culture media. The dominant algal genus was Chlorella. Initial chl a concentration was 5.5 g/m^3.

3.2.3.7 Preliminary experiment

The effect of algal density on bioflocculation was tested. Three levels of initial algal density (1.5, 4.0 and 8.0 g chl a/m^3) were applied to cultures initially dominated by Chlorella and Monodus.

Another preliminary experiment examined the growth potential of Synechocystis under mixed and unmixed conditions at 21 °C and 30 °C. Mixing (40 rev/min) was provided by the Jar test apparatus. Temperature was maintained by placing the Jar test apparatus with an electric heater in a closed fume hood. Illumination was provided by a 30 W fluorescent light located 5 cm from the lateral face of jars. Initial chl a concentration was 4.2 g/m^3.

3.3 Field Experiments

3.3.1 Description of Field System

Studies of field scale biofloccultion were conducted at the University of Florida Swine Algae System. The principal components of this system were raw waste generation, anaerobic/facultative treatment, photosynthetic oxygenation and algae harvesting (Fig. 3-1). Flushed wastes from an average population of 260 pigs housed over concrete slab or slotted floors were regularly discharged to anaerobic treatment (lagoon or digester) after temporary storage in underground pits. Average daily wastewater volume ranged from 5 to 9 m^3/day depending on the number and age of pigs in residence at the barns, the frequency of flushing, and use of cooling sprays. Total solids (TS) concentration averaged 1-2%.

The anaerobic digestion system consisted of a settling tank, a conventional, unmixed digester and a fixed bed reactor. A portion (2.8-3.2 m^3/d) of the flushed wastes from the swine confinement buildings was pumped to the 15 m^3 volume settling tank where settleable solids were separated from liquid waste. Settled solids from this tank were fed to the anaerobic digester at a rate of 0.9-1.3 m^3/d. The digester was made from a 20 m^3 polyolefin tank and was unmixed and unheated. Its loading rate ranged from 0.14-1.9 kg volatile solids (VS)/m^3d and averaged 0.67 kg VS/m^3d. The supernatant from the settling tank was fed to the fixed bed

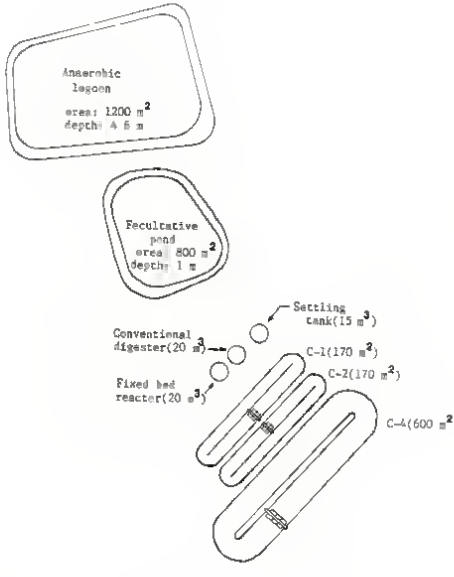

Figure 3-1 Principal components of field system.

reactor, which was operated at ambiant temperature. This unit was constructed from a 20 m^3 polyolafin tank and filled with cypress chips that acted as support media for anaarobic bacteria. Its loading rate ranged from 0.30-1.92 kg VS/m^3d and averaged 1.08 kg VS/m^3d, on an empty bed volume basis.

Excess effluant flowing from the digestar system, as well as flushed wastes not pumped to the settling tank, was discharged to the anaerobic lagoon. The anaerobic lagoon had a surface area of 1,200 m^2 and depth of 4.6 m. It received an average loading of 13 g VS/m^3d and had a detention time of 600 days. The anaerobic lagoon functioned as a continuous cultura, predominantly of the purple sulfur bacteria, Thiopedia rosea. Its overflow was received by a facultative pond which averaged 1.0 m in depth and had a surface area of 800 m^2. Effluents from the settling tank, anaerobic digester, fixed bed reactor, anaarobic lagoon, and facultative pond were used as alternative feed sources for the photosynthatic oxygenation stage (high-rate pond) and algae harvesting stage (bioflocculation channels).

The high-rate pond (designated C-4) was excavated in heavy clay soil and had a length of 46 m and surface area of 600 m^2. A racetrack configuration was imparted by a center baffle which extended for 40 m. Flow-mixing at a velocity for 20 cm/s was achieved by means of an electric motor driven paddlewheel. Mixing was carried out for 30 min three times weekly, coinciding with periods of waste loading. Operating depth of the high-rate pond varied from 0.3-0.6 m.

The bioflocculation channels (designated C-1 and C-2) were identical concrete lined channels, each 38 m long and 170 m^2 in surface area, with center baffles arranged to give a racetrack configuration. Mixing schedules and waste loading were varied according to the experiment in progress. Operating depth ranged from 0.2-0.5 m.

3.3.2 Field Scale Experimental Procedures

The general experimental procedure was to fill the paired bioflocculation channels, C-1 and C-2, with mature culture medium from the high-rate pond, apply experimental variables and conditions, monitor the channels until bioflocculation took place, then drain and clean the channels thoroughly in preparation for the next experiment. Specific procedures for each experiment are given below.

3.3.2.1 Effect of Mixing--Exp. 1, 2, 4, 12, and 13

The effect of mixing on bioflocculation was evaluated in 5 experiments. Mixed and unmixed channels were compared in exp. 1, 2, and 4. The effects of two different flow mixing velocities (14 cm/s and 30 cm/s) were examined in exp. 12 and 13.

In exp. 1, culture medium from the high-rate pond was transferred to the bioflocculation channels, providing an initial depth of 31 cm in C-1 and 33 cm in C-2. C-2 was mixed at 16 cm/s whereas C-1 was not mixed. Anaerobic digester effluent was added to each channel two times weekly. Ammonium hydroxide was added to control grazers on two occasions.

In exp. 2, C-2 was completely drained and cleaned after termination of exp. 2. Then culture medium from C-1 was transferred to C-2 and C-1 was cleaned. Culture medium from C-2 was then divided equally between the channels, giving each an initial depth of 20 cm. C-2 was mixed at 16 cm/s and C-1 was not mixed. 100 L of anaerobic digester effluent and 400 L of fixed bed reactor effluent were applied to each channel during the course of the experiment. Initial chl a concentration was 0.5 g/m^3. The dominant algal genus was Synechocystis initially.

In exp. 4, culture medium from the high-rate pond was transferred to the bioflocculation channels, giving each an initial depth of 31 cm. C-1 was mixed at 16 cm/s and C-2, the control, was not mixed. Neither channel was loaded during the experiment. Initial chl a concentrations were 10.5 and 10.7 g/m^3 in C-1 and C-2, respectively. Dominant algal genera were Synechocystis and Chlorella/Monodus. The relative proportions of these two populations were 46% and 54%, repectively.

In exp. 12, culture medium from the facultative and high-rate ponds was used in relative proportions of 58% and 42%, respectively, to fill the bioflocculation channels to an initial depth of 46 cm. C-1 was mixed at 14 cm/s and C-2 was mixed at 30 cm/s. Both channels were loaded with 1,350 L of screened fixed bed reactor effluent weekly. Dominant algal genera were Chlorella (59%) and Monodus (41%).

Initial chl a concentrations in C-1 and C-2 were 7.0 g/m^3 and 6.8 g/m^3, respectively.

In exp. 13, the bioflocculation channels were filled to a depth of 35 cm with medium from the high-rate pond. C-1 was mixed at 30 cm/s and C-2 was mixed at 14 cm/s. Both channels were loaded with 1,500 L of screened fixed bed reactor effluent weekly. Dominant algal genera were Synechocystis (83%) and Chlorella/Monodus (17%) initially. Initial chl a concentrations in C-1 and C-2 were 7.6 g/m^3 and 5.6 g/m^3, respectively.

3.3.2.2 Effect of Waste Loading--Exp. 5, 6, 10, and 11

The effect of waste loading was investigated in 4 experiments. Loaded cultures were compared to non-loaded cultures in exp. 5 and 11. Different waste loading rates were evaluated in exp. 6. The influence of waste pretreatment was studied in exp. 10.

In exp. 5, culture medium from C-4 was used to fill the bioflocculation channels to an initial depth of 37 cm. C-2 was loaded with 1,000 L of FBR effluent weekly, whereas C-1 was not loaded. FBR effluent was screened through 1 mm reinforced screen to remove solids. Both channels were mixed at 20 cm/s. The major algal genus was Synechocystis. Initial chl a concentrations in C-1 and C-2 were 4.1 g/m^3 and 4.5 g/m^3, repectively.

In exp. 11, culture medium from the high-rate pond was used to fill the bioflocculation channels to an initial depth of 37 cm. C-2 was loaded with 1,350 L of screened

fixed bed reactor effluent weekly, whereas C-1 was not loaded. Both channels were mixed at 14 cm/s. The initial photosynthetic community was composed of 58% Chlorella, 37% Monodus, and 5% Thiopedia rosea. Initial chl a concentrations in C-1 and C-2 were 15.3 g/m^3 and 17.8 g/m^3, respectively.

In exp. 6, culture medium obtained from the high-rate pond was transferred to C-1 and C-2, giving each an initial depth of 35 cm. C-1 and C-2 were loaded with 600 L and 1,800 L, respectively, of screened FBR effluent weekly. Both channels were mixed at 14 cm/s. The dominant algal genus was Synechocystis. Initial chl a concentration was 3.0 g/m^3.

In exp. 10, culture media from the facultative pond and high-rate pond were used in relative proportions of 70% (25 cm) and 30% (10 cm) to fill the bioflocculation channels to a depth of 35 cm. C-1 and C-2 were loaded three times weekly with 300 L of settled waste supernatant and with 300 L of fixed bed reactor effluent respectively. Both channels were mixed at 14 cm/s. The initial photosynthetic community in both channels was composed of 43% Chlorella, 44% Monodus and 13% Thiopedia rosea. Initial chl a concentrations in C-1 and C-2 were 5.3 g/m^3 and 5.2 g/m^3, respectively.

3.3.2.3 Effect of Algal Biofloc and Bacterial Seeding--Exp. 7, 9 and 14

Algal biofloc seeded culture and non-seeded culture in exp. 7, photosynthetic bacteria seeded culture and non-

seeded culture in exp. 9, and activated sludge seeded culture and non-seeded culture in exp. 14 were compared.

After termination of exp. 6, C-1 was completely drained and cleaned. C-2 culture medium was transferred to C-1, then C-2 was cleaned. Settling of the medium in C-1 was allowed for 24 h. C-2 was then filled to a depth of 11 cm (25% of culture volume) with supernatant from C-1. Additional C-1 supernatant was discarded until a depth of 11 cm remained, containing most of the bioflocculated algae from experiment 6. Both bioflocculation channels were then brought to a depth of 44 cm by adding culture medium from C-4. The channels were mixed at 14 cm/s. Each received 600 L of screened fixed bed reactor effluent. Dominant algal genera were Chlorella and Synechocystis initially. The relative proportions of these two genera were 35% and 65%, respectively. Initial chl a concentrations in C-1 and C-2 were 3.2 g/m^3 and 1.5 g/m^3, respectively.

Experiment 9 was begun with culture medium carried over from exp. 8. No new medium from the facultative or high-rate ponds was added. Anaerobic lagoon effluent, containing a dense population of the purple sulfur bacteria, Thiopedia rosea, was added to C-2 on two occasions during the experiment. Initial depths in C-1 and C-2 were 28 cm and 30 cm, respectively. Both channels were mixed at 14 cm/s. Each received 600 L of screened fixed bed reactor effluent weekly. Dominant algal genera were Chlorella and Monodus. The relative proportions of these two genera were 58% and

42%, respectively. Initial chl a concentrations were 6.1 g/m^3 in both channels.

Culture medium from the facultative pond was used to fill the bioflocculation channels to a depth of 24 cm at the start of experiment 14. C-2 received 380 L of activated sludge from a municipal waste treatment plant (0.7% solids). Both channels were mixed at 30 cm/s and each received 2,000 L of fixed bed reactor effluent on two occasions. Dominant algal genera were Chlorella and Monodus throughout the experiment. Initial Chl a concentration was 5.0 g/m^3 in each channel.

3.3.2.4 Effect of Carbonate-supplementation--Exp. 3

After termination of exp. 2, C-1 was completely drained and cleaned. Culture medium from C-2 was transferred to C-1, then C-2 was cleaned. Culture medium from C-1, supplemented with additional medium from the facultative pond, was split between the bioflocculation channels, giving initial depths of 26 cm and 27 cm for C-1 and C-2 respectively. The relative proportions of medium from C-2 and the facultative pond were 83% and 17%, respectively. Fixed bed reactor effluent was added to the channels on two occasions. C-2 was dosed with 22.5 kg sodium carbonate to give an added carbonate-carbon concentration of 50 g/m^3. Both channels were mixed at 19 cm/s. Initial chl a concentrations were 2.3 g/m^3 in both channels. Dominant algal genera were Chlorella and Monodus.

3.3.2.5 Effect of pH--Exp. 8

A mixture of 80% culture medium from the facultative pond and 20% medium from the high-rate pond was used to fill the bioflocculation channels to an initial depth of 30 cm. Concentrated sulfuric acid was added periodically to C-2 to reduce its pH to 6.0 or less, whereas the pH of C-1 was not controlled. Both channels were mixed at 14 cm/s. Each received 600 L of screened FBR effluent weekly. Major algal species were Chlorella (69%) and Monodus accuminata (28%). Initial chl a concentrations of C-1 and C-2 were 5.8 g/m^3 and 5.4 g/m^3, respectively.

3.4 Analytical Techniques

3.4.1 Algal Counts

Culture media samples obtained at 0.1 m depth were examined microscopically according to the following procedure. After sufficient agitation to ensure resuspension of all cells, the sample vial was subsampled with a pipette. A measured volume of 0.05 mL was placed on a glass slide and flattened with a 22 mm diameter, circular cover slip. At least 100 cells of each algal type were enumerated, or for rare types, at least 10 microscopic fields (at 400x) were scanned. Counts were expressed as number of cells per Whipple Grid. Given an average liquid layer thickness of 100 μm slip and slide and the 185 μm x 185 μm dimensions of the Whipple Grid (at 400x), a count of 1 cell per grid corresponds to 2.9 x 10^8 cells per L. Counts of algae other

than Chlorella were normalized to Chlorella equivalents by multiplying them by the appropriate biovolume ratios.

3.4.2 Chlorophyll a and Algae Removal Efficiency

The chlorophyll a measurement was a modified version of the procedure described by Talling and Driver (1963). A 10 mL sample was centrifuged for 10 min at 2,400 x g and centrate poured off. Then, 10 mL of boiling 90% methanol was added, and the pellet disrupted by 15 sec of vigorous shaking. Chlorophyll a extraction was continued by placing the centrifuge tube in a 70 °C water bath for 45 seconds. The sample was then centrifuged at 2,400 x g for 10 min and the absorbance of the supernatant measured by absorption spectrophotometry. Chlorophyll a concentration was calculated according to following equation:

$$\text{Chl a} = 13.9 \times (D_{665} - D_{750}) \times (v/V) \qquad (3\text{-}1)$$

where chl a has units of g/m^3, D_{665} = absorbance at 665 nm, D_{750} = absorbance at 750 nm to correct for turbidity, v = final solvent volume (mL) and V = sample volume (mL).

The algae removal efficiency was calculated by measuring the initial chl a concentration of the sample to the chl a of 100 mL supernatant sample taken at the end of the 24 hr settling period. Algae removal was expressed as the percent decrease in chl a.

3.4.3 Visibility and Estimation of Volatile Suspended Solids

Visibility was measured using a 15 cm diameter secchi

disc. The depth at which the disc disappeared from the sight as it was lowered in the culture was taken as the depth of visibility.

Volatile suspended solids concentration of the combined algae and bacterial biomass was calculated from visibility measurements based on the empirical formula of Lincoln and Hill (1980):

$$VSS\ (g/m^3) = 750/d \qquad (3\text{-}2)$$

where d = the depth of visibility expressed in inches.

3.4.4 Settleability

Settleability of algae was determined by retaining samples in 1 L Imhoff cone over a 24 hr period in the absence of light. Adherence of algal cells to the sides was minimized by gently swabbing the inside of the cone with a rod after an initial settling period of 2 hr. Volume of settled matter at the bottom of the cone was measured after an additional 22 hr of retention.

3.4.5 Dehydrogenase Activity

Dehydrogenase activity was measured by a modified version of the procedure described by Koopman et al. (1984). Sample pH was adjusted to 8.6 with 0.1 N NaOH. Triplicate 10 mL aliquots were amended with 1.0 mL 0.2% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5- phenyltetrazolium chloride (INT) (Eastman Kodak) and incubated at room temperature (22 ± 2 °C) for 1 hr in the dark. Mixing was

provided by a hematology mixer. Incubation was terminated by adding 1.0 mL 37% formaldehyde. INT-formazan (INTF) formed during the incubation period was extracted according to the following procedure. A 10 mL INT-treated sample was placed in a centrifuge tube and centrifuged at 2,100 x g for 20 min. Centrate was decanted, leaving a pellet approximately 0.1 mL in volume, and replaced with 10 mL mixture of 4+6 tetrachloroethylene/acetone. The tube was capped and vortexed for 20 sec. Extraction was continued in the dark for 30 min. Extract was clarified by centrifugation at 2,100 x g for 20 min and optical density of the extract was determined at 490 nm. INT-dehydrogenase activity (INT-DHA) was calculated according to the following equation:

$$\text{INT-DHA} = 1024\ D_{490}\ v\ /\ V\ t\ F \qquad (3\text{-}3)$$

where INT-DHA is expressed in units of equivalent oxygen uptake (g O_2*/m^3-d), D_{490} = absorbance at 490 nm, v = volume of extract (mL), V = volume of sample (mL), t = incubation time (min), and F = factor to account for sample dilution by INT and formaldehyde (0.833).

3.4.6 Residue, Organic Matter and Nutrients

Analyses of residue and organic matter were carried out according to APHA (1985). These included total solids (209A), volatile solids (209D), total suspended solids (209C), volatile suspended solids (209F), biochemical oxygen

demand (421B, 507), and chemical oxygen demand (508A). Total Kjeldahl nitrogen and ammonia nitrogen were measured by the distillation (00610) and digestion (00625) procedures described in the EPA (1974). Total phosphorus was determined by the persulfate digestion procedure (00665) of EPA (1974).

3.4.7 pH, Dissolved Oxygen and Optical Density

The pH was monitored by an electrode analyzer system (601A, Orion Research Inc., Cambridge, MA).

Dissolved oxygen (DO) was determined with a YSI model 54A oxygen meter and polarographic electrode.

Optical density was measured by absorption spectrometry (Spectronic 21, Baush and Lomb, Marietta, GA).

CHAPTER 4
RESULTS OF LABORATORY EXPERIMENTS

4.1 Effect of pH

The effect of pH on bioflocculation was investigated in 4 experiments. Algal genera tested were Chlorella in exp. 2 and 3 and Synechocystis in exp. 7 and 8.

Under illuminated conditions of exp. 2, the stimulatory effect on the growth of Chlorella was observed in the culture adjusted to pH 7.5. Counts of this alga in the culture adjusted to pH 9.5 fell to less than 14% of the initial count within 4 days, whereas those in the culture adjusted to pH 5.5 declined very slowly over the period of experiment. Complete bioflocculation marked by cell aggregation and rapid settling of cells occurred within 6 days and 86% removal of algae was obtained in 9 days in the culture adjusted to pH 5.5. This phenomenon was preceded by the rapid decay in algal cells and increase in bacterial activity. Microscopic examination showed that small flocs colored tan-green became visible after 6 days. A substantial fraction of the bacterial component was associated with these flocs. In contrast, the cultures adjusted to pH 7.5 and 9.5 showed no sign of flocculation.

An inverse relationship between algal and bacterial growth activities was shown: as algal growth increased,

bacterial activities decreased and vice versa (Fig. 4-1).

In exp. 3, complete bioflocculation was repeated in the cultures adjusted to pH 5.5 and 6.5. In contrast, the culture adjusted to pH 7.5 and control showed no sign of flocculation. Macroscopic flocs became visible after 7 days and these were more compacted than those developed in exp. 2. They appeared as a predominantly tan-green to orange-brown matrix of Chlorella cells, but bleached noticeably with time. A substantial bacterial component was present in these flocs. Maximum algal removals by 2 h sedimentation showed 82% on day 9 in the culture adjusted to pH 6.5 and 79% on day 11 in the culture adjusted to pH 5.5 (Fig. 4-2). The time when this phenomenon occurred was well correlated with the declining growth phase of the cultures.

The stimulatory effect of pH on the growth of Chlorella was observed in the culture adjusted to pH 7.5 as observed in exp. 2. The control grew well for first 12 days then declined rapidly. Declines in cell numbers in the cultures adjusted to pH 5.5 and 6.5 were most rapid between 2 and 10 days.

Bacterial activity in all the cultures tested generally declined progressively over the period of the experiment.

In exp. 7 and 8, the effect of pH on bioflocculation was investigated with the blue green, Synechocystis. Under mixed conditions, the inhibitory effect of elevated pH on the growth of Synechocystis was noticeable (Fig. 4-3,

4-4). The extent of cell decline in the culture adjusted to pH 5.5 both in exp. 7 and 8 was somewhat less than those in the other cultures. However, the cultures adjusted to pH 5.5 and 6.5 became dominated by Chlorella after 14 days. In exp. 7, floc particles became visible after 6 days and appeared to have a ten-green to orange-brown matrix with Synechocystis cells as the major component. Chlorella and Monodus contributed approximately 5% of the total biovolume. Maximum algal removal by 2 h sedimentation showed 40% and 28% in the cultures adjusted to pH 6.5 and 7.5, respectively, on day 8 (Fig. 4-4). DHA decreased as the algal growth decreased.

Effect of pH on maximum algae removal and corresponding DHA and chl a levels in cultures initially dominated by Chlorella (exp. 2, 3) and Synechocystis (exp. 7, 8) is summarized in Table 4-1.

4.2 Effect of Mixing

The effect of mixing on bioflocculation was investigated in exp. 5 and exp. 6. Three levels of mixing intensities, 20 rev/min, 40 rev/min, and 80 rev/min were compared to the control without mixing.

In exp. 5, the control showed a stationary phase of growth throughout the experiment but the other cultures manifested an initial stimulatory effect of mixing on the growth of Chlorella and Monodus, then declined gradually after 8 days.

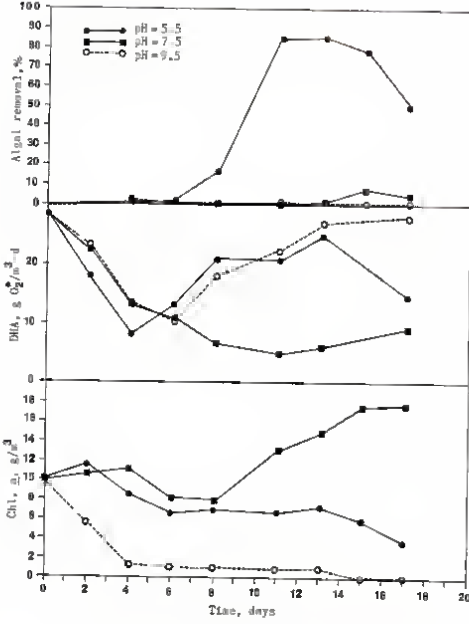

Figure 4-1 Effect of pH on temporal variation of settleability, DHA and chl a in a culture initially dominated by Chlorella, exp. 2.

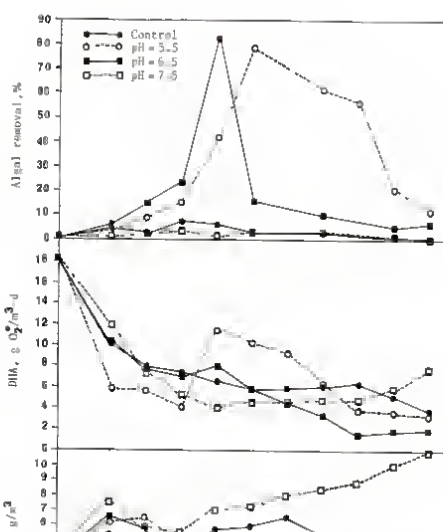

Figure 4-2 Effect of pH on temporal variation of settleability, DHA and chl a in a culture initially dominated by Chlorella, exp. 3.

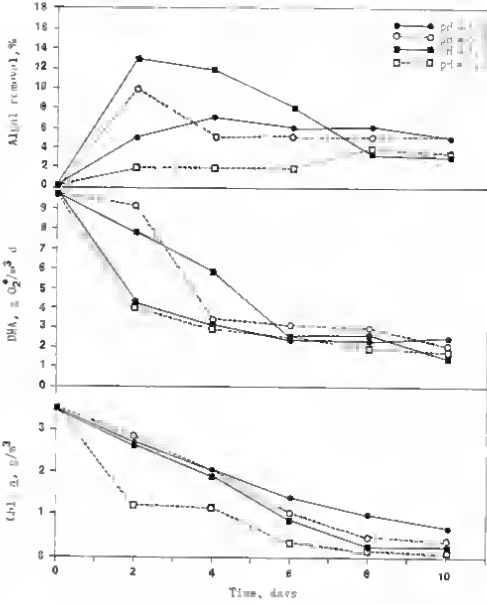

Figure 4-3 Effect of pH on temporal variation of settleability, DHA and chl a in a culture initially dominated by Synechocystis, exp. 7.

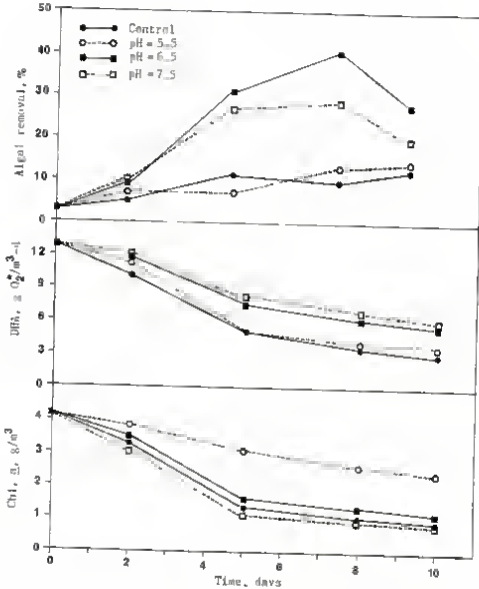

Figure 4-4 Effect of pH on temporal variation of settleability, DHA and chl a in a culture initially dominated by Synechocystis, exp. 5.

Table 4-1 Effect of pH on maximum algae removal and corresponding levels of DHA and chl a levels in cultures initially dominated by Chlorella (exp. 2, 3) and Synechocystis (exp. 7, 8)

Exp.	pH	Max. algae removal, %	DHA, g O_2*/m^3-d	Chl a, g/m^3
2	5.5	86.0	24.92	7.39
	7.5	7.4	6.00	17.68
	9.5	2.0	13.52	1.38
3	8.7**	7.2	7.34	2.93
	5.5	79.0	10.16	1.65
	6.5	82.0	7.96	2.72
	7.5	3.5	4.53	7.38
7	5.5	7.0	3.17	2.09
	6.5	10.0	9.15	2.89
	7.5	13.0	7.90	2.68
	8.5	4.0	2.01	0.20
8	7.5**	12.0	10.00	0.93
	5.5	13.2	5.30	2.45
	6.5	40.0	6.30	1.35
	7.5	27.5	7.42	0.95

**pH of the control when maximum algae removal was obtained

The best growth rate with a maximum chl a concentration of 23 g/m^3 was observed in the culture mixed at 80 rev/min, whereas 15 g chl a/m^3 was the maximum in the control after 8 days.

Complete bioflocculation marked by cell aggregation and rapid settling occurred within 4 days in the mixed cultures. In contrast, the control showed no sign of flocculation. Macroscopic flocs which were larger than 100 microns in diameter appeared and compacted after 8 days in all the cultures. They were composed entirely of Chlorella and Monodus with little or no bacterial component. As mixing intensity increased, floc density increased, resulted in much smaller flocs.

The production of settleable solids in the mixed cultures increased progressively in the order of mixing intensities, 20 rev/min, 80 rev/min, and 40 rev/min, whereas this parameter in the control increased very slowly or remained stationary. Algal removal and settled solids volume in the culture mixed at 40 rev/min were greater than those in the control (max. 90% vs 55% and 65 mL/L vs 24 mL/L) (Fig. 4-5).

The pH dropped gradually in all the cultures, initially, but declined to pH 6.0 rapidly in the cultures mixed at 40 rev/min and 80 rev/min after 8 days. The time when pH dropped coincided with the period of the extensive bioflocculation (Fig. 4-5, 4-6).

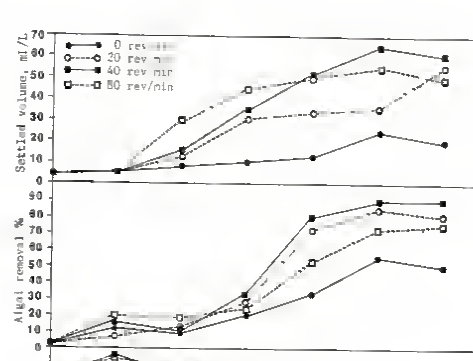

Figure 4-5 Effect of mixing on temporal variation of settleability, pH and chl a in cultures initially dominated by Chlorella and Monodus, exp. 5.

In exp. 6, complete bioflocculation was repeated but changes in the characteristics of the mixed cultures were less pronounced, compared to those in exp. 5. The bright-green matrices of Chlorella and Monodus cells were less compacted than those that appeared in the previous experiment. The culture mixed at 40 rev/min showed the highest algal removal, compared to the control on day 14 (max. 60% vs 28%). A higher growth rate was also observed in the culture mixed at 40 rev/min. Maximum chl a concentration of 28 g/m^3 was obtained in the culture mixed at 40 rev/min, whereas the maximum chl a was 15 g/m^3 in the control (Fig. 4-6).

Dissolved oxygen concentration declined progressively in all the cultures but the extent of decline in the control was much less than in the mixed cultures which had a DO below saturation after 6 days. The pH in the mixed cultures showed a rapid decline from 8.5 to 6.2 over days 0-3 but they remained at a pH below 6.0 after 10 days (Fig. 4-6). It was observed that the extensive bioflocculation occurred when DO dropped below saturation and pH went down below 6.0

Growth characteristics of Synechocystis at different mixing intensities were investigated (data are not presented here). The inhibitory effect of mixing on the growth of this alga was pronounced. Counts of this alga in all the cultures declined progressively throughout the experiment. The extent of decline in unmixed culture was much less than in mixed cultures. Decline in bacterial activities was also observed

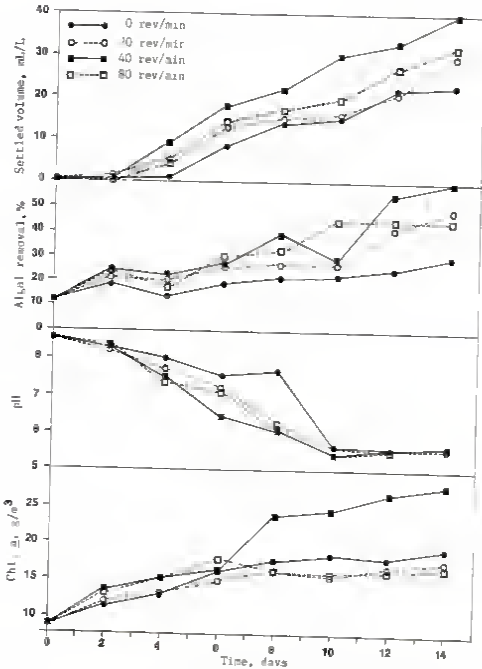

Figure 4-6 Effect of mixing on temporal variation of settleability, pH and chl a in cultures initially dominated by Chlorella and Monodus, exp. 6.

in all cultures.

Effect of mixing intensity on maximum settleability and corresponding levels of pH and chl a in cultures initially dominated by Chlorella and Monodus (exp. 5, 6) was summarized in Table 4-2.

4.3 Effect of Waste Loading

The effect of waste loading was investigated in 3 experiments. Different waste loading rates were evaluated in exp. 4 and 9. The influence of waste type was studied in exp. 11. Algal genera dominent during investigations were Chlorella and Monodus in exp. 4, 11 and Synechocystis in exp. 9.

4.3.1 Waste loading rate

In exp. 4, no significant difference in growth rates among the cultures was observed. The cultures loaded with FBR effluent weekly at 2% and 4% of media volume grew better than the control. Waste loaded cultures generally formed flocs earlier than the control. Flocs less than 100 microns in diameters were visible after 6 days in waste loaded cultures, but these showed poor settleability. A substantial bacterial component was identified in the floc structure. After 10 days, 42% algal removal was obtained in the culture loaded with FBR effluent weekly at 4% of the media volume, compared to 15% removal achieved in the control on day 10 (Fig. 4-7).

The pH dropped gradually in all the cultures initially, declined rapidly over days 4-8, then remained near pH 6.0

Table 4-2 Effect of mixing on maximum settleability and corresponding levels of pH and chl a in cultures initially dominated by Chlorella and Monodus, exp. 5, 6

Exp.	Mixing speed, rev/min	Max. settled volume, mL/L	Max algae removal, %	pH	Chl a, g/m³
5	0	24	55	8.01	12.78
	20	36	85	7.07	14.62
	40	65	90	6.36	18.02
	80	55	75	6.27	19.14
6	0	23	29	5.69	20.38
	20	30	51	5.61	17.62
	40	40	60	5.55	27.94
	80	32	47	5.55	16.77

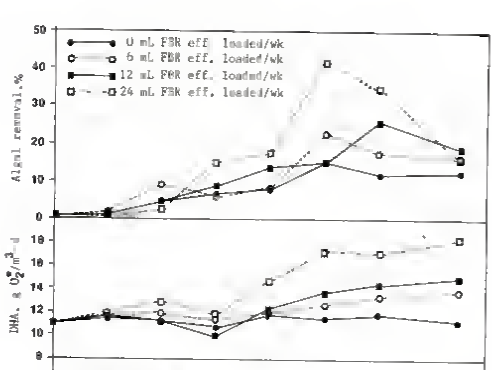

Figure 4-7 Effect of waste loading on temporal variation of settleability, DHA, pH and chl a in cultures initially dominated by Chlorella, and Monodus, exp. 4.

until the end of the experiment. DHA showed no big differences among cultures but values for heavily waste loaded cultures were somewhat greater than the control (Fig. 4-7).

In exp. 9, the effect of waste loading rate on bioflocculation was investigated with Synechocystis. Counts of this alga in all the cultures declined gradually during the experiment. The extent of decline in heavily waste loaded culture (5% of culture volume) was somewhat less than those in other cultures. Tan-green to orange-brown colored flocs became visible after 5 days but were not fully developed and not closely compacted. After 8 days, 40% algal removal was obtained in heavily waste-loaded culture, compared to 15% achieved in the control on day 8. DHA was also declined progressively (Fig. 4-8).

Effect of waste loading rate on maximum algae removal and correspondlng levels of DHA, pH and chl a in cultures initially dominated by Chlorella and Monodus (exp 4) and Synechocystis (exp. 9) was summarized in Table 4-3.

4 3.2 Waste Pretreatment

In exp. 11, two different types of wastes, settled waste supernatant and PBR effluent were pretreated and loaded to each culture. Generally, waste loaded cultures grew better than the control. The culture loaded with settled waste supernatant showed somewhat better growth potential than the culture loaded with fixed bed reactor effluent.

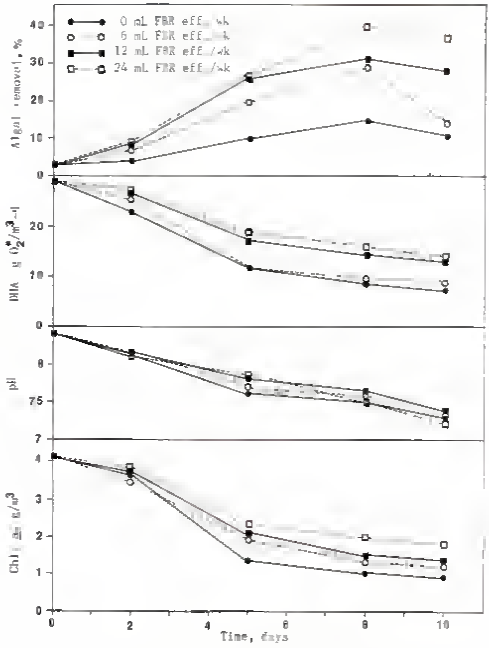

Figure 4-8 Effect of waste loading on temporal variation of settleability, DHA, pH and chl a in cultures initially dominated by Synechocystis, exp. 9.

Table 4.3 Effect of waste loading on maximum algae removal and corresponding levels of DHA, pH and chl *a* in cultures initially dominated by *Chlorella* and *Monodus* (exp. 4) and *Synechocystis* (exp. 9)

Exp.	Waste loading rate, mL FBR/wk	Max. algae removal, %	DHA g O_2*/m^3-d	pH	Chl *a*, g/m^3
4	0	15	11.72	5.95	9.75
	6	23	12.78	5.97	10.23
	12	26	14.55	5.95	9.91
	24	42	17.52	5.90	9.12
9	0	15	8.71	7.52	1.00
	6	29	9.68	7.60	1.35
	12	31	14.12	7.65	1.46
	24	40	15.72	7.52	2.00

Flocs became visible after 4 days in waste loaded cultures and 7 days in the control. The floc size and density in the waste loaded cultures were almost the same but much greater than that of the control. Flocs were composed almost entirely of Chlorella and Monodus with a small bacterial component. Oscillatoria and Spirulina sometimes were associated with flocs formed. They were easily identified by INT uptake. Pin flocs were developed but they did not grow further and showed poor settleability. Maximum algal removal of the cultures loaded with FBR effluent and settled waste supernatant were 46% and 43%, respectively on day 10. In contrast, 14% algal removal was obtained in the control (Fig. 4-9, Table 4-4).

DHA of the culture loaded with settled waste supernatant was somewhat greater than those of other cultures throughout the experiment.

4.4 Effect of Activated Sludge Seeding

In exp. 10, each culture received different levels of activated sludge as 5%, 10% and 20% of algal weight, whereas the control did not receive any. Chlorella and Monodus were co-dominant initially. Counts of these algae decreased in all the cultures during the experiment.

Microscopic examination showed that bacterial matrices surrounded by attached algal cells were present in the seeded cultures within 2 days and macroscopic flocs became visible after 4 days. Flocs did grow further but remained as pin flocs, showing poor settleability. A small contribution

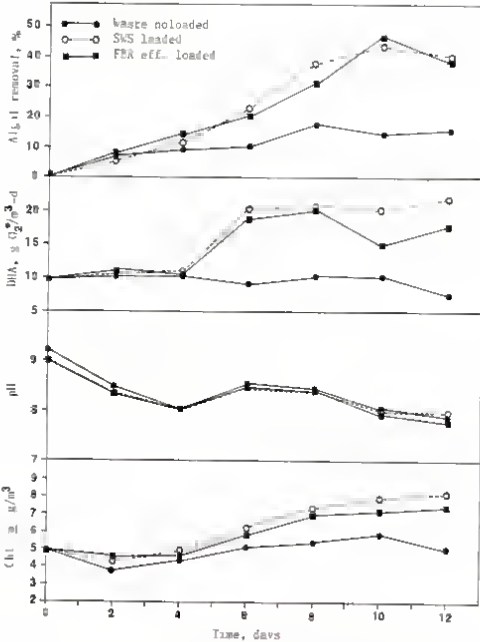

Figure 4-9 Effect of waste pretreatment on temporal variation of settleability, DHA, pH and chl a in waste loaded cultures initially dominated by Chlorella and Monodus, exp. 11.

Table 4-4 Effect of waste pretreatment on maximum algae removal and corresponding levels of DHA, pH and Chl *a* in cultures initially dominated by *Chlorella* and *Monodus*, exp. 11

Exp.**	Waste pretreatment	Max. algae removal, %	DHA, g O_2*/m^3-d	pH	Chl *a*, g/m^3
11	control	17	10.03	8.39	5.37
	SWS	43	20.04	8.01	7.92
	FBR	46	14.63	7.95	7.18

**Control: waste not loaded
SWS- settled waste supernatant
FBR- fixed bed reactor effluent

of Oscillatoria and Spirulina to floc was noticeable. Though full bioflocculation was not achieved, 49% algal removal was obtained in the culture seeded at 10% of algal weight on day 8. In contrast, the control showed 26% algal removal (Fig. 4-10, Table 4-5)

The pH was gradually decreased in all the cultures. DHA was proportionately higher in the heavily seeded cultures. Maximum DHA obtained was 37 g O_2*/m^3-d.

4.5 Effect of Other Variables

4.5.1 Effect of Carbon Addition

In exp. 1, the effect of carbon concentration on bioflocculation was investigated. Three levels of carbon concentration (50 g/m^3, 100 g/m^3, and 1,000 g/m^3) were compared to the control without carbon addition. No difference in growth rates among the cultures was observed. Complete bioflocculation marked by cell aggregation and rapid settling of cells occurred within 5 days. Algae removals of 93% and 81% were obtained in cultures added to 50 g carbon/m3 and 100 g carbon/m^3, respectively after 7 days. In contrast, the control and the culture added to 1,000 g carbon/m^3 showed no sign of flocculation. Macroscopic flocs which were larger than 100 microns in diameter appeared after 5 days. They were composed entirely of Chlorella with a substantial fraction of the bacterial component.

4.5.2 Effect of Algal Density

Three different initial algal concentrations (low: 1-5

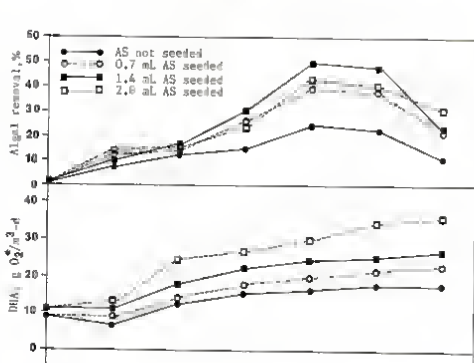

Figura 4-10 Effect of activated sludge seeding on temporal variation of settleability, DNA, pH and chl a in cultures initially dominated by Chlorella and Monodus, exp. 10.

Table 4-5 Effect of activated sludge seeding on maximum algae removal and corresponding levels of DHA, pH and chl a in cultures initially dominated by Chlorella and Monodus, exp. 10

Exp.**	AS seeding, % of algae dry weight	Max. algae removal, %	DHA g O_2*/m^3 d	pH	Chl a g/m^3
10	0	24	16.72	8.51	4.06
	5	39	19.82	8.45	3.92
	10	49	26.43	8.44	3.75
	20	43	30.09	8.43	3.61

**AS: Activated sludge

g chl a/m^3, intermediate: 4.0 g chl a/m^3, and high: 8.0 g chl a/m^3) were employed in cultures dominated by Chlorella and Monodus. The culture with high algal density showed very slow growth rate initially, then declined rapidly after 6 days. Cell numbers in the culture with intermediate algal density increased rapidly up to one doubling, then decreased progressively. The culture with low algal-density showed a gradual increase in cell counts throughout the experiment.

Microscopic examination showed that algal cells clumped and closely compacted in the cultures with high and intermediate algal-density after 8 days. The floc density was much greater in the high algal-density culture. No sign of flocculation in the low algal-density culture was observed. Settling test showed that algal removal by 2-h sedimentation was significantly greater in the high algal density culture than in the low one on day 10 (max. 62% vs 19%).

The pH was gradually decreasing in all the cultures. DHA was much higher in the high algal density culture:

CHAPTER 5
RESULTS OF FIELD EXPERIMENTS

5.1 Integrated System Operation

The quality characteristics of waste streams added to the algal cultures and operational data for the field system are given in this section. Climatological data are given in section 5.2. Results of field scale bioflocculation experiments are described in section 5.3.

5.1.1 Settling Tank and Digesters

Quality characteristics of settled waste solids and settled waste supernatant from the settling tank are given in Tables 5-1 and 5-2, respectively. The total solids (TS) content of settled waste solids was variable with season. Concentrations were highest in winter (e.g., 47,500 g/m^3 in January 1984) and lowest in summer (e.g., 4,000 g/m^3 in June 1985). This tendency was attributed to the use of cooling sprays for the animals with concomitant dilution of the raw waste stream during the summer. The TS of settled waste solids averaged 17,552 g/m^3. Volatile solids (VS) were approximately 74% of TS. Chemical oxygen demand (COD) and total kjeldahl nitrogen (TKN) averaged 32,740 g/m^3 and 1,877 g/m^3, respectively, giving a COD:TKN ratio of 17.5.

TS of settled waste supernatant also varied on a seasonal basis. On the average, the supernatant fraction was

Table 5-1. Monthly average characteristics of settled waste solids*

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Oct 83	19 830	14 940	27 650	1 840	1 200
Nov	24 050	16 730	52 880	3 580	3 220
Dec	--	--	--	--	--
Jan 84	47 500	38 110	36 900	2 880	2 520
Feb	17 030	12 650	38 980	2 470	1 640
Mar	18 550	14 300	42 380	2 170	1 650
Apr	15 290	11 570	42 650	1 810	1 570
May	8 080	5 350	14 850	1 100	913
Jun	7 140	4 110	15 840	1 250	983
Jul	13 460	9 890	27 070	1 350	980
Aug	9 680	6 640	19 000	924	699
Sep	13 500	10 010	27 180	1 225	723
Oct	25 140	19 850	51 670	2 000	1 350
Nov	23 800	18 980	48 470	2 030	1 520
Dec	24 640	18 520	50 040	2 755	2 100
Jan 85	32 480	24 810	66 810	3 330	2 780
Feb	18 870	13 750	43 250	2 370	1 750
Mar	26 420	20 090	50 290	2 900	2 130
Apr	25 320	18 870	49 350	2 625	1 760
May	13 820	10 160	26 150	1 470	940
Jun	4 000	2 600	8 910	479	339
Jul	8 140	5 573	14 335	970	658
Aug	5 815	3 613	7 495	886	700
Sep	10 878	7 009	14 646	1 285	893
Oct**	7 821	4 989	8 959	1 313	1 152
Mean	17 552	13 030	32 740	1 877	1 424
S.D.	10 038	8 203	17 165	841	727

*Values in g/m^3
**Anaerobic digester atop running at Oct 27, 1985

Source. R. Nordstedt, Personal communication, 1986, unpublished data.

Table 5-2. Monthly average characteristics of settled waste supernatant*

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb 84	10 670	6 530	22 680	1 970	1 600
Mar	10 620	7 010	32 840	2 220	1 440
Apr	10 510	7 230	37 980	1 560	1 340
May	10 940	8 120	25 480	1 460	1 210
Jun	12 210	8 600	20 350	1 400	1 000
Jul	6 760	4 100	8 670	1 110	963
Aug	3 910	1 940	5 610	927	808
Sep	7 010	4 605	20 940	945	749
Oct	11 010	7 715	28 740	1 480	1 180
Nov	15 210	11 600	37 740	1 610	1 180
Dec	18 030	12 820	40 510	2 470	1 900
Jan 85	15 850	11 110	43 120	2 470	2 300
Feb	18 420	13 070	33 550	2 920	1 925
Mar	16 470	11 420	41 880	2 660	1 995
Apr	17 670	12 480	39 050	2 490	1 880
May	4 750	4 460	15 100	1 130	850
Jun	3 400	2 170	4 355	479	353
Jul	5 349	3 439	8 106	830	641
Aug	3 933	2 178	5 423	809	693
Sep	4 827	2 655	5 775	1 000	806
Oct	4 869	2 781	5 161	1 064	865
Nov	5 112	3 299	5 868	789	559
Dec	7 465	5 066	12 601	1 225	911
Mean	9 782	6 713	21 806	1 522	1 180
S.D.	5 107	3 818	14 235	704	530

*Values in g/m^3

Source. R. Nordstedt, Personal communication, 1986, unpublished data.

approximately 56% and 52%, respectively, of the settled solids fraction in terms of TS and VS. Its average concentrations of COD and TKN were 67% and 81%, respectively, of those in the solids fraction. The average COD:TKN ratio of settled waste supernatant was 14.3.

The volumetric feed rate of settled waste solids to the conventional digester was initially 1.0 m^3/d (Oct. 83-Jun. 84) and was raised subsequently to 1.3 m^3/d (Jul. 84-Oct. 85). Corresponding liquid detention times were 20 and 15 days, respectively. Mean organic loading was 0.52 kg VS added daily per m^3 of liquid volume (kg VS_a/m^3d), as shown in Table 5-3. Digester temperature ranged from 13.5-29.7 °C, averaging 22.8 °C.

The fixed bed reactor (FBR) was started in Jan. 1984. The volumetric feed rate of settled waste supernatant to the FBR on an empty bed volume basis was 1.90 m^3/d during Jan. 1984-Oct. 1985 and 3.8 m^3/d during Nov.-Dec. 1985, giving corresponding liquid detention times of 10.5 days and 5.3 days, respectively. Organic loading rates ranged from 0.300-1.917 kg VS_a/m^3d, averaging 0.95 kg VS_a/m^3d (Table 5-3). The operating temperature ranged from 15.6-30.4 °C, averaging 23.9 °C.

Monthly average characteristics of effluents from the anaerobic digester and fixed bed reactor are given in Tables 5-4 and 5-5. Pollutant levels in both waste streams were least in the summer and greatest in winter, reflecting the

Table 5-3. Loading and temperature of anaerobic digester and fixed bed reactor.

Month	Anaerobic digester Loading, kg VS_a/m^3 d	Temp., C	Fixed bed reactor Loading,[a] kg VS_a/m^3 d	Temp., C
Oct 83	0.751	24.6	--	--
Nov	0.870	19.4	--	--
Dec	(b)	13.6[c]	--	--
Jan 84	1.980	13.5	--	--
Feb	0.657	16.9	1.166	15.6[c]
Mar	0.714	19.0	1.212	17.2
Apr	0.541	21.4	1.162	24.2
May	0.242	26.7	1.263	27.1
Jun	0.144	28.5	0.973	29.5
Jul	0.658	28.8	0.709	29.8
Aug	0.395	29.5	0.300	30.4
Sep	0.567	27.4	0.658	28.3
Oct	1.134	25.4	1.156	27.0
Nov	1.076	19.8	1.588	22.1
Dec	0.578	17.8	1.772	19.0
Jan 85	0.544	15.0	1.152	16.3
Feb	0.427	15.2	1.917	17.4
Mar	0.661	21.1	0.940	22.4
Apr	0.670	24.1	1.438	25.5
May	0.604	27.8	0.668	28.8
Jun	0.178	29.7	0.375	30.0
Jul	0.378	27.2	0.429	27.2
Aug	0.253	27.4	0.274	27.4
Sep	0.435	25.8	0.308	26.8
Oct	0.320	25.0	0.321	24.9
Nov	(b)	--	0.885	21.6
Dec	(b)	--	1.245	12.7
Mean	0.615	22.8	0.953	23.9
S.D.	0.377	5.2	0.476	5.2

[a]Empty bed volume basis

[b]Digester not operated during this period

[c]Ambient temperature

Source: R. Nordstedt, Personal communication, 1986, unpublished data.

Table 5-4. Monthly average characteristics of anaerobic digester affluent*

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjaldahl Nitrogen	Ammonia Nitrogen
Oct 83	32 060	23 770	34 620	2 450	1 620
Nov	31 030	22 090	38 580	3 220	2 380
Dec	--	--	--	--	--
Jan 84	24 960	17 720	27 240	3 520	2 550
Feb	38 410	29 730	50 980	3 690	2 740
Mar	25 990	19 770	41 920	2 430	1 950
Apr	16 390	12 360	38 390	1 915	1 290
May	10 720	8 195	21 920	1 350	1 180
Jun	8 960	5 495	11 680	1 305	1 040
Jul	8 030	4 870	9 730	1 180	930
Aug	6 630	3 970	8 080	1 180	962
Sep	7 550	4 810	7 360	987	686
Oct	12 180	8 600	14 540	1 690	1 055
Nov	13 160	10 480	18 950	1 620	1 160
Dec	18 120	13 330	26 670	2 200	1 490
Jan 85	16 320	11 570	19 880	2 440	1 820
Feb	20 010	14 370	30 350	2 660	1 980
Mar	21 320	14 780	23 510	2 980	2 120
Apr	19 910	13 460	22 660	2 890	2 030
May	13 590	8 980	16 080	1 995	1 390
Jun	5 550	3 430	4 630	835	570
Jul	5 690	3 412	5 576	911	711
Aug	6 017	3 538	5 479	900	749
Sep	7 442	4 399	8 185	1 215	1 145
Oct	6 084	4 422	5 552	1 505	1 274
Mean	15 672	11 148	20 513	1 962	1 453
S.D.	9 425	7 309	13 340	874	616

*Values in g/m^3

Source. R. Nordstedt, Personal communication, 1986, unpublished data.

Table 5-5. Monthly average characteristics of fixed bed reactor effluent*

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb	10 660	5 830	23 110	1 970	1 700
Mar 84	10 620	7 010	33 090	2 220	1 440
Apr	10 510	7 230	35 980	1 560	1 340
May	10 940	8 120	25 480	1 460	1 210
Jun	12 210	8 600	20 350	1 400	1 000
Jul	6 760	4 100	8 670	1 110	963
Aug	3 910	1 940	5 610	927	808
Sep	3 220	1 645	2 870	731	668
Oct	7 580	5 100	10 720	1 380	1 130
Nov	10 300	7 500	14 210	1 490	1 070
Dec	13 620	9 810	17 950	2 170	1 620
Jan 85	11 450	7 855	23 420	2 580	2 340
Feb	10 370	6 825	17 840	2 250	1 750
Mar	11 540	7 565	16 050	2 550	1 850
Apr	14 090	9 400	22 730	2 550	1 925
May	4 080	2 160	9 510	1 590	1 260
Jun	2 060	981	5 840	584	514
Jul	2 646	2 346	1 797	749	650
Aug	3 082	1 496	2 424	774	693
Sep	3 099	1 391	2 922	893	826
Oct	3 439	1 580	2 759	1 022	907
Nov	3 129	1 778	3 387	662	546
Dec	6 100	3 989	8 253	1 278	1 064
Mean	7 627	4 924	13 696	1 474	1 188
S.D.	4 070	3 066	10 221	657	496

*Values in g/m^3

Source: R. Nordstedt, Personal communication, 1986, unpublished data.

variation of influent streams. TS ranged from 5,550-38,410 g/m^3 in digester effluent and from 2,060-14,090 g/m^3 in FBR effluent, averaging 15,672 g/m^3 and 7,627 g/m^3, respectively. COD and TKN in digester effluent averaged 20,513 g/m^3 and 1,962 g/m^3, respectively. The corresponding two parameters in FBR effluent averaged 13,697 g/m^3 and 1,474 g/m^3, respectively. Mean COD:TKN ratios were 10.5 in digester effluent and 9.3 in FBR effluent.

5.1.2 Anaerobic Lagoon and Facultative Pond

The anaerobic lagoon functioned as a continuous culture, predominantly of the purple sulfur bacterium (Chromatiaceae), Thiopedia rosea. This culture has been established for several years and was a central part of the waste treatment facility. It had distinctly pink color in the summer and a brownish-pink color during the winter. An absence of odor nuisance is attributed to the oxidation of H_2S to elemental sulfur by this T. rosea.

As shown in Table 5-6, mean COD and TKN concentrations were reduced to 1,300 g/m^3 and 450 g/m^3, respectively. Mean effluent phosphorus concentration was 120 g/m^3.

The photosynthetic microbial population of the facultative pond was dominated by microalgae during summer and fall and by purple sulfur bacteria during winter and early spring. Further reduction of pollutant levels was achieved in the facultative pond (Table 5-6).

5.1.3 Bioflocculation Channels

On a volumetric basis, high-rate pond effluent was the

Table 5-6 Mean annual characteristics of anaerobic lagoon and facultative pond effluents*

Stage	Chemical oxygen demand, g/m^3	Biochemical oxygen demand, g/m^3	Total susp. solids, g/m^3	Volatile susp. solids, g/m^3	Total Kjeldahl nitrogen, g/m^3	Total phos-phorus, g/m^3	No. samples
Anaer. 1	350 (33)	291 (49)	1 220 (33)	897 (29)	448 (17)	118 (40)	19
Facul.	361 (48)	--	--	--	131 (47)	51 (27)	27

*Coefficient of variation given in ( )

most significant feed source for bioflocculation channels. Waste loading to the bioflocculation channels was varied according to the experiment in progress (Table 5-7). In some cases the rate of loading or the type of waste added was the experimental variable examined. The type of waste most commonly utilized was FBR effluent.

Zooplankton populations were controlled by addition of equal volume of ammonium hydroxide to the channel on an as-needed basis (Table 5-8). In experiment 8, ammonium hydroxide was not added to the experimental culture (C-2) in order to avoid raising its pH. Although the volumes of ammonium hydroxide added to each channel were generally identical, differences in the resulting free ammonia of each channel were caused by variations in channel depth and culture pH, as indicated in Table 5-8, as well as variations in the initial total ammonia concentration, which were not assured.

During exp. 8, concentrated sulfuric acid was added to channel C-2 on an intermittent basis in order to reduce the culture pH to a target value of 6.0 (Table 5-9). The target pH was sometimes undershot, however, dropping the pH below 4.5 in several instances.

5.1.4 High-rate Pond

Alternative feed sources for the high-rate pond (C-4) included effluents from the facultative pond, anaerobic digester, and fixed bed reactor (Table 5-10). Facultative pond (F-2) effluent (average 2.29 m^3/d) was the most

Table 5-7. Waste loading to bioflocculation channels*

Exp.	C-1 Source	C-1 Rate, L/d	C-2 Source	C-2 Rate, L/d
1	STR	21	STR	21
2	STR FBR	2 9	STR FBR	2 9
3	FBR	17	FBR	17
4	--	0	--	0
5	--	0	FBR	147
6	FBR	72	FBR	217
7	FBR	85	FBR	85
8	FBR	38	FBR	38
9	FBR	9	P-1 FBR	772 25
10	SWS	105	FBR	105
11	FBR	180	--	0
12	FBR	190	FBR	190
13	FBR	82	FBR	82
14	FBR	67	FBR	67

*Abbreviations for waste sources:
SWS = settled waste supernatant
STR = anaerobic digester
FBR = fixed bed reactor
P-1 = anaerobic lagoon
P-2 = facultative lagoon

Table 5-8. Ammonium hydroxide additions to bioflocculation channels for grazer control

Exp.	Day	C-1			C-2		
		Added NH_4OH g N/m3	pH	Free NH_3* g N/m3	Added NH_4OH g N/m3	pH	Free NH_3* g N/m3
1	10	56	9.3	29	65	9.2	30
	28	37	8.6	7	37	9.4	21
2	11	25	9.7	18	24	9.9	19
	41	56	10.1	49	41	10.5	39
3	16	33	9.0	12	35	9.3	18
4	-1	20	9.0	7	20	9.0	7
	1	20	9.0	7	20	9.1	8
	18	38	9.2	18	39	9.8	30
5	1	35	8.9	11	37	8.9	11
	10	38	9.4	22	38	8.9	12
	20	54	9.4	31	57	9.0	20
6	5	63	8.5	9	66	8.3	7
8	-2	50	9.1	21	50	9.1	21
	12	150	9.3	79	--	--	--
11	4	45	9.0	16	45	9.1	18
12	-1	37	9.1	15	37	9.2	17
13	8	32	8.5	5	32	8.5	5
14	5	44	9.1	18	47	9.1	19

*Contributed directly by added NH_4OH

Table 5-9. Sulfuric acid additions to channel C-2 during experiment 8

Day	Volume, l	Initial pH	Final pH
0	11.0	8.7	6.3
1	4.0	7.9	6.4
3	4.0	7.6	6.1
4	4.0	7.6	3.5
6	2.0	7.1	4.1
9	1.3	6.9	4.3
13	0.7	7.5	6.5
16	0.6	7.2	6.3
17	1.5	6.6	3.9
21	0.4	7.1	--
30	7.5	8.1	4.5

Table 5-10. Waste loading to high-rate pond[a]

Month	Waste source			
	Facul. pond	Anaer. dig.	Fixed bed react.	Other
Oct 83	--	0.13	--	--
Nov	6.17	0.27	--	--
Dec	--	--	--	--
Jan 84	--	0.12	0.31	--
Feb	--	--	0.88	--
Mar	--	--	0.71	0.10[b]
Apr	--	--	0.33	--
May	7.55	--	0.45	--
Jun	5.60	--	0.47	--
Jul	--	--	0.74	--
Aug	--	--	0.53	--
Sep	9.00	--	0.43	--
Oct	9.87	--	0.27	--
Nov	4.00	--	0.25	0.40[c]
Dec	1.06	--	0.45	--
Jan 85	0.97	--	0.55	0.01[d]
Feb	0.21	--	0.54	--
Mar	0.97	--	0.38	--
Apr	1.20	--	0.05	--
May	--	0.74	1.05	3.00[c]
Jun	--	--	0.03	--
Jul	8.81	--	0.84	--
Aug	--	0.05	0.73	--
Sep	--	0.36	0.57	--
Oct	6.17	0.55	0.70	--
Nov	1.60	--	1.43	--
Dec	--	--	2.02	--
Mean	2.33	0.08	0.55	0.13

[a] m^3/d

[b] c_{-1}

[c] Anaerobic lagoon

[d] Settled waste supernatant

important feed source as a volumetric basis followed by FBR effluent (0.85 m^3/d). When organic loading rate in terms of COD is considered, FBR effluent (average 7.4 kg COD/d) was the most significant feed source, followed by facultative pond effluent (average 0.8 kg COD/d).

Infestations of rotifers (Brachionus rubens) and cladocerans (Diaphanosoma brachyurum) in the high-rate pond were controlled by temporarily raising the free ammonia concentration to a target value of 20 g/m^3 (Lincoln et al., 1983) by addition of NH_4OH solution (Table 5-11). Algal growth is not inhibited by ammonium hydroxide dosages equal to or somewhat higher than those lethal to zooplankton.

Without mechanical mixing, the major algal genera dominating in high-rate pond medium were Chlorella, Monodus, and Synechocystis. As shown in Table 5-12, Chlorella and Monodus coexisted but Chlorella was dominant during the winter and early spring. Monodus gradually began increasing as spring progressed and declined in late spring or early summer and was succeeded by Synechocystis. Synechocystis populations remained dominant throughout the summer and fall seasons. On a biovolume basis, Chlorella was dominant during 18 months of the 25 month period monitored, whereas Synechocystis was dominant in the other 7 months. Monodus, although second in overall importance to Chlorella, did not predominate during any of months studied. Phototrophic bacteria (Thiopedia rosea) were commonly observed, but were not significant on a biovolume basis.

Table 5-11. Ammonium hydroxide additions to high-rate pond for grazer control

Date	NH_4OH added g N/m^3	pH	Free NH_3* g N/m^3
5 Oct 83	32	8.6	6
14 Oct	39	9.1	16
25 Oct	25	9.4	17
16 Mar 84	29	8.5	4
18 Apr	37	8.7	8
3 Jul	62	8.5	9
26 Jul	43	9.5	27
7 Sep	103	9.5	66
3 Oct	97	9.2	45
15 Oct	41	8.7	9
21 Jun 85	51	8.6	9
23 Jul	164	9.3	82
8 Aug	31	9.4	18
22 Aug	15	9.2	7
20 Sep	24	8.7	6
24 Sep	23	8.8	6
4 Nov	34	8.9	11

*Contributed directly by added NH_4OH

Table 5-12. Photosynthetic community composition of high-rate pond*

Month	Normalized cell count (#/grid)			
	Chlor.	Monod.	Synec.	Thiop.
Oct 83	1.7	0.2	47.9	1.3
Nov	47.4	0.9	7.1	3.4
Dec	25.7	1.4	0.5	0.1
Jan 84	4.3	0.6	0.1	tr
Feb	29.6	0.3	0.2	3.7
Mar	58.9	10.3	tr	0.2
Apr	20.9	1.5	45.7	7.1
May	41.4	14.6	21.1	15.8
Jun	30.4	19.6	44.5	4.6
Jul	4.5	1.3	68.1	6.1
Aug	0.6	0.1	46.1	0.9
Sep	73.6	22.5	19.8	1.0
Oct	45.3	31.7	1.2	3.6
Nov	59.3	10.2	tr	4.3
Dec	73.2	29.0	tr	4.3
Jan 85	103.0	56.0	tr	7.3
Feb	102.7	73.0	tr	7.7
Mar	181.5	61.5	tr	10.0
Apr	106.7	46.7	tr	3.0
May	66.0	29.3	2.3	41.1
Jun	133.5	16.0	2.2	1.4
Jul	80.2	21.1	0.5	1.2
Aug	60.0	23.9	0.4	5.4
Sep	9.2	4.6	37.3	tr
Oct	19.7	4.8	107.0	0.5
Nov	69.1	20.7	15.7	1.1
Dec	89.5	40.3	0.1	5.6
Mean	56.2	20.9	17.3	5.2

*Abbreviations: Chlor. = Chlorella spp.
Monod. = Monodus spp.
Synec. = Synechocystis spp.
Thiop. = Thiopedia rosea
tr = trace

5.2 Climatological Information

Climatological data on minimum and maximum air temperatures, rainfall, pan evaporation, wind and photosynthatically active radiation (PAR) were obtained from readings taken at the Agronomy Farm Weather Station of University of Florida, Gainesville.

Monthly average maximum and minimum temperatures are shown in Figure 5-1. The highest monthly average maximum temperature was 33.6 °C in June 1985. The lowest monthly average minimum temperature was 2.9 °C in January 1985. The variation of monthly average photosynthetically active radiation (PAR) was similar to that of temperature. The highest monthly average PAR was in May 1985 (1345 Einsteins/m^2-d) whereas the lowest was in December 1984 (408 Einsteins/m^2-d) (Fig. 5-2B).

Wind movement was greatest in the spring and fall and least in the summer (Fig. 5-2A).

Monthly rainfall was greater in the late spring and summer than in the late fall and winter. Highest monthly rainfall was in August 1985 (17.8 cm) and the lowest in December 1984 (1.2 cm) (Fig. 5-3A).

The variation of monthly evaporation was similar to that of PAR and temperature. Monthly evaporation was higher in the late spring and summer than in the fall and winter. Highest monthly evaporation was in May 1985 (20 cm) and the lowest in December 1983 (4.3 cm) (Fig. 5-3B).

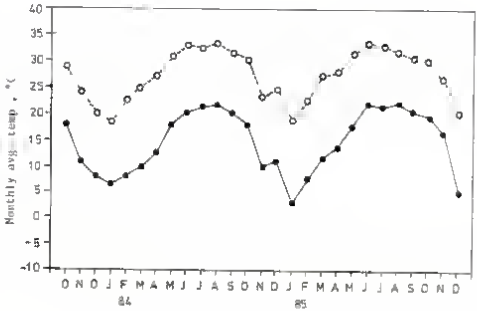

Figure 5-1 Monthly average maximum and minimum air temperature

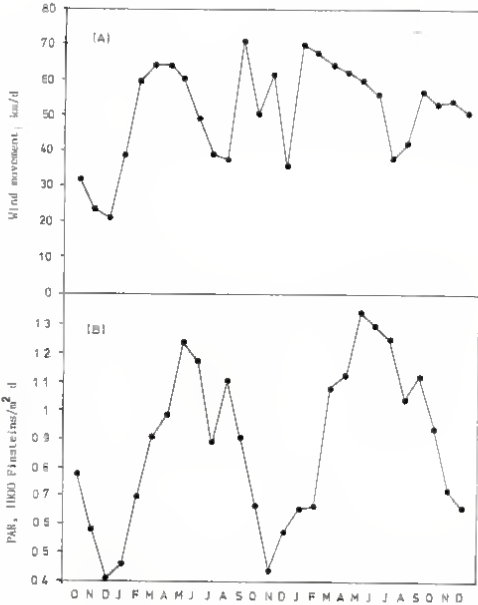

Figure 5-2 Monthly average wind movement at 60 cm above ground level (A) and monthly average photosynthetically active radiation (B).

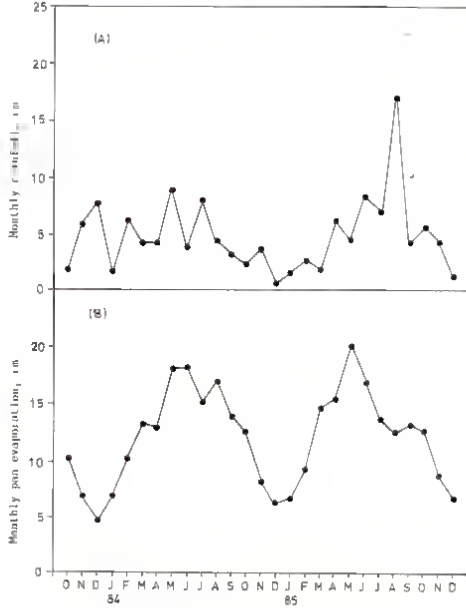

Figure 5-3 Monthly rainfall (A) and monthly pan evaporation (B).

5.3 Field Trials

5.3.1 Effect of Mixing

The effect of mixing on biofloccuiation was investigated in 5 experiments. Mixed cultures and unmixed controls were compared in exp. 1, 2, and 4. The relative effects of two different mixing velocities (14 cm/s and 30 cm/s) were examined in exp. 12 and 13.

5.3.1.1 Mixing versus Nonmixing

Algal genus tested in exp. 1 and 2 was Synechocystis (Tables 5-13, 5-14). Counts of this alga suspended in mixed cultures fell to less than 2% (exp. 1) and 15% (exp. 2) of those in unmixed controls over periods of one month in each trial. Declines in cell numbers were most rapid between the third and fourth weeks.

In exp. 1 (Fig. 5-4), characteristics of the mixed culture (C-2) changed dramatically during the first three weeks. Complete biofloccuiation marked by aggregation and rapid settling of cells occurred within 22 days. Settling tests showed 95% removal of the algae in 4 min with a settling rate of 8 cm/s and settled volume of 35 mL/L. No settling occurred in the samples from C-1. This phenomenon was preceded by a decline in DO, from an average concentration of 18 g/m^3 on days 0-7 to 5 g/m^3 on days 8-22. The pH in the mixed culture showed a progressive decline, from 8.0 to 6.8 over days 0-22. In contrast, the unmixed culture (C-1) showed no signs of floccuiation.

Table 5-13. Effect of mixing on photosynthetic community composition in bioflocculation channels, exp. 1*

Day	Pond	Normalized cell count (#/grid)			
		Chlor.	Monad.	Synec.	Thiop.
0	C-1	1.8	0.4	82.0	0.0
	C-2	1.4	0.4	80.0	0.0
4	C-1	1.8	1.4	88.3	0.0
	C-2	1.6	1.0	84.7	0.0
11	C-1	1.4	0.6	72.8	1.2
	C-2	1.2	0.4	74.4	0.2
18	C-1	1.0	0.8	63.4	0.0
	C-2	1.0	0.8	64.0	0.0
26	C-1	1.8	0.8	61.4	0.0
	C-2	0.6	0.0	6.0	0.0
32	C-1	1.6	0.8	53.4	0.0
	C-2	0.4	0.2	0.8	0.0
38	C-1	1.2	0.4	48.2	0.0
	C-2	1.4	0.0	0.9	0.0
42	C-1	0.2	0.0	34.6	0.0
	C-2	0.0	0.0	0.0	0.0
45	C-1	1.0	1.1	45.4	0.0
	C-2	1.5	1.7	0.2	0.0
53	C-1	0.3	1.4	32.8	0.0
	C-2	0.8	1.1	0.9	0.0

*C-1 Cotrol (not mixed)
C-2 Mixed

Table 5-14. Effect of mixing on photosynthetic community composition in bioflocculation channels, exp. 2*

Day	Pond	Normalized cell count (#/grid)		
		Chlor.	Monod.	Synec.
0	C-1	0.3	0.7	24.2
	C-2	0.3	1.1	23.2
1	C-1	0.7	1.4	22.5
	C-2	0.8	1.4	22.3
4	C-1	0.0	0.4	14.6
	C-2	0.0	0.6	14.6
5	C-1	0.7	1.0	18.1
	C-2	0.8	1.3	17.4
7	C-1	0.6	1.1	21.4
	C-2	0.8	2.4	19.8
9	C-1	0.4	1.1	21.4
	C-2	0.6	2.5	18.5
10	C-1	1.4	1.6	24.2
	C-2	2.0	4.8	15.6
13	C-1	1.4	1.6	21.6
	C-2	2.5	3.4	17.7
16	C-1	1.0	2.4	20.9
	C-2	5.7	8.4	14.3
19	C-1	5.4	3.9	20.5
	C-2	7.5	12.1	16.1
20	C-1	8.9	7.9	22.0
	C-2	8.1	16.2	13.4
21	C-1	11.4	7.5	23.1
	C-2	3.9	16.1	11.5
23	C-1	13.0	8.6	23.3
	C-2	13.8	24.7	9.7
26	C-1	11.9	8.6	23.8
	C-2	12.5	24.7	4.2
28	C-1	11.5	9.1	21.6
	C-2	11.3	20.0	6.8
32	C-1	14.8	9.5	24.5
	C-2	15.1	27.1	3.6
35	C-1	14.9	9.1	23.1
	C-2	14.2	24.5	2.9
38	C-1	17.0	10.2	21.5
	C-2	23.5	35.6	1.5
42	C-1	15.8	10.2	18.0
	C-2	23.0	29.7	1.0

*C-1 Control (not mixed)
C-2 Mixed

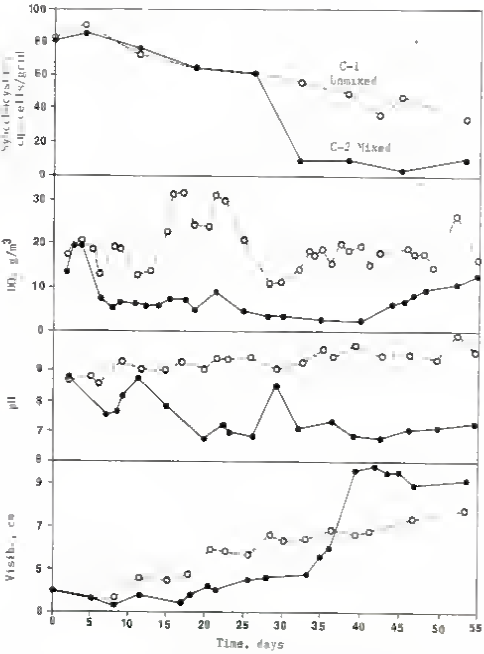

Figure 5-4 Temporal variation of Synechocystis, DO, pH and visibility in mixed (C-2) and unmixed (C-1) cultures. Exp. 1.

The pH rose to 9.6-9.8 and DO consistently reached peak values in excess of 35 g/m^3. Averages for these two parameters were 18 g/m^3 and 9.0, respectively.

Microscopic examination showed that flocs of Synechocystis were present after 4 days. Macroscopic floc became visible after 13 days. Flocs in the mixed culture consisted of Synechocystis cells with no visible bacterial component. They ranged from 50 to 400 microns in diameter. The flocs at first retained the blue-green coloration typical of Synechocystis, but bleached noticeably with time.

In exp. 2, changes in the characteristics of the mixed (C-1) culture were less pronounced. Microscopic examination showed that Synechocystis cells became aggregated after 9 days. Floc grew to diameter up to 50 microns diameter after 13 days. Floc particles of 200 to 300 microns diameter were abundant in 22 days (density of 1.2 flocs/field at 100 x). Settleability of the culture was poor, showing 25% removal of Chl a by sedimentation at day 23 (Fig. 5-5).

Chlorella and Monodus, though initially negligible, developed significant populations by the mid-point of the experiment (Table 5-14). These algae showed no tendency to flocculate. DO and pH levels in the mixed culture were somewhat less than those in the unmixed culture (Fig. 5-6).

Experiment 4 started with co-dominant cultures of Synechocystis and Chlorella (Table 5-15, top). Synechocystis counts declined rapidly in both the mixed (C-1) and unmixed

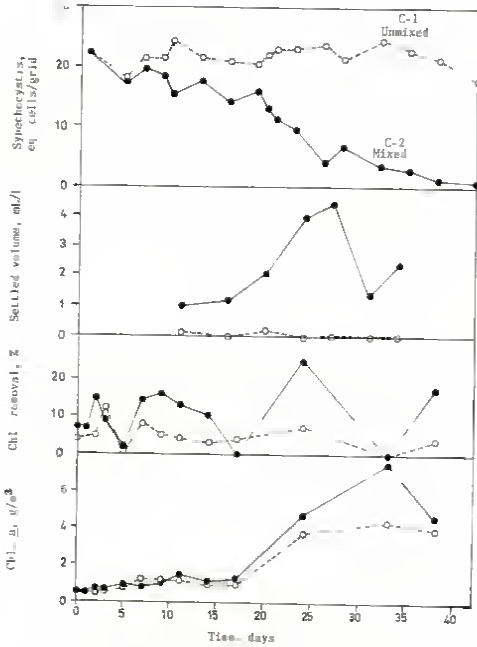

Figure 5-5 Temporal variation of Synechocystis, settleability and chl. a in mixed (C-2) and unmixed (C-1) cultures. Exp. 2.

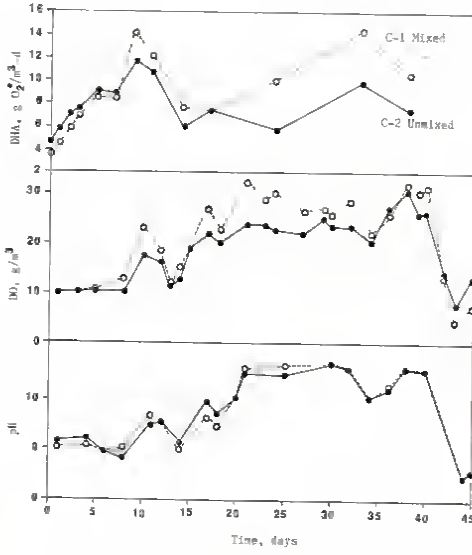

Figure 5-6 Temporal variation of DHA, DO and pH in mixed (C-1) and unmixed (C-2) cultures initially dominated by Synechocystis. Exp. 2.

Table 5-15. Effect of mixing on photosynthetic community composition in bioflocculation channels, exp. 4, 12, 13

Exp.*	Day	Pond	Normalized cell count (#/grid)			
			Chlor.	Monad.	Synec.	Thiop.
4	4	C-1	30.2	6.2	38.8	4.8
		C-2	29.9	6.4	40.0	0.4
	8	C-1	33.5	5.7	32.4	0.4
		C-2	29.8	7.2	40.6	0.0
	13	C-1	40.4	29.8	23.4	0.0
		C-2	44.4	6.9	26.8	0.0
	18	C-1	35.7	7.7	3.4	0.4
		C-2	34.8	3.1	4.4	0.1
12	-1	C-1	92.2	63.4	0.3	0.3
		C-2	90.4	63.4	0.3	tr
	6	C-1	107.8	78.0	0.5	0.0
		C-2	107.9	75.7	5.4	0.0
	13	C-1	106.6	45.0	0.6	0.2
		C-2	118.2	39.8	0.1	0.0
	24	C-1	75.2	32.0	0.4	0.0
		C-2	49.6	16.6	0.0	0.0
13	-2	C-1	17.2	3.8	112.8	0.0
		C-2	16.5	3.5	110.0	0.0
	7	C-1	12.4	0.0	77.0	0.0
		C-2	3.6	0.0	184.4	0.0
	13	C-1	30.6	5.2	14.3	0.4
		C-2	10.1	1.8	1.7	0.0

*Exp. 4: C-1 Mixed
C-2 Control (not mixed)

Exp. 12: C-1 Slow mixed
C-2 Fast mixed

Exp. 13: C-1 Fast mixed
C-2 Slow mixed

(C-2) culture after two weeks, whereas Chlorella and Monodus cell counts increased. Bioflocs of 20 to 80 microns in diameter were noticed in the mixed, C-1 medium within one week and progressively increased in number and size, whereas flocs were not observed in the C-2 medium. Microscopic examination showed that algal cells clustered with a density of 20-100 cells/grid and were closely compacted at 13 days. They were composed predominantly of Synechocystis (50-75%) with some Chlorella and Monodus (10-20%) entrapped. Straight, long chains of filamentous bacteria with a density of approximately 5 filaments/grid contributed.

The difference in visible flocculation characteristics was reflected in the respective settleable solids contents of the two cultures (Fig. 5-7). A maximum settled solids volume of 17 mL/L was measured in the mixed culture, whereas this parameter remained near zero in the unmixed culture. Despite the flocculation of C-1 medium, algae removal obtained by 24-h sedimentation declined from 12% to 3% during the course of the experiment. In contrast, up to 25% algae removal was attained with the non-flocculent C-2 medium.

The growth pattern and DO of the mixed culture were similar to those of the unmixed culture. The mixed culture grew faster initially, but was overtaken after 12 days by the unmixed culture (Fig. 5-8). Dehydrogenase activity was generally much greater in the mixed culture (avg. 25 vs. 17 g O_2*/m^3d) whereas pH was less (avg. 9.0 vs. 10.3).

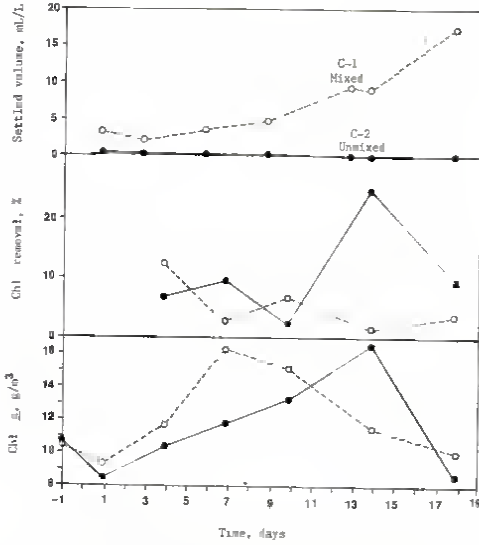

Figure 5-7 Temporal variation of settleability and chl a in mixed (C-1) and unmixed (C-2) cultures initially dominated by Synechocystis and Chlorella. Exp. 4.

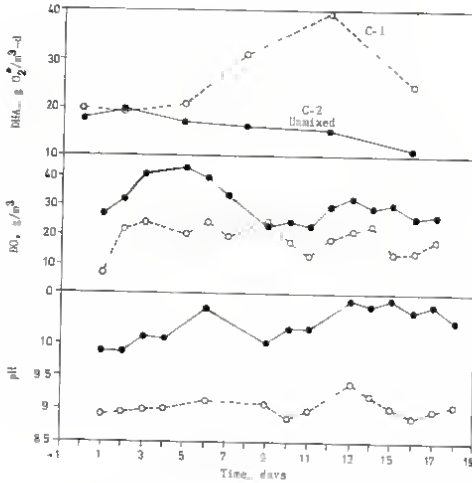

Figure 5-8 Temporal variation of DHA, DO and pH in mixed (C-1) and unmixed cultures (C-2) initially dominated by Synechocystis and Chlorella, Exp. 4.

5.3.1.2 Flow Mixing Velocity

Two different mixing intensities, 14 cm/s and 30 cm/s, were employed during exp. 12 and 13.

In exp. 12, Chlorella and Monodus were co-dominant initially and remained so throughout the experiment (Table 5-15, middle). Flocs became visible after 12 days in both C-1 and C-2. The floc size and density was greater in the fast-mixed culture (C-2). Flocs in C-2 medium were 50 to 200 microns in diameter with a density of 2.1 flocs/field at 400x. Flocs in C-1 medium were 50 to 80 microns with a density of 1.1 flocs/field. A maximum settled solids volume of 53 mL/L was measured in C-2 compared to 10 mL/L in C-1. Algae removals by 24 h sedimentation were similar in both cultures through day 14, after which they became significantly greater in C-2 (max. 56% vs. 36%) (Fig. 5-9).

DO and pH were generally greater in the slow-mixed culture, whereas OHA and algae concentration were greater in the fast-mixed culture (Fig. 5-9, 5-10).

In exp. 13, Synechocystis was dominant initially but declined rapidly in both cultures after the first week. Counts of this alga in both cultures fell to less then 12% of the initial counts over two weeks in each trial (Table 5-15, bottom). Beside the inhibitory effect of mixing on Synechocystis, significant populations of the protozoan, Fleuromonas, also affected this rapid decay.

Flocs became visible after 7 days in both cultures. Floc particles appeared as a predominately

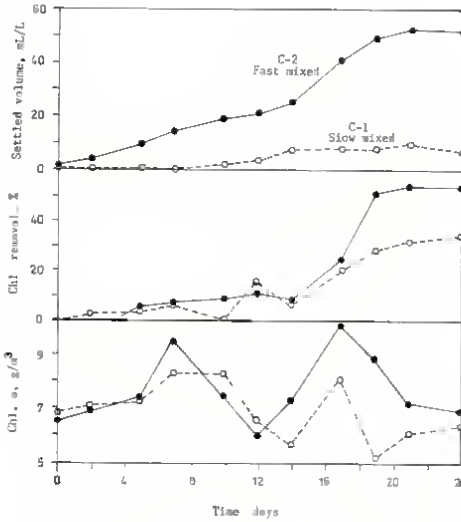

Figure 5-9 Temporal variation of settleability and chl a in slow mixed (14 cm/s, C-1) and fast mixed (30 cm/s, C-2) cultures initially dominated by Chlorella and Monodus. Exp. 12.

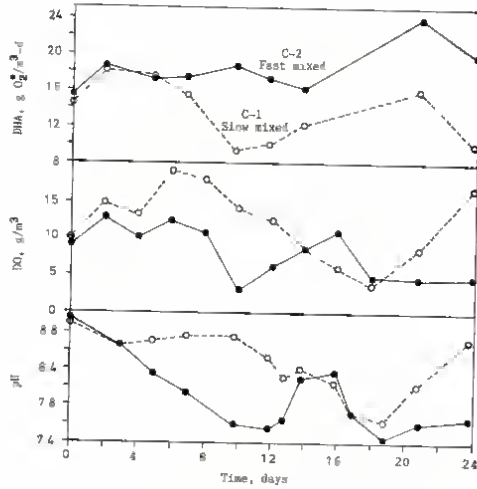

Figure 5-10 Temporal variation of DHA, DO and pH in slow mixed (C-1) and fast mixed (C-2) cultures initially dominated Chlorella and Monodus. Exp. 12.

tan-green to orange-brown matrix, Synechocystis cells being the major component. A substantial bacterial component was also present in this matrix. Chlorella and Monodus composed approximately 10% of the total biovolume of flocs.

The production of settleable solids progressively increased in the fast mixed culture (C-1), whereas this parameter remained near zero in the slow mixed culture (C-2) (Fig. 5-11). Algae removal by 24-h sedimentation was significantly greater in C-1 (max 44% vs. 25%). Algae concentration and DHA were similar in both cultures (Fig. 5-11, 5-12). DO and pH were generally greater in C-2.

5.3.2 Effect of Waste Loading

The effect of waste loading was investigated in 4 experiments. Loaded cultures were compared to cultures not loaded in exp. 5 and 11. Different waste loading rates were evaluated in exp. 6. The influence of waste type was studied in exp. 10. Algal genera dominant initially were Synechocystis in exp. 5 and 6 and Chlorella and Monodus in exp. 10 and 11.

5.3.2.1 Loading versus Noloading

In exp. 5, Synechocystis counts increased for the first 10 days, then declined gradually in both cultures. The extent of decline was somewhat greater in the waste loaded culture (C-2) than in the control (C-1) (terminal counts of 52 vs. 73 cells/grid) (Table 5-16, top). Synechocystis was still the dominant genus in both cultures at the end of the experiment.

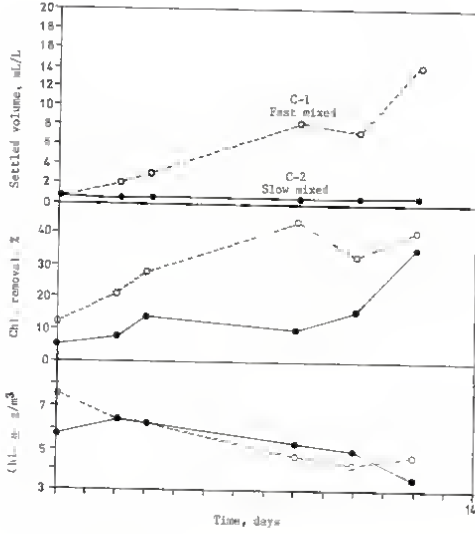

Figure 5-11 Temporal variation of settleability and chl a in fast mixed (30 cm/s, C-1) and slow mixed (14 cm/s, C-2) cultures initially dominated by Synechocystis. Exp. 13.

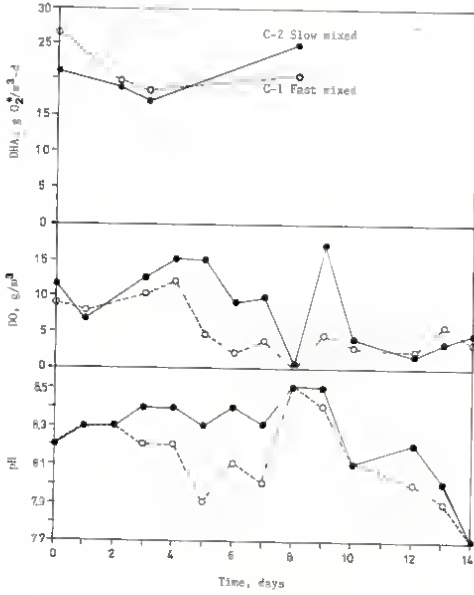

Figure 5-12 Temporal variation of DHA, DO and pH in fast mixed (C-1) and slow mixed (C-2) cultures initially dominated by Synechocystis.Exp. 13

Table 5-16. Effect of waste loading on photosynthatic community composition in bioflocculation channels, exps. 5, 11

Exp.*	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monad.	Synec.	Thiop	Micra.**
5	~1	C-1	11.3	1.8	69.8	1.9	0.0
		C-2	10.6	0.0	69.6	1.9	0.0
	3	C-1	9.8	3.8	109.4	0.0	0.0
		C-2	10.6	3.7	109.8	0.0	0.0
	10	C-1	8.5	3.2	118.2	0.0	0.0
		C-2	8.2	2.4	118.8	0.0	0.0
	18	C-1	7.6	4.1	50.6	0.0	0.0
		C-2	8.8	3.6	111.6	0.0	0.0
	25	C-1	3.8	1.3	61.2	0.0	0.0
		C-2	9.3	1.5	77.4	0.0	0.8
	30	C-1	1.6	0.7	73.0	0.0	0.0
		C-2	19.2	4.4	52.0	0.0	1.6
11	0	C-1	122.4	77.8	0.0	3.0	0.0
		C-2	129.0	75.8	0.0	6.2	0.0
	9	C-1	149.6	68.2	0.0	4.9	0.0
		C-2	146.8	71.4	0.0	4.7	0.0
	15	C-1	126.0	83.2	0.0	2.9	0.0
		C-2	111.8	85.6	0.0	1.5	0.0
	23	C-1	210.0	91.2	tr	2.8	0.0
		C-2	209.0	84.8	0.0	0.0	0.3
	30	C-1	172.8	70.0	0.0	0.0	0.0
		C-2	143.0	64.0	0.0	0.0	0.0

*Exp. 5: C-2 Waste loaded
C-1 Control (not loaded)

Exp. 11: C-1 Waste loaded
C-2 Control (not loaded)

**Micractinium

The waste-loaded C-2 medium became visibly flocculent after 5 days, whereas flocs were not observed in C-1 medium. Microscopic examination showed that flocs in C-2 medium ranged from hundreds to thousands of microns in diameter. They consisted almost exclusively of bright green Synechocystis cells (95%). Smaller flocs less than 100 microns in diameter were observed in C-1 medium. Additional observations made using a stereoscopic dissecting microscope showed the C-2 flocs to be irregularly or stellate shaped, with distinct lobes. C-1 flocs were roughly spherical in shape. Filamentous bacteria with a density of approximately 4 filaments/grid contributed to C-2 flocs. Filaments at a density of 2 filaments/grid contributed to C-1 flocs. Figure 5-13 indicates production of settleable solids was greater in the waste-loaded (C-2) culture. A maximum settleable solids volume of 35 mL/L was measured in C-2 compared to 4.5 mL/L in C-1. Algae removal by 24-h sedimentation was initially similar in both cultures, but became significantly greater in the waste-loaded culture after 20 days. Maximum removals in C-2 and C-1 media were 43% and 20%, respectively. Growth patterns of the two cultures were similar. DO and DHA were greater in the loaded culture (avg. 31 vs. 19 g O_2*/m^3d), whereas pH was less (avg. 8.3 vs. 8.4) (Fig. 5-14).

In exp. 11, changes in characteristics between the waste-loaded (C-1) and control (C-2) cultures were moderate. The algal communities in both cultures consisted

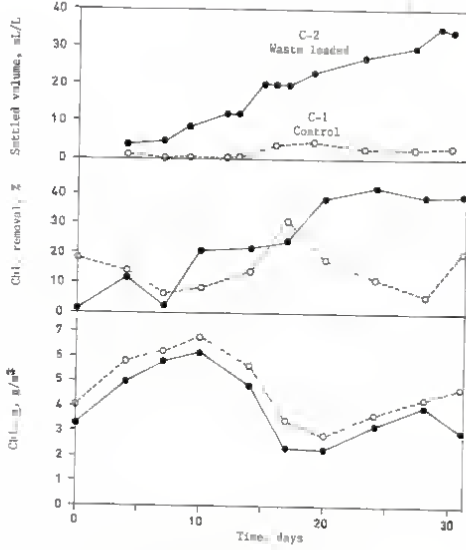

Figure 5-13 Temporal variation of settleability and chl a in waste-loaded (C-2) and control(C-1) cultures initially dominated by Synechocystis. Exp. 5.

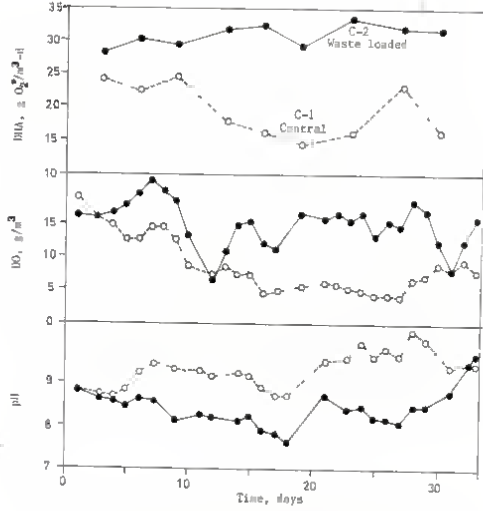

Figura 5-14 Temporal variation of DHA, DO and pH in waste-loaded (C-2) and control (C-1) cultures initially dominated by Synechocystis. Exp. 5.

almost entirely of Chlorella and to a lesser extent Monodus throughout the experiment (Table 5-16, bottom). Macroscopic flocs appeared after approximately one week in both cultures. Floc size was greater than 400 microns in both cultures by day 22. They were composed of Chlorella and Monodus cells without a substantial bacterial matrix. Production rate of settleable solids was similar for the first three weeks, then became somewhat greater in non-loaded C-2 than in C-1 (max. 54 vs 40 mL/L) (Fig. 5-15). Algal removals by 24-h sedimentation in both cultures were nearly equal (max. 33% vs. 32%) throughout the experiment. DO and pH were generally greater than in the non-loaded (C-2) culture. Algae grew better in the waste-loaded culture. DHA was similar in both cultures (Fig. 5-16).

5.3.2.2 Waste Loading Rate

Two different waste loading rates of FBR effluent, 72 L/d in C-1 and 217 L/d in C-2, were employed during exp. 6 with Synechocystis dominant initially. Counts of this alga declined progressively in both cultures throughout the experiment, ending near zero. There was a partial replacement of this alga by non-flocculent Chlorella in both cultures (Table 5-16). Flocs became visible after 6 days in both cultures. They were similar in size but more numerous in C-2 than in C-1 (avg. 3.6 vs. 2.5 flocs/field, 100x). The flocs were composed predominantly of Synechocystis (90%), with Chlorella and bacteria making up the balance. Floc

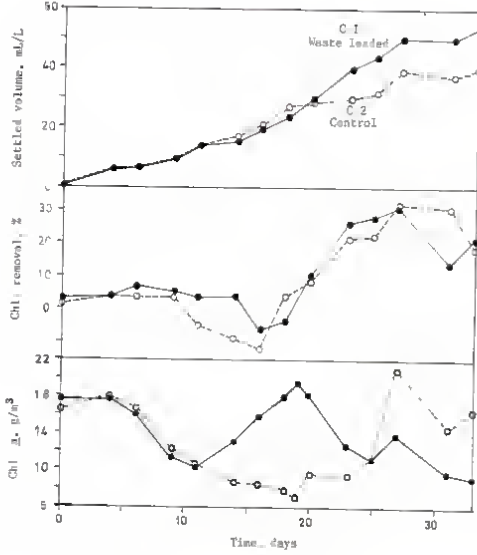

Figure 5-15 Temporal variation of settleability and chl a in waste-loaded (C-1) and control (C-2) cultures initially dominated by Chlorella and Monodus. Exp. 11.

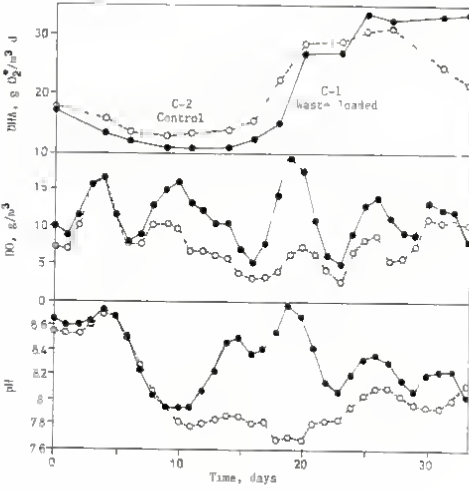

Figure 5-16 Temporal variation of DHA, DO and pH in waste-loaded (C-1) and control (C-2) cultures initially dominated by Chlorella and Monodus, Exp. 11.

were distinct lobes in shape. At the end of the experiment, Micractinium cells were attached to flocs as a dense outer layer.

Production of settleable solids was almost identical in the two cultures. Algae removals were similar throughout the first 25 days, then became greater in C-1 medium (Fig. 5-17). Maximum algae removals were 90% in C-1 and 74% in C-2. Algae concentration and DHA were greater in the more heavily loaded culture. DO and pH were similar in both cultures (Fig. 5-18).

5.3.2.3 Waste Pretreatment

Two different waste types, settled waste supernatant in C-1 and FSR effluent in C-2, were employed at the same loading rate of 105 L/d during exp. 10. Chlorella and Monodus co-dominant initially. Settled waste supernatant had a stimulatory effect on Chlorella, as indicated by an increase in the relative proportion of this alga during the experiment. This waste also sustained a relatively high popuiation of Thiopedia rosea in C-1. The abundance of Chlorella and Thiopedia rosea decreased in C-2 during the experiment (Table 5-17).

Flocs became visible after 10 days in both cultures. The floc size and density in C-2 medium was greater than in C-1 medium. Flocs in C-2 medium were 50 to 200 microns in diameter with a density of 10 flocs/field at 400x. In C-1 medium flocs were 50 to 80 microns in diameter and were present at a density of 0.9 floc/field. C-2 flocs were

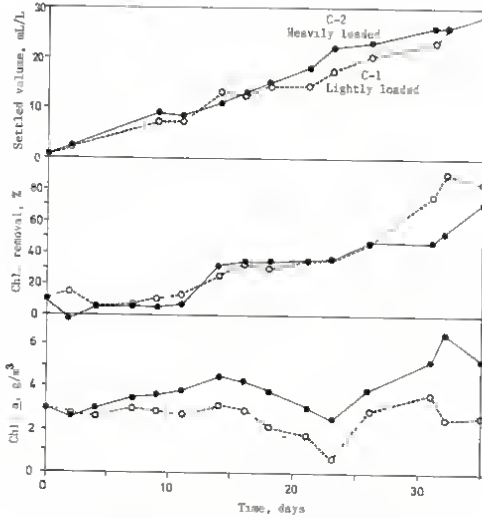

Figure 5-17 Temporal variation of settleability and chl a in lightly loaded (72 L/d, C-1) and heavily loaded (217 L/d, C-2) cultures initially dominated by Synechocystis. exp. 6.

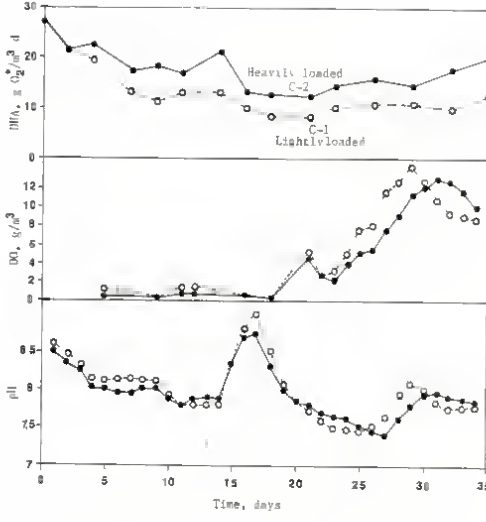

Figure 5-18 Temporal variation of DHA, DO and pH in lightly loaded (C-1) and heavily loaded (C-2) cultures initially dominated by Synechocystis. Exp. 6.

Table 5-17. Effect of waste loading on photosynthetic community composition in bioflocculation channels, exp. 6, 10

Exp.*	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monad.	Synec.	Thiop.	Micra.
6	4	C-1	3.5	0.1	70.2	7.5	0.0
		C-2	3.4	0.0	70.0	8.1	0.0
	7	C-1	4.1	0.0	57.2	0.0	0.0
		C-2	3.8	0.0	59.4	0.0	0.0
	11	C-1	3.8	0.0	68.6	0.1	0.0
		C-2	4.0	0.0	71.4	0.4	0.0
	16	C-1	8.3	0.7	46.0	0.0	0.0
		C-2	10.8	0.1	47.8	0.0	0.0
	18	C-1	7.7	0.5	52.8	tr	0.0
		C-2	10.7	0.2	60.0	tr	0.0
	21	C-1	3.4	0.5	48.8	0.0	0.0
		C-2	5.5	0.0	43.0	0.0	0.0
	26	C-1	11.4	tr	14.6	0.0	2.9
		C-2	21.6	tr	12.4	0.0	0.6
	33	C-1	5.5	0.9	0.6	tr	0.8
		C-2	12.4	0.2	0.5.	0.0	3.5
10	0	C-1	44.0	43.0	0.0	11.0	0.0
		C-2	42.0	43.0	0.0	13.5	0.0
	6	C-1	63.0	44.0	0.0	19.5	0.0
		C-2	65.0	47.0	0.0	17.5	0.0
	12	C-1	86.0	39.0	0.0	17.0	0.0
		C-2	84.0	41.0	0.0	7.5	0.0
	19	C-1	94.0	44.0	0.0	11.5	0.0
		C-2	80.0	44.0	0.0	4.0	0.0
	26	C-1	85.0	66.0	0.0	11.0	0.0
		C-2	27.0	26.0	0.0	0.3	0.0
	34	C-1	109.0	92.0	0.0	5.0	0.0
		C-2	17.0	50.0	0.0	0.5	0.0
	40	C-1	125.0	84.0	0.0	4.5	0.0
		C-2	32.0	33.0	0.0	0.0	0.0

*Exp. 6: C-1 Lightly loaded
C-2 Heavily loaded

Exp. 10: C-1 Settled waste supernatant
C-2 PBR effluent

composed entirely of Chlorella and Monodus (55%). C-1 flocs consisted of Chlorella and Monodus (40%) and Thiopedia rosea (60%).

Maximum settleable solids volume and algae removal in C-2 medium were 30 mL/L and 42%, respectively. Maxima for these parameters in C-1 medium were 15 mL/L and 14%, respectively (Fig. 5-19). DHA was consistently greater in C-1 than in C-2 (Fig. 5-20). Algae concentration, DO and pH were initially somewhat greater than those of C-1, but were similar initially. Values of these parameters eventually became greater in C-1 medium.

5.3.3 Effect of Algal Biofloc and Bacterial Seeding

5.3.3.1 Algal Biofloc Seeding

In exp. 7, the culture (C-1) seeded with Synechocystis biofloc developed in the previous experiment was compared with a control culture (C-2). Chlorella and Monodus were dominant initially. Counts of these algae declined progressively in both cultures throughout the experiment, ending near zero (Table 5-18). Settleable solids in C-1 increased for the first 9 days, leveled out, then eventually diminished (Fig. 5-21). The quantity of settleable matter remained greater in C-1. Algae removals in C-1 medium were greater than in C-2 initially, but were eventually exceeded by those in C-2 medium. Floc size and density were similar in both cultures. Maximum algae removals of 95% in C-2 and 81% in C-1 were obtained. Algae concentration, DO and pH were similar in the two cultures (Fig. 5-22).

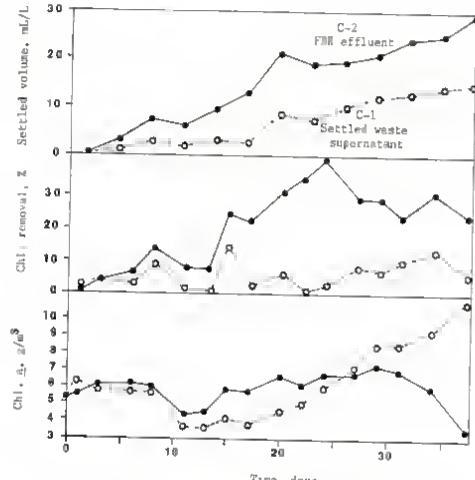

Figure 5-19 Temporal variation of settleability and chl a in cultures loaded with settled waste supernatant (C-1) and FBR effluent (C-2) Chlorella and Monodus were dominant initially. Exp. 10.

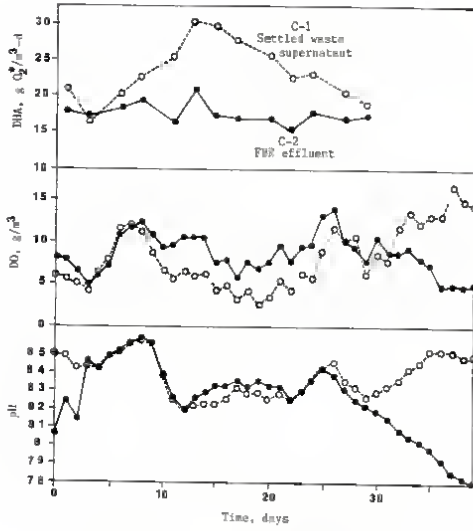

Figure 5-20 Temporal variation of DHA, DO and pH in cultures loaded with settled waste supernant (C-1) and FSR effluent (C-2) Chlorella and Monodus were dominant initially. Exp. 10.

Table 5-18. Effect of algal biofloc and bacterial seeding on photosynthetic community composition in bioflocculation channels, exp. 7, 9

Exp.*	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monod.	Synec.	Thlop.	Micra
7	0	C-1	16.1	0.2	31.0	0.7	0.0
		C-2	10.2	0.2	37.8	0.5	0.0
	6	C-1	31.0	0.1	19.2	0.0	0.3
		C-2	28.7	0.1	24.8	0.4	0.3
	11	C-1	13.5	0.0	7.5	0.0	0.4
		C-2	15.7	0.5	5.3	0.0	0.0
	19	C-1	9.7	0.0	0.2	0.0	0.0
		C-2	7.2	0.0	0.0	0.0	0.5
	26	C-1	0.0	0.0	0.0	0.0	1.5
		C-2	0.0	0.0	0.0	0.0	0.0
9	1	C-1	31.0	23.0	0.0	0.3	0.0
		C-2	75.0	18.0	0.0	21.8	0.0
	8	C-1	19.0	14.0	0.0	0.0	tr
		C-2	76.0	22.0	0.0	0.0	tr
	11	C-2	77.0	26.0	0.0	89.0	0.0
	15	C-2	69.0	8.0	0.0	64.3	0.0
	23	C-2	76.0	29.0	0.0	53.5	0.0
	28	C-2	70.0	40.0	0.0	39.0	0.0

*Exp. 7: C-1 Algal biofloc seeded
C-2 Control (not seeded)

Exp. 9: C-1 Control
C-2 Seeded with photosynthetic bacteria

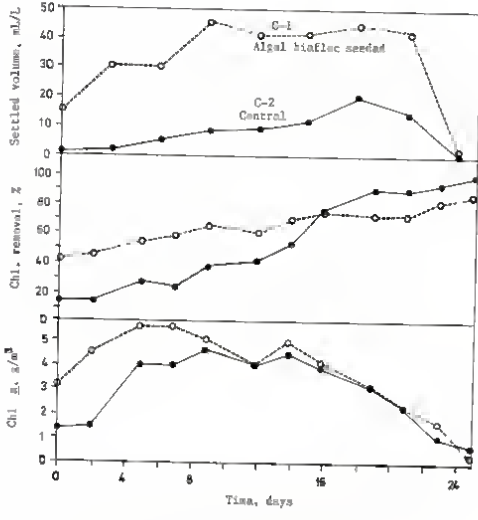

Figure 5-21 Temporal variation of settleability and chl a in algal biofloc-seeded (C-1) and control (C-2) cultures initially dominated by Chlorella and Monodus. Exp. 7.

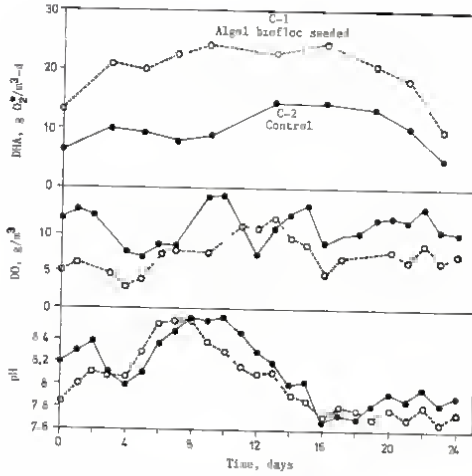

Figure 5-22 Temporal variation of DHA, DO and pH in algal biofloc-seeded (C-1) and control (C-2) cultures initially dominated by Chlorella and Monodus. Exp. 7.

DHA was consistently greater in C-1 medium.

5.3.3.2 Photosynthetic Bacteria Seeding

In exp. 9, culture (C-2) seeded with anaerobic lagoon effluent containing purple sulfur bacteria, Thiopedia rosea was compared to a culture which was not seeded (C-1). Chlorella and Monodus which were co-dominant initially, remained at significant levels throughout the experiment (Table 5-18, bottom). After seeding, Thiopedia rosea declined progressively but still remained abundant. Microscopic examination of C-2 revealed the presence of flocs consisting largely of Thiopedia rosea cells. Approximately 20% of the flocs consisted of Chlorella and Monodus cells. The culture seeded with photosynthetic bacteria exhibited a progressive increase in settleable matter but algae removals decreased (Fig. 5-23). DHA, DO and pH dropped off towards the end of the experiment (Fig. 5-24).

5.3.3.3 Activated Sludge Seeding

In exp. 14, Chlorella and Monodus were co-dominant initially. Counts of these algae increased progressively in both cultures throughout the experiment (Table 5-19). Microscopic examination of C-2 medium showed flocs consisting of a bacterial matrix surrounded by algal cells in C-2 within 2 days. Small flocs became visible in C-1 after 6 days. These were small, loose aggregates consisting of Chlorella and Monodus. Complete bioflocculation had occurred in both cultures by day 23. This phenomenon was

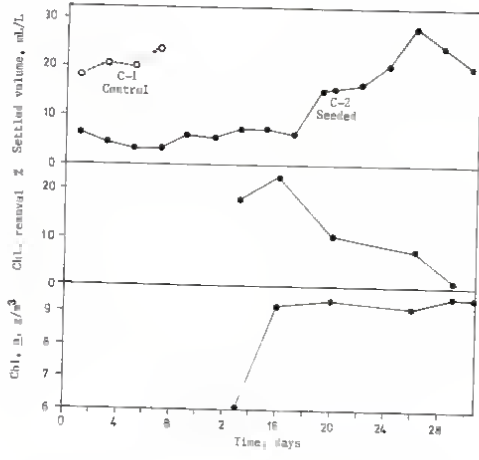

Figure 5-23 Temporal variation of settleability and chl a in a culture (C-2) seeded with photosynthetic bacteria. Chlorella and Monodus were dominant initially. Exp. 9.

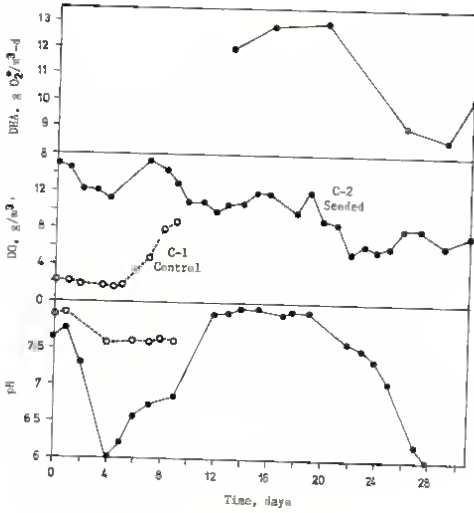

Figure 5-24 Temporal variation of DHA, DO and pH in a culture (C-2) seeded with photosynthetic bacteria. Chlorella and Monodus were dominant initially. Exp. 9.

Table 5-19 Effect of activated sludge addition on photosynthetic community in bioflocculation channels, exp 14*

Day	Channel	Normalized cell count (#/grid)				
		Chlor.	Monod.	Synec	Thiop.	Other
-2	C-1	77.0	53.6	0.0	0.0	0.0
	C-2	77.8	54.0	0.0	0.0	0.8
3	C-1	155.0	85.8	0.0	0.0	0.0
	C-2	162.2	85.6	0.0	0.0	0.0
9	C-1	155.2	87.0	0.1	0.0	0.0
	C-2	158.6	97.8	0.1	0.0	0.1
17	C-1	171.2	90.8	0.0	0.0	0.0
	C-2	172.0	93.4	0.0	0.0	0.0
23	C-1	188.8	75.2	0.0	0.0	0.0
	C-2	172.4	96.4	0.0	0.0	0.0

*C-1 Control
C-2 Seeded with activated sludge

preceded by an increase in the settled volume of C-1 and C-2 to 10 mL and 22 mL, respectively (on day 20) and 29 mL and 50 mL, respectively, on day 23 (Fig. 5-25). Maximum algae removals were relatively low; 46% in C-2 and 28% in C-1. Algae concentrations were similar in the two ponds. DHA consistently was higher in C-2. The pH and DO were similar in both cultures (Fig. 5-26).

5.3.4 Effect of carbonate-supplementation

In exp. 3, Chlorella and Monodus were co-dominant initially and remained so throughout the experiment (Table 5-20). The carbonate supplemented culture, C-2, began browning after 12 days and the cultures were olive brown from day 14 on. The first distinct sign of flocculation was seen in both cultures on day 20. Flocs consisted of an olive green matrix of Chlorella, Monodus, and Thiopedia rosea. Extensive flocculation was evident in both cultures by day 20, but settling of the cultures did not leave clear supernatants. Settled solids volume in both cultures decreased gradually for the first 12 days, then increased up to day 20 and then eventually diminished. The quantity of settleable matter remained greater in C-2. The maximum settled volume (on day 20) of C-1 and C-2 were 19 mL/L and 14 mL/L, respectively (Fig. 5-27).

Carbonate addition did not affect the photosynthetic community in terms of succession or bioflocculation. The DHA was greater in the carbonate supplemented culture (Fig. 5-28). The pH, DO and algae concentration were virtually

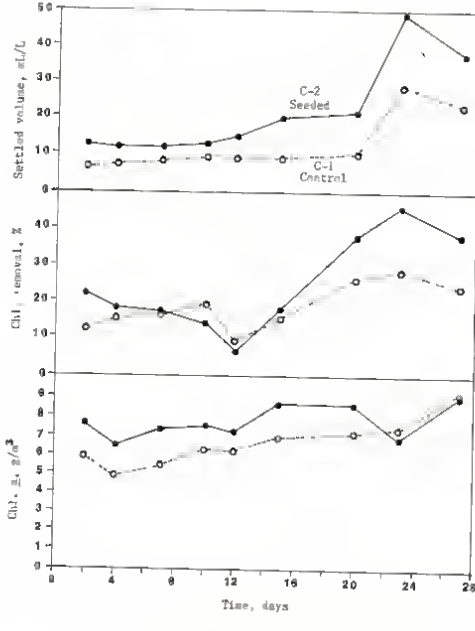

Figure 5-25 Temporal variation of settleability and Chl a in activated sludge seeded (C-2) and control (C-1) cultures initially dominated by Chlorella and Monodus. Exp. 14.

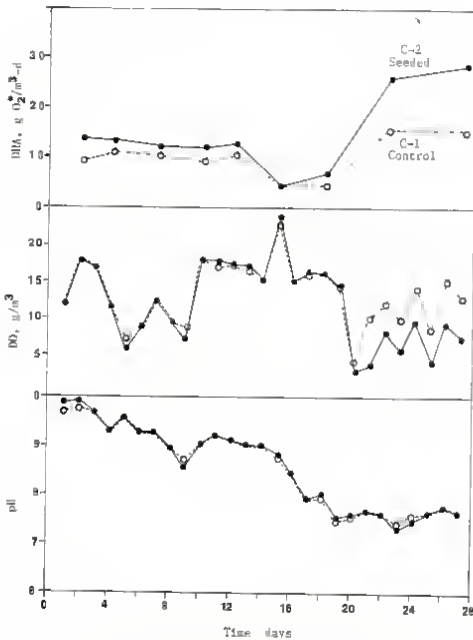

Figura 5-26 Temporal variation of DHA, DO and pH in activated sludge seeded (C-2) and control (C-1) culturas initially dominated by Chlorella and Monodus. Exp. 14.

Table 5-20. Effect of carbonate supplementation and pH reduction on photosynthetic community composition in bioflocculation channels, exp. 3, 8

Exp.*	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monad.	Synec.	Thiop.	Micra.
3	0	C-1	26.9	19.6	0.0	15.5	0.0
		C-2	27.0	19.4	0.0	15.4	0.0
	2	C-1	25.1	17.8	0.5	13.7	0.0
		C-2	20.9	17.4	0.5	14.4	0.0
	3	C-1	29.9	17.6	0.0	11.8	0.0
		C-2	21.6	16.7	0.0	12.1	0.0
	9	C-1	29.6	29.4	0.0	8.7	0.0
		C-2	28.7	18.4	0.0	3.4	0.0
	14	C-1	25.2	39.9	0.0	2.6	0.0
		C-2	26.0	23.0	0.0	2.2	0.0
	20	C-1	11.5	57.3	0.0	1.8	0.0
		C-2	28.1	30.6	0.0	2.2	0.0
	24	C-1	5.8	76.5	0.0	0.0	0.0
		C-2	15.9	45.7	0.0	0.0	0.0
	30	C-1	0.4	71.4	tr	0.8	0.0
		C-2	21.0	43.9	tr	0.7	0.0
	36	C-1	0.2	0.4	tr	0.0	1.9
		C-2	2.5	7.4	0.0	0.0	0.0
	40	C-1	1.6	0.5	tr	0.0	0.8
		C-2	1.4	5.0	0.1	0.0	0.2
	45	C-1	7.8	6.8	0.0	0.0	0.2
		C-2	8.6	6.5	0.0	0.0	0.5
	49	C-1	5.5	21.4	0.0	0.0	1.1
		C-2	5.6	13.3	0.0	0.0	2.3
	56	C-1	1.7	19.9	0.0	0.0	1.3
		C-2	1.6	2.6	0.0	0.0	1.0
8	2	C-1	59.0	24.0	0.0	2.5	0.8
		C-2	62.0	22.0	0.0	2.2	1.1
	5	C-1	73.0	12.0	tr	2.1	0.9
		C-2	61.0	27.0	tr	1.8	2.1
	15	C-1	40.0	29.0	0.0	0.6	0.3
		C-2	50.0	44.0	tr	0.0	4.0
	23	C-1	32.0	25.0	0.0	0.0	0.0
		C-2	80.0	17.0	0.0	0.4	0.7
	28	C-2	83.0	13.0	0.0	0.7	0.0

*Exp. 3: C-1 Control (not supplemented)
C-2 Carbonate supplemented

Exp. 8: C-1 Control (not adjusted)
C-2 Acidified

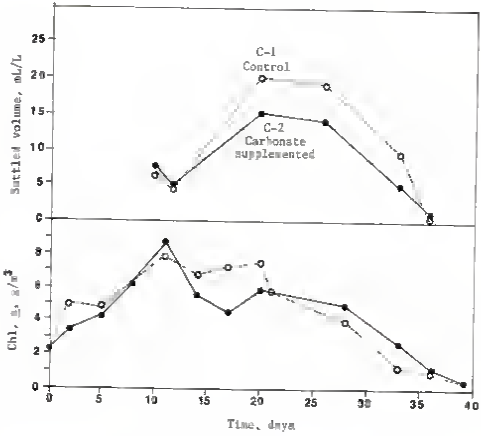

Figure 5-27 Temporal variation of settleability and Chl a in carbonate supplemented (C-2) and control (C-1) cultures initially dominated by Chlorella and Monodus. Exp. 3.

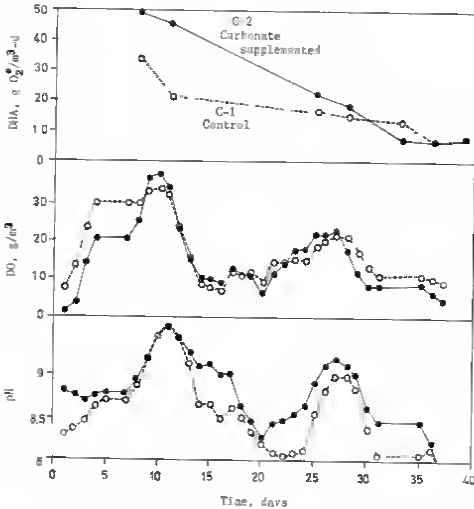

Figure 5-28 Temporal variation of DNA, DO and pH in carbonate supplemented (C-2) and control (C-1) cultures initially dominated by Chlorella and Monodus. Exp. 3.

identical in the cultures throughout the experiment.

5.3.5 Effect of pH Reduction

In exp. 8, the effect of intermittently acidifying a culture dominated initially by Chlorella and Monodus was evaluated. Concentrated sulfuric acid was used to reduce pH of the experimental culture (C-2) to the target value of 6.0. Sometimes pH values significantly less than this were experienced (Table 5-9). Despite extremes in pH, algal density in C-2 was greater than the control (C-1) (Table 5-20, bottom). Settleable solids and algae removals were similar in the cultures until day 13, just after C-1 was treated heavily with ammonium hydroxide (Fig. 5-29). Beyond this point, these parameters became greater in C-1 than in C-2. Floc size and density were greater in C-2. They were composed of Chlorella and Monodus (approx. 40-60%) and Thiopedia rosea and other bacterial components (approx. 40%). Maximum algae removals in C-1 and C-2 were 76% and 28% respectively. Algae concentration and DO were consistently greater in C-2 throughout the experiment (Fig. 5-30). No significant difference in bacterial activity was observed. A decrease in algal cell numbers coincided with an increase in active bacterial populations between the second and fourth weeks. A rapid drop of DO in C-1 followed closely the heavy ammonium hydroxide dosage on day 12 which resulted in extensive algal cell death.

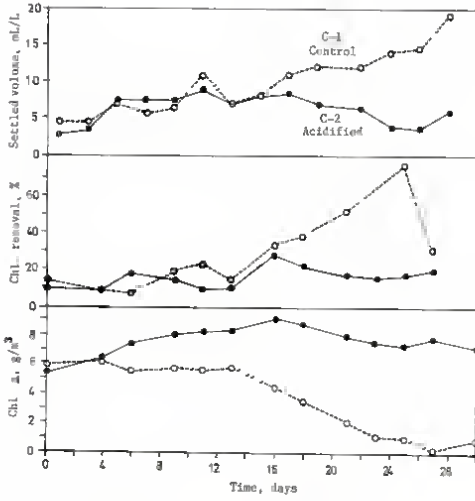

Figure 5-29 Temporal variation of settleability and Chl a in acidified (C-2) and control (C-1) cultures initially dominated by Chlorella and Monodus. Exp. 8.

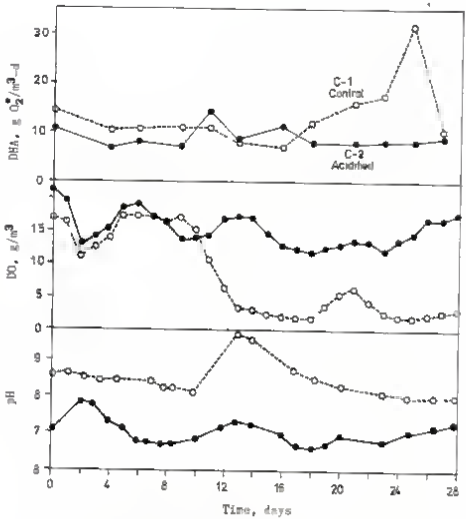

Figure 5-30 Temporal variation of DHA, DO and in acidified (C-2) and control (C-1) cultures initially dominated by Chlorella and Monodus. Exp. 8.

CHAPTER 6
DISCUSSION

6.1 Effect of Mixing

6.1.1 Algal Species Control to Favorable Bioflocculation

The dominant algal genera encountered initially in field experiments included Synechocystis, Chlorella, and Monodus. Without mechanical mixing, algal species changes experienced in the high-rate pond in the present research were dependent on season. During winter and early spring, Chlorella and Monodus coexisted in roughly equal numbers. Monodus gradually become dominant in late spring and declined in early summer. The minute unicellular cyanobacterium, Synechocystis, remained dominant through the summer and fall seasons, sometimes persisting well into winter. Intermittent mixing did not appreciably change this pattern of algal species succession (Lincoln and Koopman, 1982, 1983).

Complete bioflocculation marked by cell aggregation was achieved in the field experiment 1. Microscopic examination revealed that Synechocystis flocs composed entirely of algal cells with small bacterial component. Low bacterial dehydrogenase activity also supported this observation. In this case, the stress of continuous flow mixing is believed to have induced the release of exocellular polymers by the algae themselves in contrast to the bioflocculation

associated with Micractinium which is dependent largely on its genetic tendency to form canobia, along with a substantial bacterial contribution (Eisenberg et al., 1981, Lincoln and Koopman, 1982). Absence of bacterial interaction in Synechocystis bioflocculation does not necessarily contradict the hypothesis that bacterial exopolymers play an important role in algal bioflocculation. Instead, it may simply indicate that certain cyanobacteria have exocellular polymer production characteristics similar to those of bacteria. This type of bioflocculation observed in the unicellular, blue green algae would be a preferable alternative because of the higher removal efficiency and the abundance of this alga in summer and fall.

Inhibition of Synechocystis by continuous flow mixing was observed in the field exp. 1, 2, 4 and 13 (Fig. 5-4, 5-5, 5-7, 5-11) and laboratory exp. 7, 8 and 9 (Fig. 4-3, 4-4, 4-8). This response contrasts with that of bioflocculating Micractinium cultures, which retained high photosynthetic capacities under continously mixed conditions. Synechocystis counts also declined rapidly in field exp. 4 with an initially co-dominant culture of Synechocystis and Chlorella while Chlorella and Monodus cell counts kept increasing in the mixed culture. The stimulatory effect of mixing on the growth of Chlorella and Monodus was also confirmed in the laboratory experiments (Fig 4-5, 4-6).

6.1.2 Polymer Bridging Interaction

Another very important role of mixing might be to keep

algae and bacteria in suspension so that they could have more chance to interact with the polymer, thus forming three dimensional, bridged structures. This hypothesis was verified in the series of field experiments 1, 2, and 4, which showed that the production of settleable solids in the mixed cultures was significantly higher than that in the unmixed cultures (Fig. 5-5, 5-7). Generally, bioflocs became visible after an initial 4-6 days in the mixed cultures, but no sign of biofloccculation was evident in unmixed cultures. Therefore it is concluded that mixing is required for induction of the bioflocculation process.

Settleable matter production and algal removal were found to be proportional to mixing intensities in field experiments 12 and 13 (Fig. 5-9, 5-11). In other words, the rate of floc formation was directly proportional to the mean velocity gradient (G). The time of floc formation should decrease with increasing value of G (Table 6-1). This phenomena was confirmed by the results of laboratory experiments 7 and 8. As G value increased, floc density increased with the occurance of much smaller flocs. It is obvious that as velocity gradient increases, shear stress increases, then floc particles become weaker and are subject to being torn apart, reducing the extended length and number of adsorbed polymers. Eventually, the flocs showed poorer settleability (Fig. 4-5). Therefore, there is a maximum size of floc particle associated with each mean velocity gradient

Table 6-1. Mean velocity gradient (G) applied in mixing experiments

Experiment No.	Channel	Operating depth (cm)	flow velocity (cm/sec)	G (sec^{-1})	Days required for initial floc formed
1	C-1	31	0	0.0	15
	C-2	33	16	6.5	4
2	C-1	20	0	0.0	13
	C-2	20	16	8.5	9
4	C-1	31	19	3.6	7
	C-2	31	0	0.0	13
12	C-1	46	14	4.3	7
	C-2	46	30	14.4	7
13	C-1	35	30	16.4	7
	C-2	35	14	4.9	7
7 & 8**			0	0.0	10
			20*	13	4
			40*	35	4
			80*	90	4

*rev/min
**Laboratory experiment with Jar test apparatus

and velocity gradient should be selected with this in mind. Values of G between 10 sec^{-1} and 75 sec^{-1} have been found to promote floc growth without destruction of the floc particles (Fair et al., 1968).

Bacterial activities were much higher in the mixed cultures particularly in fast-mixed cultures than in unmixed and slow-mixed cultures (Fig. 5-6, 5-8, 5-10). This might be due to the fact that the fast-mixed cultures could stress bacterial metabolism, thus resulting higher bacterial activity. During most of the field experiments, floc particles normally became visible within 7 days and semi-flocculent cultures developed as pin floc less than 500 microns in diameter. Usually these pin floc did not grow bigger but remained in a flocculent state and persisted until the culture entered the declining or endogenous growth phase. This phenomenon might be explained as follows. Maximum activity of autolytic enzymes occurs during the early phase of logarithmic growth, releasing low molecular weight polymers into the medium. These polymers show little ability to promote aggregation of microorganisms (Gules et al., 1979). Consequently, partial stabilization or restabilization between algal cells, bacterial cells, and biopolymers occurs at lower or higher surface coverage ratios. Therefore, environmental or biological stresses which lead to release of high molecular weight polymers and induce rapid and complete bioflocculation should be employed.

It was generally observed that at high specific growth rates, nonflocculant growth is usually present, less bioflocculating tendency, whereas low specific growth rates tend to create conditions which promote bioflocculation (Fig. 4-5, 4-7, 5-11, 5-17, 5-19, 5-21, 5-25, 5-29).

The fact that most of bioflocculation in the field experiments occurred during the declining or the endogenous phase of algal growth (Fig. 5-11, 5-13, 5-15, 5-19, 5-21, 5-29) supports earlier findings that the extracellular biopolymers synthesized during the logarithmic growth phase are slowly modified to more insoluble forms so that they remain in the vicinity of the cells and can entangle cells and suspended solids.

8.2 Effect of Waste Loading

One of the principal bases for algal flocculation is the symbiotic relationship existing between algae and bacteria. The algae provide oxygen for aerobic bacteria while the bacteria supply carbon dioxide to the algae. In addition, the photosynthetic production of carbohydrate provides new carbon skeletons for both algae and bacteria. Since swine waste has a very low carbon to nitrogen ratio, 4:1 or less, the development of new carbon sources is essential if the nitrogen is to be converted into biomass and conserved as organic nitrogen (Nye and Rivera-Negron, 1980).

Algal growth rates of both waste loaded and unloaded cultures were found to be similar in field experiment 5

(Fig. 5-13). However in field experiment 11, the waste-loaded culture exhibited a large increase in algal growth potential (Fig 5-15). The more heavily loaded culture showed a moderate increase in algal growth activity but the difference was not noticeable in field experiment 6 (Fig. 5-17). This result might be due to difference in initial nutrient conditions in each experiment. With enriched nutrient levels initially (232 kg COD load/ha-d) (Table 6-2) as in field experiment 5, loading of the culture had no appreciable effect on growth while a big difference in algal growth potential was shown in culture medium which was lightly loaded with nutrients initially (134 kg COD load/ha-d) as in field experiment 11. There were no big differences in algal growth rates between cultures loaded with settled waste supernatant and FBR effluent both in laboratory and field experiments (Fig. 4-9, 5-19). Average nutrient characteristics of wastes loaded to bioflocculation channels during these experiments were similar, representing COD values of 24,940 g/m^3 and 20,720 g/m^3 and TKN values of 2,680 g/m^3 and 2,540 g/m^3 for anaerobic digester effluent and fixed bed reactor effluent, repectively.

DHA was substantially greater in the waste-loaded than the non-loaded culture, as expected, in field experiment 5 (Fig. 5-14), while DHA was not stimulated by the waste addition in field experiment 11 (Fig. 5-16). The heavily loaded cultures exibited only a small increase in DHA in both field and laboratory experiments (Fig. 4-7, 5-16).

Table 6-2. Avarage COD loading to high-rate pond prior to transfers of medium to bioflocculation channels

Experiment No.	Experiment Variables	Avarage COD loading (kg COD/ha-d)
1	± mixing	75
2	± mixing	34
3	± $CO_3^{=}$	*
4	± mixing	158
5	± waste loading	232
6	waste loading rate	130
7	± biofloc (algae) seeding	50
8	pH	106
9	± purple sulfur bacteria seeding	**
10	waste pretreatment	205
11	± waste loading	134
12	flow mixing velocity	26
13	flow mixing velocity	71
14	± activated sludge addition	*

*Facultative pond affluent was used
**Culture medium from the previous experiment were used

From the above mixed results of algal and bacterial activities, it is very hard to attribute floc formation entirely to the symbiotic association between algae and bacteria, which is considered one of the principal bases for algal bioflocculation.

If bacteria can play a role in algal bioflocculation in wastewater cultures, then it should be beneficial to provide suitable organic substrates for their growth. This hypothesis was examined in experiments on waste loaded and non-loaded cultures and on different rates of waste loading. Results were mixed. In field experiment 5, conducted with Synechocystis initially dominant, FBR effluent-loading had a pronounced, positive effect on both settleable solids production and algal removal (Fig. 5-13). However, with Chlorella and Monodus co-dominant, FBR effluent-loading showed a slightly negative effect on settleable solids production, while not effecting algal removal (Fig. 5-15). Also, there was little difference between high and low loading with FBR effluent in terms of settleable matter production or algal removal (Fig. 5-17). On the other hand, in laboratory experiments 6 and 12, algal removal was found to be proportional to waste loading rates with Chlorella and Monodus (Fig. 4-7) and with Synechocystis (Fig. 4-8) dominant initially.

The mixed results of the FBR effluent loading experiments could perhaps have been due to several reasons. First, mature culture medium of bioflocculation channels,

C-1 and C-2, had received enriched nutrients before transfer from the growth pond (C-4) which received 0.54 m^3 of PBR effluent on an average daily basis (Table 4-10).
No correlation was shown between bioflocculation potential and average COD loading to the high-rate pond prior to transfer to bioflocculation channels (Table 5-2). There was a slight tendency to achieve a better bioflocculation potential with low COD loading to the high-rate pond. It was also found that the amount of loading in excess of 3% per day (as NH_3-N) was generally not critical to bioflocculation (Lincoln et al., 1984).

If floc forming bacteria can significantly contribute to algal bioflocculation, effort should be directed toward developing good populations of floc forming bacteria. It would be preferable that a large population of these bacteria be maintained in C-4 before transferring. However the presence of an anaerobic sludge layer of approximately 10-15 cm and mixing 3 times per week make it unlikely that extensive environments for floc forming bacteria are available in C-4. Moreover, a high DO content (25-35 mg/L) during the day time might be toxic to these aerobic bacteria, preventing symbiosis. Likewise in the experimental channels, with slow mixing (flow velocity: 14 cm/sec), certain portions of channel bottom would not be mixed well and would develop anaerobiosis. This might explain the result that the fast mixed cultures (flow velocity: 30 cm/sec) promoted substantially higher bacterial activity,

presumably producing more exocellular biopolymer by elevated bacteria metabolism, thus accelerating bioflocculation.

Second, floc forming aerobic bacteria need biodegradable organics as their substrates but much of the biodegradable matter in FBR effluent is removed through the anaerobic digestion process, thus limiting the nutrient value to these bacteria. Undigested waste should contain a higher fraction of biodegradable organic matter and promote greater production of floc forming bacteria. This hypothesis was tested in the field experiment 10. Contrary to the hypothsis, it was found that the culture loaded with FBR effluent has a much higher bioflocculation potential than that loaded with settled waste supernatant. As expected, bacterial activity was greater in the culture loaded with settled waste supernatant. However, most of this activity was apparently due to proliferation of purple sulfur bacteria. Two completely different types of floc were noticeable. In the culture loaded with FBR effluent, flocs were composed entirely of algal cells, primarily Chlorella and Monodus, while in the culture loaded with settled waste supernatant, flocs consisted almost entirely of purple sulfur bacteria, Thiopedia rosea. In contrast to the Micractinium predominance resulting from continuous mixing and settled raw waste loading (Lincoln and Koopman, 1983), there was no algal species change.

As with the bioflocculation experiments conducted at Richmond, California, by Eisenberg et al. (1981), the

relative importance of flow mixing velocities turned out to be greater than type of algal growth medium. It has been observed in conventional wastewater treatment systems that concentrated wastes tend to inhibit bioflocculation. It might be concluded that concentrated swine waste medium needs a faster mixing velocity to reach the same degree of bioflocculation through the action of the floc forming bacterial population than did the sewage effluent medium of the Richmond experiment. Dilution of mature culture medium of C-4 with potable water would not be economically feasible, but recycling of treated effluent from the harvesting operation has been shown to be practicable. The relatively high nutrient content of this effluent also prevents unwanted reduction in algal density.

6.3 Effect of Algal Biofloc and Bacterial Seeding

Seeding with bioflocculated algal floc should be one way to accelerate the bioflocculation process. It was assumed that a certain portion of the biopolymers of flocs developed in the previous experiment still could have vacant sites so that algae and bacters could attach onto them. Field experiment 7, conducted with Synechocystis and Chlorella co-dominant initially, showed that algal removal was initially higher in the biofloc seeded culture, but was overtaken by the non-seeded culture after 16 days (Fig. 5-21). Extended mixing might have reduced the length and number of adsorbed polymers in the seeded culture, permitting restabilization. On the other hand, it was

inferred that 25% of the non-seeded culture volume, consisting of supernatant recovered at the end of previous experiment, started to enter endogenous respiration and produced large amounts of biopolymer from leaky cells, thus giving large surface active sites. However, contrary to the hypothesis, total biopolymer concentrations between the two cultures were similar.

It was found that photosynthetic purple sulfur bacteria (Thiopedia rosea) introduced into either the high-rate pond or bioflocculation channels with anaerobic lagoon effluent bioflocculated readily under continuous mixing within relatively short periods of time. The question was asked whether these bacteria could interact with algal cells in an aerobic environment, triggering algal bioflocculation concurrently. Field experiment 9, conducted with Chlorella and Monodus co-dominant initially showed that photosynthetic bacteria bioflocculated as predicted from previous observations, but there was only moderate interaction with algal cells. Floc consisted of about 80% Thiopedia and 20% Chlorella and Monodus on a biovolume basis. Biopolymer concentration increased in parallel with settleable solids volume (Fig. 5-23). This result indicates that bacterial exopolymers can be quite specific to bacteria, limiting the degree of algal-bacterial interaction in floc formation.

Activated sludge has successfully been introduced in algal culture medium to develop algal-bacterial bioflocculating systems (McKinney et al., 1971, Humenik and

Hanna, 1971; McGriff and McKinney, 1972; John and Bokil, 1979). The mechanism of flocculation in this system has been thought to be bridging and entrapment of algae by activated sludge. The dry weight ratios of algae and bacteria were reported to be 7:13 (McGriff and McKinney, 1972) and 3:2 (John and Bokil, 1979). A 10:1 ratio was employed in field experiment 14, but no effect on bioflocculation was shown. However, in laboratory experiment 13, a culture loaded at the algae/bacteria ratio of 10:1 exhibited substantially greater algal removal than those cultures loaded at ratios of 5:1 and 20:1 (Fig. 4-10). Bacterial activity was found to be proportional to the amount of activated sludge seeding in both laboratory and field experiments (Fig. 4-10, 5-26).

6.4 Effect of pH

A critical factor is pH in autoflocculation, sometimes occurred in high-rate ponds at elevated pH (10-11) as a results of the consumption of CO_2 by algal photosynthesis. Azov et al. (1980) found that Coelastrum and Spirulina are capable of autoflocculation, forming aggregates which settle down when not agitated.

Several chemical reactions such as phosphate precipitation, calcium and magnesium hydroxide formation, and complexation were probably responsible for spontaneous algal autoflocculation at pH values greater than 9.0, the minimum pH value at which phosphate precipitation will occur (Golueke and Oswald, 1965; Gorden and Chapman, 1979; Sukenik et al., 1985). However, the efficiency of algal settling was

also found to be independent of pH at levels as low as 7.9 (McGriff and McKinney, 1972). Their observation of spontaneous algal flocculation in activated algae systems was more probably due to exopolymer interaction.

Several findings support the assumption that bioflocculation achieved in the present experiments did not involve autoflocculation. First, complete bioflocculation was shown in the culture having the lower pH in field experiment 1, 6, 12 and 14 and laboratory experiments 2, 3, 4, 5 and 6. On many occasions in field and laboratory experiments, a general increase in settleable matter was accompanied by declining pH trends. Second, cultures having almost the same pH sometimes differed in settleability (field experiment 2, 3, 7, and 10). Finally, microscopic examination revealed that bio-flocs were consisted largely of Synechocystis or Chlorella/Monodus with appropriate proportions of bacterial component. No inorganic chemical matrix was found. These flocs were quite different from chemical flocs formed with aluminum sulfate in which algal cells made up less than half the floc volume.

In laboratory experiments 2 and 3, conducted with Chlorella dominant initially, complete bioflocculation was observed within 8 days (Fig. 4-1, 4-2). At the same time, these cultures entered the declining growth phase. The combined influence of electrical double layer thickness and polymeric ionization caused by lowering the culture pH was perhaps responsible for this phenomenon.

An inverse relationship between algal and bacterial growth was noticeable in laboratory experiments 2 and 3 with Chlorella. As algal growth increases, bacterial activity decreases and vice versa. A slight trend in this direction was shown in field experiments due to changes in variables such as algal species composition, climate condition, grazers, etc. Fogg (1965) and Dor and Benzion (1980) have reported algal-bacterial antagonism in which certain algal species such as Chlorella, Scenedesmus, and Chlamydomonas produced substances inhibitory to bacterial activity. However this view of antagonism is in sharp contrast to the generally accepted one that bacterial-algal interaction in high-rate ponds is essentially synergistic.

Contrary to the laboratory results, it was found that lowering of pH did inhibit flocculation in field experiments. Algal growth, however, was stimulated at the lower pH and reduction of pH limited bacterial activity relative to the control culture.

It was generally observed that pH in laboratory cultures tended to decline below 6 within 5-7 days. This occurred even after adjusting culture to pH 8.0 every day (laboratory experiment 4, 5 and 6; Fig. 4-5, 4-6, 4-7). The declining pH effect might be explained by the influence of nitrification in which 3.55 Kg of bicarbonate alkalinity (as $CaCO_3$) was reduced for each Kg of nitrate produced. Further research is recommended to determine whether or not this nitrification would affect algal bioflocculation.

6.5 Effect of Algal Species

The bioflocculation tendency of different algal species has not yet been fully studied. However, their morphological characteristics can significantly effect the extent of bioflocculation because their shape and size or total available surface area can determine the optimal polymer concentration for bridging. For example, Micractinium and Scenedesmus have prominent setae or spines which facilitate anchoring to surrounding cells, biopolymers or other colloids, while the regular spherical to ellipsoidal shapes of Chlorella and Monodus make them less prone to attach to other cell surfaces or polymeric chains.

Microalgal genera previously identified in the bioflocculation experiments include Ankistrodesmus (Koopman et al., 1978) Micractinium (Eisenberg et al., 1981) Chlorella (McKinney et al., 1971), Scenedesmus (Oswald et al., 1978), and Synechocystis (Lincoln et al., 1984). The principal algal genera encountered in the present research were Synechocystis, Chlorella and Monodus. Of these three, the former genus had the greatest bioflocculation tendency. This alga, because of its small size, requires high flocculant doses for chemical flocculation. However, with no chemical addition, bioflocculation of Synechocystis through continuous flow mixing was highly effective. Complete bioflocculation marked by 90% cell aggregation was achieved in the field experiment 1. Settling tests showed 95% removal of the algae in 4 min with a settling rate of 8 cm/sec.

Maximum algal removals achieved in all field experiments in which Synechocystis was dominant or co-dominant averaged 55%. The bioflocculating tendency when Chlorella and Monodus were co-dominant was less, but still led to significant algal removal. Maximum algal removals obtained in all field experiments in which Chlorella and Monodus were co-dominant averaged 44%. This may be much better demonstrated in terms of biomass recovery. For examples, 35 Kg and 46 Kg dry algal solids were harvested after experiments 10 and 11, respectively.

6.6 Effect of Other Variables

Excocellular polymer production has been shown to be influenced by the relative concentrations of carbon and nitrogen in the growth medium (Saviour and Kristiansen, 1983). A similar result was reported by Rudd et al. (1984). A high C:N ratio could enhance the polymer production of capsular rather than soluble materials.

Addition of 50 g carbon/m^3 to the culture medium induced bioflocculation in laboratory experiment 1, inoculated with Chlorella from the high-rate pond (C-4) system at Gainesville. Such chemical supplementation would not degrade the quality of the algal biomass harvested, because the bicarbonate serves as a nutrient, not a flocculant. However, it would represent a significant, additional expense.

In another trial conducted in the field (experiment 3) with Chlorella and Monodus co-dominent initially, an

increase in carbon/nitrogen ratio by the addition of carbonate supplement (50 g/m^3 as C), showed a somewhat negative effect on bioflocculation. Interestingly, bacterial activity was stimulated by carbonate addition, even though culture pH was not appreciably changed (Fig. 5-28).

Pavoni et al. (1972) have shown that the production of extracellular biopolymer of unicellular algae increased during carbon limiting conditions. During late summer and early fall 1984, alum flocculated, anaerobic lagoon effluent of the University of Florida Swine Algae Research System was found to be carbon limiting (Kang, 1984), representing long lag period and finally death phase. The observed high pH caused by CO_2 extraction from carbonate-bicarbonate system could be an indication of carbon limitation. Carbon limitation of the anaerobic lagoon effluent was probably due to the excess carbon utilization by the higher activity of purple sulfur bacteria (Thiopedia rosea) during the warmest seasonal period. It was also reported that carbon losses incurred in methane production could lead to subsequent carbon limitation (Maddox et al., 1982). Further research is recommended to determine whether or not this carbon limiting would affect algal bioflocculation.

High algal removal was obtained in a culture with high algal density (initial chl a : 8 g/m^3). Settleable matter increase coincided well with declining growth phase. In general, as algal density increases, there is more chance for algal cells to collide, eventually increasing polymer

adsorption onto algal cell surfaces. If there is, however, a maximum floc formation associated with the optimum algae and bacteria concentration, an algal concentration should be selected with this in mind before transferring mature medium to bioflocculation channels.

6.7 Preliminary Cost Evaluation

The energy cost of waste ponds system are low since the stabilization of waste organics is supported by photosyntically derived oxygen, thereby obviating the need for mechnical aeration which is one of the principal energy requirement source in a conventional activated sludge system. Culp et al. (1978) estimated the direct energy requirement for aeration itself to be 532 kWh ($31.92) per million gallons treated in a diffused aeration system. The energy cost of triokling filter plants has also been estimated to be 158 kWh ($9.48) per one million gallons treated. It can be seen that energy costs for high-rate activated algae systems estimated by McKinney et al. (1971) and conventional activated sludge system are compatible. The energy cost of the photosynthetic oxygenation system utilized in the present research is estimated to be 130 kWh per million gallons of high-rate pond effluent treated by the bioflocculation process.

An estimation of the energy cost of the bioflocculation process can be compared to the cost of chemicals for alum flocculation. It was found that the AC motor driving the paddlewheel of the high-rate pond utilized 360 watts at a

flow mixing velocity of 20 cm/s. With continuous mixing, energy consumption was 9-12 kWh/d. At $0.06/kWh, the energy cost would be $0.55/d. At an average flocculation time of 21 days, the cost of mixing would thus be $11.55 for 300 m^3 of culture volume. Experimental harvests of the bioflocculating cultures yielded approximately 0.5 kg/m^3 air-dry solids on three occations. With 300 m^3 of culture volume, a total of 150 kg algal solids could be produced. The corresponding mixing energy cost would be $0.08 per kg of algal solids. Using alum priced at $200/tonne, and a typical alum/TSS ratio of 1.2 (Freedman et al., 1983), the alum cost would be $0.24 per kg of algal solids. When using chitosan, a synthetic biodegradable organic polymer, priced at $17.50 per kg, with a typical chitosan/TSS ratio of 0.02 (Lee, 1987), chitosan cost would be $0.35 per kg of algal solids. Thus, the energy cost for the bioflocculation process actually obtained under field conditions would be one-third of that for the alum flocculation.

CHAPTER 7
CONCLUSIONS

Laboratory and field scale investigations of algal bioflocculation have produced the following conclusions:

1. Continuous flow mixing was an essential factor in the induction of algal bioflocculation. Algal removal and settleable matter production were found to be proportional to flow mixing velocity. A mixing intensity of 40 rev/min was optimum for the maximum yield of settleable matter in laboratory cultures. Continuous flow mixing was inhibitory to the photosynthetic capacity of Synechocystis, leading to bioflocculation.
2. Spontaneous algal bioflocculation observed in the present research was due to extracellular polymer mediated flocculation, not entrapment in chemical precipitation (autoflocculation).
3. Algal species composition is the strongest parameter in bioflocculation of microalgae grown in anaerobically treated swine waste, e.g., Synechocystis showed the greatest bioflocculation tendancy.
4. Nutrient composition in wastewater influences the types of microalgae and bacteria which grow in the

culture medium. Concentrated wastewaters tend to inhibit the bioflocculation process.

5. Extracellular polymer produced by algae and bacteria can be specific to species, i.e., bioflocculation of specific microbial types in mixed cultures is possible.
6. It was observed that at high specific growth rates, nonflocculent growth is usually present whereas low specific growth rates create conditions for the optimum flocculation.
7. A sharp increase in settleable matter was accompanied by pH decline and by the declining or endogenous phase of algal growth.
8. Waste loading on bioflocculation had a moderate effect in the laboratory and a slight, positive effect in the field.
9. Dehydrogenase activities were highest in mixed or fast mixed, waste-loaded or heavily waste-loaded, and algal biofloc or activated sludge-seeded cultures.

APPENDIX
CALCULATION FOR G VALUE

The velocity gradient, $G = (P/V\mu)^{1/2} = (\text{Power/Volume} \times \text{Viscosity})^{1/2}$. P/V = Flow x Pressure/ (A x L) = $QWh/AL = vWh/L$. Therefore, $G = (vWS/\mu)^{1/2}$. Where, A = cross sectional area, L = legth in ft, Q = flow in cfs, W = specific weight in 62.4 lb/cu-ft, h = head loss in ft, v = velocity in fps and μ = absolute viscosity in 2.106×10^{-5} lb-sec/ft^2. From Manning equation, $v = (1.49/n)\, R^{2/3}\, S^{1/2}$. Where, v = velocity of flow in fps, n = roughness coefficient (n=0.013 for concrete), R = hydraulic radius, ft and S = slope of energy grade line. Thus, $S = (v \times n/1.49 \times R^{2/3})^2$. The width of the bioflocculation channel is 7.33 ft. When the depth is 1.08 ft (33 cm), $R^{2/3} = 0.89$. v = 0.525 fps then $S^{1/2} = 2.7 \times 10^{-5}$. Accordingly $G = (vWS/\mu)^{1/2} = (0.525 \times 62.4 \times 2.7 \times 10^{-5}/2.106 \times 10^{-5})^{1/2} = 6.5$ for G value of channel (C-2) when Exp. 1 was conducted

REFERENCES

Allen, M.D.B. and M.K. Garrett. 1975. Bacterial changes occurring the cultures of algae in the liquid phase of animal slurry. Jour. Appl. Bacteriol., 42: 27-43.

APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 15th. Ed. Am. Pub. Health Assoc., Washington, D.C.

Avnimelech, Y., B.W. Troeger and L.W. Reed. 1982. Mutual flocculation of algae and clay. Science, 216: 63-65.

Azov, Y., G. Shelef, R. Moraine and A. Levi. 1980. Controlling algal genera in high-rate wastewater oxidation ponds. In: Algae Biomass, 245-253. G. Shelef and C.J. Soeder (Eds.). Elsevier/North-Holland Biomedical Press, Amsterdam.

Bishop, C.T., G.A. Adams and E.O. Hughes. 1954. A polysaccharide from the blue-green algae Anabaena cylindrica. Canadian Journal of Chemistry, 32: 999.

Bitton, G. and B. Koopman. 1982. Tetrazolium reduction-malachite green method for assessing the viability of filamentous bacteria in activated sludge. Appl. Environ Microbiology, 43: 964.

Bogan, R.H., O.E. Albertson and J.C. Pluntze. 1960. Use of algae in removing phosphorus from sewage. J. San. Engrg. Div., Proc. Am. Soc. Civil Eng., 86: 1-20.

Bokil, S.D. and U.L. John. 1981. Treatment of domestic wastewater by flocculating algal-bacterial system. Indian J. Environ. Health, 23: 142-146.

Busch, P.L. and W. Stumm. 1968. Chemical interactions in the aggregation of bacteria. Environ. Sci. and Technol., 2: 49-53.

Clarke, A.B. and C.F. Forster. 1982. Biopolymer yields from activated sludge and their relation to the operation of treatment plant. Biotechnol. Letters, 4. 655-660.

Cohen, J.M., G.A. Rourke and R.L. Woodward. 1958. Natural and synthetic polyelectrolytes as coagulant aids. Jour. AWWA, 50: 463-478.

Cornwell, R.A. and M.M. Bishop. 1983. Determining velocity gradients in laboratory and full-scale systems. Jour. AWWA, 75: 470-474.

Costerton, J.W., G.G. Geesey and K.J. Cheng. 1978. How bacteria stick. Sci. American, 238: 86-95.

Culp, R., G. Wesner and G. Culp. 1978. Handbook of advanced wastewater treatment. Van Nostrand Reinfold Co., New York.

De Pauw, N. and E. Van Vaeenberg. 1981. Microalgal treatment system: Potential and limits. Paper presented at the conference phytodepuration and the biomass produced. Institute di Ecologia, University of Parma, Italy.

Dor, I. and S. Benzion. 1980. Effect of heterotrophic bacteria on the green algae in wastewater. In: Algae Biomass, 431-436. G. Shelef and C.J. Soeder (Eds.). Elsevier/North-Holland Biomedical Press, Amsterdam.

Dugan, P.R. and H.M. Pickrum. 1972. Removal of minerml ions from water by microbially produced polymers. In: Proc. 27th Industrial Waste Conference. Purdue University, Lafayette, IN.

Dugid, J.P. and J.P. Wilkinson. 1953. The influence of cultural condition on polysaccheride production by Aerobacter aerogenes. J. Gen. Microbiol., 9: 174-189.

Eisenberg, D.M., B. Koopman, J.R. Benemann and W.J. Oswald. 1981. Algal bioflocculation and energy conservation in microalgal sewage ponds. Biotechnol. Bioengrg. Symp., 11: 429-448.

Endo, T., K. Nakamura and H. Takahashi. 1976. Pronase-susceptible floc forming bacteria: Relationship between flocculation and calcium ion. Agric. Biol. Chem., 40: 2289-2295.

Endo, T. and M. Takahashi. 1981. Partial purification and some properties of aggregation factor from pronase-succeptible floc forming Flavobacterium Strain B. Agric. Biol. Chem., 45: 513-516.

EPA. 1974. Methods for Chemical Analysis of Water and Wastes. U.S. Environ. Prot. Agency, Washington, D.C.

Fair, G.M., J.C. Gayer and D.A. Okun. 1968. Water and Wastewater Engineering. Vol. 2. John Wiley and Sons, Inc., New York.

Friedman, D., B. Koopman and E.P. Lincoln. 1983. Chemical and biological flocculation of purple sulfur bacteria in anarobic lagoon effluent. Jour. Agr. Eng. Resear., 28: 115-125.

Friedman, S.A. and P.R. Dugan. 1968. Identification of Zoogloea species and the relationship to zoogloeal matrix and floc formation. J. Bacteriol., 95: 1903-1909.

Friedman, S.A., P.R. Dugan, R.M. Pfister and C.C Remsen. 1968. Fine structure and composition of the Zoogloeal matrix surrounding Zoogloea ramigera. J. Bacteriol., 96 2144-2156.

Friedman, S.A., P.R Dugan, R.M. Pfister and C.C. Remsen. 1969. Structure of excellular polymers and their relationship to bacterial flocculation. J. Bacteriol., 98: 1328-1334.

Gauthier, J.J. 1981. Improving Municipal and Industrial Waste Treatment Processes: Nutritional and Physical Conditions that Regulate Floc Formation in Bacteria Isolated from Activated Sludge. Project A-068ALA. U.S. Department of the Interior, Washington, D.C.

Gehr, R. and J.G. Henry. 1983. Removal of extracellular material: Techniques and pitfalls. Water Res., 17: 1743-1748.

Goldman, J.C. 1979. Outdoor algal mass cultues. II. Photosynthetic yield limitations. Water Res., 13: 119-136.

Golueke, C.G. and W.J Oswald. 1965. Harvesting and processing sewage-grown planktonic algae. Jour. WPCF, 37: 471-498.

Golueke, C.G. and W.J. Oswald. 1980. Surface properties and ion exchange in algae removal. Jour. WPCF, 42: R304-R314.

Gorden, M.S. and D.J. Chapman. 1979. Aquacultural Approaches to Wastewater Nutrient Recycling. Department of Biology, Univ. of California, Los Angeles, CA.

Gulas, V., M. Bond and L Benefield. 1979. Use of exocellular polymers for thickening and dewatering activated sludge. Jour. WPCF, 51: 798-807.

Gupta, S.K. and A.V.S.P. Rao. 1980. Treatment of urea by algae, activated sludge and floccualating algal bacterial system-A comparative study. Indian J. Environ. Health, 22: 103-112.

Harris, R.H. and R. Mitchell. 1973. The role of polymers in microbial aggregation. Ann. Rev. Microbiol., 27: 27-50.

Hemens, J. and M.H. Mason. 1968. Sewage nutrient removal by a shallow algal stream. Water Res., 2: 277-287

Hiatt, R.L., W. Kolb and M. Horgan. 1977. Unpublished paper. Woodland Public Works Department, Woodland, CA.

Humenik, F.J. and G.F. Banna. 1971. Algal-bacterial symbiosis for removal and conservation of wastewater nutrients. Jour. WPCF, 43: 580-594.

John, U.L. and S.D. Bokil. 1979. Flocculating algal-bacterial system: A new method of wastewater treatment. Indian J. Environ. Health, 21: 1-9.

Kang, R. 1984. Algal growth potential of anaerobically treated swine waste. ME. Thesis. University of Florida-Gainesville, FL.

Kiff, R.J. 1978. A study of the factors affecting bioflocculation in the activated sludge process. Jour. WPCF, 77: 464-470.

Kiff, R.J. and R. Thompson. 1979. Physical methods for the extraction of bacterial exopolymers from activated sludge biomass. Biotechnol. Letters, 1: 183-186.

Koopman, B. 1981. Factors affecting algal separation in batch-operated secondary ponds. Ph D. Dissertation. University of California, Berkley, CA.

Koopman, B., R. Thomson, R. Yackzan, J. Benemann and W. Oswald. 1978. Investigation of the Pond Isolation Process for Microalgae Separation from Woodland's Waste Pond Effluents. Univ. of California, Berkeley, CA.

Koopman, B., J.R. Benemann and W.J. Oswald. 1981. In-pond separation of algae from treated effluents. In: Proc. 1981 National Conf. on Environ. Engrg., 434-442. F.M. Saunders (Ed). Am. Soc. Civil Engrs., Atlanta, GA.

Koopman, B., G. Bitton, C.L. Logue, J.M. Boscart and J.M. Lopez. 1984. Validity of tetrazolium reduction assays for assessing toxic inhibition of filamentous bacteria in activated sludge. In: Toxicity Screening Procedures Using Bacterial Systems, 147-162. D. Liu and B.J. Dutka (Eds.) Marcel Dekker, New York.

Koopman, B. and E.P. Lincoln. 1983. Autoflotation harvesting of algae from high-rate pond effluents. Agricultural Wastes, 5: 231-245.

Koopman, B., E.P. Lincoln, S.-I. Lee, H. Kang and C.W. Kim. 1984. Biological flocculation in waste-grown algal cultures. First annual report to the Florida Water Resource Research Center. University of Florida, Gainesville, FL.

Koopman, B., E.P. Lincoln and R.A. Nordstedt. 1982. Anaerobic photosynthetic reclamation of swine waste. Proceeding of the Water Reuse Symposium II, 2: 924-934.

Lai, R., M. Hudson and J. Singley. 1975. Velocity gradient calibration of jar-test equipment. Jour. AWWA, 67: 553-557.

LaMer, V.K. and T. Healy. 1963. Adsorption-flocculation reactions of macromolecules at the solid-liquid interface. Rev. Pure Appl. Chem., 13: 112-113.

Lee, S.-I. 1987. Flocculation and flotation of waste-grown microalgae. Ph.D. Dissertation, University of Florida, Gainesville, FL.

Lewin, R.A. 1956. Extracellular polysaccharides of green algae. Canadian J. Microbiol., 2: 665-672.

Lincoln, E.P. and B. Koopman. 1982. Microalgae for biomass production and treatment of anaerobic digester effluent First annual report, Project No. AGE-2157. GRI/IFAS Methane from Biomass Program. University of Florida, Gainesville, FL.

Lincoln, E.P. and B. Koopman. 1983. Microalgae production for the treatment of anaerobic digester effluent Second annual report, Project No. AGE-2157 GRI/IFAS Methane from Biomass Program. University of Florida, Gainesville, FL.

Lincoln, E.P., T.W. Hall and B. Koopman. 1983. Zooplankton control in mass algal cultures. Aquaculture, 32. 331-337.

Lincoln, E.P. and T.D. Hill. 1980. An integrated microalgae system. In: Algae Biomass, 229-244. G. Shelef and C.J. Soeder (Eds.). Elsevier/North-Holland Biomedical Press, Amsterdam.

Lincoln, E.P. and B. Koopman. 1984. Bioflocculation of microalgae in mass cultures. Paper presented at Algal Biomass Workshop, Univ. of Colorado, Boulder, 5-7 April 1984.

Lincoln, E.P., B. Koopman and T. Hall. 1984. Control of a unicellular, blue-green alga, Synechocystis sp., in mass algal culture. Aquaculture, 42: 349-358.

Logue, C., B. Koopman and G. Bitton. 1983. INT reduction assays and control of sludge bulking. J. Environ. Engrg., 109: 915-923.

Maddox, J.J., L.L. Behrends, D.W. Burch, J.B. Kingsley and E.L. Waddell. 1982. Optimization of biological recycling of plant nutrients in livestock waste using waste heat from cooling water. EPA-800/7-82-041. Muscle Shoals, AL.

Malis-Arad, S., R. Friedlander, R. Ben-Arie and A.E. Richmond. 1980. Alkalinity-induced aggregation in Chlorella vulgaris I. Changes in cell volume and cell-wall structure. Plant & Cell Physiol., 21 (1): 27-35.

Malis-Arad, S. and R.E. McGowan. 1982. Alkalinity-induced aggregation in Chlorella vulgaris II. Changes in the cell wall during the cell cycle. Plant & Cell Physiol., 23: 11-17.

McGriff, E.C. 1970. Nutrient removal by activated algae. Ph.D. Dissertation. University of Kansas, Lawrence, KS.

McGriff, E.C. and R.E. McKinney. 1972. The removal of nutrients and organics by activated algae. Water Res., 6: 1155-1164.

McKinney, R.E. 1952. A fundamental approach to the activated sludge process-a proposed theory of floc formation. Sewage Indust. Wastes., 24: 3.

McKinney, R.E., R.J. Sherwood, V.N. Wahbah and D.W. Newport. 1971. Ahead: Activated algae? Water Wastes Engng., 8 (9): 51.

Merz, R.C., R.G. Zahnpfenning and J.R. Klima. 1962. Chromatographic assay of extracellular products of algal metabolism. Jour. WPCF, 34: 103.

Moore, B.G. and R.G. Tischer. 1964. Extracellular polysaccharides of algae: Effects on life-support systems. Science, 145: 586-587.

Nambiar, K.R. and S.D. Sokil. 1981. Luxury uptake of nitrogen in flocculating algal-bacterial system. Water Res., 15: 667-669.

Nishikawa, S. and M. Kuriyama. 1968. Nucleic acid as a component of euciliaga in activated sludge. Water Res., 2: 811-812.

Northrop, J.H. and P.R. Dekruif. 1921. The stability of bacterial suspensions III: Agglutination in the presence of protains, normal serum, and immune serum. Journal of General Physiology, 4: 655-667.

Novak, J.T. and B.-E. Haugan. 1981. Polymer extraction from activated sludge. Jour. WPCF, 53: 1420-1424.

Nye, I.C. and F.R. Rivera-Negron. 1980. Symbiotic growth of algae and bacteria on swine waste. In: Livestock Waste, 154-159. Fourth international symposium on livestock waste. ASAE, St. Joseph, MI.

O'Melia, C.R. 1972. Coagulation and flocculation. In: Physicochemical Processes for Water Quality Control, W.J. Weber (Ed.). Wiley-Interscience, New York, NY.

Oron, G., G. Shelef, A. Meydan and Y. Azov. 1979. Algae/bacteria ratio in high-rate ponds used for waste treatment Applied and Environmental Microbiology, 38: 570-575.

Oswald, W.J. 1965. Advances in anaerobic pond system design. In: Advances in Water Quality Improvement, 409. E F. Gloyne and W.W. Eckenfelder (Eds.). University of Texas Press, Austin, TX.

Oswald, W.J., E.W. Lee, S. Aden and K.H. Yao. 1978. New wastewater treatment method yields a harvest of saleable algae. World Health Organiz. Chron., 32: 348-350.

Parker, D.S., W.J. Kaufman and D.Jenkins. 1971. Physical conditioning of activated sludge flocs. Jour. WPCF, 43: 1817-1833.

Parker, D.S., W.J. Kaufman and D. Jenkins. 1972. Floc breakup in turbulent flocculation processes. J. San. Eng. Div., Proc Am. Soc. Civ. Engrs., 98: 79-99.

Parsons, A.S. and P.R. Dugan. 1971. Production of extracelluler polysaccharide matrix by Zoogloea ramigera. Appl. Microbiol., 21: 657-661.

Pavoni, J.L., M.W. Tenney and W.F. Echelberger. 1971. The relationship of algal exocellular polymers to biological flocculation. In: Proc. 26th Industrial Waste Conference, 957-962. Purdue Univ., Lafeyette, IN.

Pavoni, J.L., M.W. Tenney and W.F. Echelberger. 1972. Bacterial exocellular polymers and biological flocculation. Jour. WPCF, 44: 414-431.

Pavoni, J.L., S.W. Keiber and G.T. Roblitt. 1974. The Harvesting of algae as a food source from wastewater using natural and induced flocculation techniques. In: Proc. Conf. on Use of Wastewater in Product. of Food and Fiber, Oklahoma City, OK.

Regan, R.W. 1972. Light-depth relationship for activated algae. Ph.D. Dissertation. University of Kansas, Lawrence. KS.

Regen, R.W. and R.E. McKinney. 1977. Nitrogen oxidation and removal efficiency using activated algae. Proc. Wat. Tech., 8: 451.

Rudd, T., R.M. Sterritt and J.N. Lester. 1983. Extraction of extracellular polymers from activated sludge. Biotechnol. Letters, 5: 327-332.

Rudd, T., R.M. Sterritt and J.N. Lester. 1984. Complexation of heavy metals by extracellular polymers in the activated sludge process. Jour. WPCF, 56: 1260-1268.

Sakka, K., T. Endo, M. Watanabe, S. Okuda and H. Takahashi. 1981. Deoxyribonuclease-susceptible floc forming Pseudomonas sp. Agric. Biol. Chem., 45: 497-504.

Sakka, K. and H. Takahashi. 1981. DNA as a flocculation factor in Pseudomonas sp. Agric. Biol. Chem., 45: 2869-2876.

Salanitro, J.P., P.T. Sun and J.B. Thornton. 1983. Effects of ammonia and phosphate limitation on the activated sludge treatment of calcium-containing chemical waste. Biotechnol. Bioengrg., 25: 513-523.

Sato, T. and Y. Osa. 1975. Studies on floc-forming substances in activated sludge - II. Floc-forming action by the viscous substance extracted from activated sludge by alkaline solution. J. Hyg. Chem. (Japan), 22: 55-59.

Sato, T. and Y. Osa. 1980. Floc-forming substances extracted from activated sludge by sodium hydroxide solution. Water Res., 14: 333-338.

Saviour, R.J. and B. Kristiansen. 1983. Effect of ammonium ion concentration on polysaccharide production by Aureobasidium pullulans in batch culture. European J. Appl. Microbiol. Biotechnol., 17: 178-181.

Sharwood, R. 1966. Start-up of an activated algae field unit. M.S. Thesis. University of Kansas, Lawrence, KS.

Stahl, U., U. Kuss and K. Esser. 1983. Flocculation in yeast, an assay on the inhibition of cell aggregation. European J. Appl. Microbiol. Biotechnol., 17: 199-202.

Starkey, J.E. and P.R. Karr. 1984. Effect of low dissolved oxygen concentration of effluent turbidity. Jour. WPCF, 56: 837-843.

Sukenik, A., W. Schrodar, J. Lauer, G. Shelef and C.J. Soeder. 1985. Coprecipitation of microalgal biomass with calcium and phosphate ions. Water Res., 19: 127-129.

Tago, Y. and K. Aida. 1975. The deflocculating enzyme produced by a floc-forming bacterium. J. Gen. Appl. Microbiol., 21: 365-374.

Talling, M. and D. Driver 1963. Some problems in the estimation of chlorophyll a in phytoplankton. In: Proc. Conf. Productivity. Meas., Marine and Fresh water, U.S. Atomic Energy Commission. Report TID-7633.

Tanney, M.W., W.F. Echelberger, R.G. Schuessler and J.L. Pevoni. 1969. Algel flocculetion with synthetic organic polyelectrolytes. Appl. Microbiol., 18: 965-971.

Tenney, M.W. and W. Stumm. 1965. Chemical flocculation of microorganisms in biological waste treatment. Jour. WPCF, 37: 1370-1384.

Tezuka, Y. 1973. A Zoogloea bacterium with gelatinous mucopolysaccharide matrix. Jour. WPCF, 45: 531-536.

Udea, S., K. Fujita, K. Komatsu and Z. Nakashima. 1963. Polysaccharide produced by genue Pullularia. I. Production of polysaccharide by growing celle. Appl. Microbiol., 11: 211-215.

Van Dyne, D.C. and C.B. Gilbertson. 1978. Estimating livestock and poultry manure and nutrient production. U.S. Department of Agriculture, Washington, D.C. Report ESCS-12.

Wahbeh, V. 1965. Activated Algae. M.S. Thesis. University of Kansas, Lawrence, KS.

Wallen, L.L. and E.N. Davis. 1972. Biopolymers of activated sludge. Environ. Sci. Technol., 6: 161-164.

Warren, G.H. and J. Gray. 1955. Studies on the properties of a polysaccharide constituent produced by Pseudomonas aeruginosa. J. Bacteriol., 70: 152-157.

Weber, W.J. 1972. Physicochemical processes for water quality control. Wiley-Interaciance, New York, NY.

Wilkinson, J.F. 1958. The extracellular polysaccharidee of bacteria. Bacteriol. Rev., 22: 42-73.

Wilkinson, J.F., J.F. Deguid and P.N. Edmunde. 1954. The distribution of polysaccharide production in Aerobacter and Escherichia strains and its relation to antigenic character. J.Gen. Microbiol., 11: 59-72.

BIOGRAPHICAL SKETCH

Ho Kang was born on December 5, 1951, in Korea, the son of Byungki and Gumsoon Kang. He prepared for college at Daejon High School, Korea, and graduated in 1970. He enrolled at Chungnam National University, Korea, in March, 1970, and was graduated with a B.E. degree in chemical engineering in February, 1974. He served in the military for three years as a first lieutenant. He accepted a research assistantship in the Department of Chemical Engineering at Chungnam National University and enrolled in graduate school in March, 1977. He received the M.E. degree in chemical engineering in February, 1980.

He entered the University of Florida in the fall of 1981 and was awarded the M.E. in environmental engineering in August 1984, majoring in wastewater treatment.

He is a member of Korean Scientists and Engineers Association in America, the American Water Works Association and the Water Pollution Control Federation.

He is married to Kinam Lee and has two children, Byn and Grace.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Koopman, Chairman
Associate Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward P. Lincoln, Co-chairman
Associate Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John Zoltek, Jr.
Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Roger A. Nordstedt
Associate Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dinesh O. Shah

Dinesh O. Shah
Professor of Chemical Engineering

This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1987

Hubert A. Benn

Dean, College of Engineering

Dean, Graduate School