BIOLOGICAL FLUORESCENCE OF MICROALGAE

By

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TO MY WIFE
STEAM LEE BANG

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BIOLOGICAL FLOCCULATION OF MICROALGAE

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The effect of environmental, biological and operational variables on biological flocculation of microalgae grown on artificially treated waste water was investigated in laboratory and field scale systems. Environmental variables considered were season, rainfall, temperature and solar irradiance. The biological variables considered were algal species composition. Operational variables included flow mixing, waste loading, waste pretreatment, algal biofilm 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.

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 *Skeletonema* 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 bioflocculation; flocculation in laboratory cultures was inhibited in 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 aged biotank or activated sludge seeded.

CHAPTER 1 Introduction

1.1 Exhibit 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 most effective because this process combines wastewater treatment and feed production. Waste organic are stabilized by bacteria using photosynthetically produced oxygen, thus obviating the need for mechanical aeration. Waste nutrients are converted to the form of

algae 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 10 microns in any dimension), dispersed nature (cell suspensions are stable, showing little tendency to agglomerate), 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 (Conrad, 1968). 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 cellular 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 irradiation. The biological variable considered was algal species composition. Operational variables included flow mixing,

waste loading, waste pretreatment, algal biofilm 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 aqueous polyelectrolytes results from bridging between extended polymer lengths and various 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 secretion, and extracellular synthesis. Accumulation of extracellular biopolymers is closely related to the growth phases of microorganisms as shown in Fig. 2-2. The results of several investigators (Lewin, 1954; Moore and Fisher, 1964; Sakka et al., 1961) indicate that the quantity of extracellular polysaccharides of unicellular algae increases during the late logarithmic phase of growth and reaches a peak in the early stationary phase. Maximal polysaccharide production has been observed to occur under carbon, nitrogen, sulfur or phosphorus limiting conditions (Groni et al., 1970; Stevens and Kristiansen, 1963; Salcedo et al., 1965; Segal and Wilkinson, 1959; Rood et al., 1964). It was suggested that a higher C/N ratio could enhance the polymer production of

Figure 2-1 Schematic representation of the building model for the denaturation of signal cells by polymers, adapted from O'Keeffe (1972).

Figure 3-3 Relationship between algal mass and extracellular polymer production (B,D) and their mass ratio (C). Adapted from Farnini et al. (1992).

suspender rather than soluble materials.

3.2 Algal Biofloculation Experiments in Laboratory-Scale Systems

Prevoni et al. (1981) 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 biofloculation was successfully achieved in activated algae systems developed by McQuinn and his co-workers (Robb, 1966; Sherman, 1966; McGriff, 1970; Samadik and Sogon, 1971; Sogon, 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 Figures 2-1 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 (Robb, 1966; Samadik and Sogon, 1971; Sogon, 1972; McGriff (1970). Using 28 connected channels in the light chamber, achieved 97% DO, 10% nitrogen and 74% phosphorus removal. The laboratory research of McGriff (1970) and Sogon (1972) established that (1) a variable light-dark relationship consisted of 3.3 minutes in the light and 2.4 minutes in the dark;

Figure 3-3 Flow diagrams for activated sludge systems used by (1) Mabbett (1961) and McGuffey (1970), (2) Garwood (1966), (3) Ragsdale and Ragsdale (1971) and (4) Ragsdale (1972).

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

Year	Investigator	Settleable Sludge Flux	Reactor Surface Area (m ²)	Capacity (kg)	Volume of Aerating Column (L)	Flow Rate Feed rate (m ³ /hr)	Fluid Velocity in Aeration Column (ft/min)	Liquid Detention Time- Estimated
1905	Twiss ¹	5	200	5	1.5	400	0.75	—
1915	Harwood	8	100, 100	6, 750	1, 200	10.5 - 105	3	1, 200
1920	Reese & Barnard	5	—	5	1.5	500	—	1, 200
1927	McIntire	5	6, 100	15-25.5	—	5, 500	0.30-0.50	200-400
1929	Reese	5 ²⁰	5, 100	20	1.5	60-65	—	100-150

¹ American Type Building²⁰ Institute of Phase 3, Inc. 7

(2) a detention time of 18 hours and a flow range of 1,400 to 1,480 g/s² were workable operating parameters; (3) at the light-depth ratio of 44 ft-candle/yd, satisfactory operation resulted when systems were loaded at a carbon input rate of 1,150 g/s²-d; (4) nutrient removal for activated sludge operated at 1.0-1.5 kg BOD/day/kg BOD₅ 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 (Kerwood, 1964; 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.

Boyer et al. (1960) utilized a similar activated sludge system in which sludge was recycled between a separator and a growth unit, with species of *Chlorella* and *Limnodinium* predominant. A well-flocculated culture was observed even when the effluent pH fell below 6 (the lower limit for phosphate precipitation).

Sakai and Jahn (1961) reported that the optimum ratio of algae to bacteria for flocculation to take place was 40:40 (w/v). The optimum algal bacterial biomass concentration fell within a range of 1,400-1,600 g/s² with a detention time of 4-8 hours. Contrary to the results of McKinney et al. (1971), they mentioned that complicated light-dark cycles were not necessary in developing flocculating

algae-bacterial system.

3.3 Algal Bioflocculation Experiences in Field Scale Systems

Orwell et al. (1988) reported that *Scenedesmus* grown in continuously mixed (at 5 cycles or 12 cm/min) high-overt ponds treating domestic wastewater in Manila, the Philippines, settled quickly when transferred to quiescent conditions. The experimental regime 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 fast-flow drawdowns. The growth media was very dilute, normally contained sewage with an average BOD of only 41 g/m³ and ammonia concentration of 2 g/m³. The average algal solids removal of 73.4% was observed with detention times of 0.18-1.4 days.

A facultative pond system treating domestic sewage from the city of Richmond, California, was the first mass culture system to utilize bioflocculation for algae removal (Kiani et al., 1977). Hoopes 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 90%. In work conducted at Richmond, California, Kienberg et al. (1981) discovered that continuous flow mixing with paddle wheels promoted

dominance of bi-flocculating *Microcystis* sp. in high-rate ponds treating domestic sewage. Mixing power requirements were low (approximately 18 kW_h/ha-d) because flow velocities of only 12-18 cm/sec were needed. The algae removal efficiency achieved by settling flocculant cultures averaged 12-22%.

Algal bioflocculation in a mass culture system treating wine waste was first demonstrated by Lincoln and Knapton (1981) at Gainesville, Florida. In this system, continuous paddle wheel mixing at a velocity of 18 cm/sec promoted dominance of bi-flocculating *Microcystis* sp. with the loading of raw wine waste. Settled volumes increased to over 180 mL/L with a fixed volume of 11 mL/L. It was found that heavy (pales) loading with raw wine waste enhanced floc formation and that this media was associated with the best growth of *Microcystis*.

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

CHAPTER 2

MATERIALS AND METHODS

2.1 Summary of Experiments

A total of 11 laboratory experiments and 14 field experiments were conducted between November 1983 and February 1984. Tables 2-1 and 2-2 provide summaries of the laboratory and field experiments, respectively.

2.2 Laboratory Experiments

2.2.1 Growth Media

Three kinds of waste media were utilized in the laboratory experiments: slow flocculated anaerobic laguna effluent, centrifuged anaerobic laguna effluent and mature high-rate pond culture medium. Descriptions of the anaerobic laguna and high-rate pond are given in section 2.2.1.

Slow flocculated anaerobic laguna effluent was used in experiment 1. It was prepared by flocculating anaerobic laguna effluent with aluminum sulfate ($Al_2(SO_4)_3 \cdot 14H_2O$) to remove *Hydrobia* eggs and other suspended solids. Dosage was 1.1 g $Al_2(SO_4)_3$ /g TSS (Freedman et al., 1981). The jar test procedure consisted of 48 sec of rapid mixing at 115 rev/min ($\dot{\gamma} = 300 \text{ s}^{-1}$), 1 hour of slow mixing at 15 rev/min ($\dot{\gamma} = 2 \text{ s}^{-1}$) and 4 hours of quiescent settling. Values of mean velocity gradient (G) were calculated from stirred speed (15 rev/min) using the calibration of Cornwell and

Table 3-1. Summary of laboratory experiments

Exp. no.	Date	Experimental variable	Algal species dominant initially
1	1/3-4/28(88)	carbon addition	<u>Chlorella</u>
2	1/5-4/28(88)	pH	<u>Chlorella</u>
3	1/17-2/5(88)	pH	<u>Chlorella</u>
4	3/18-4/28(88)	waste loading	<u>Chlorella</u> / <u>Scenedesmus</u>
5	4/18-4/28(88)	mixing	<u>Chlorella</u> / <u>Scenedesmus</u>
6	1/5-7/13(88)	mixing	<u>Chlorella</u> / <u>Scenedesmus</u>
7	9/11-10/10(88)	pH	<u>Scenedesmus</u>
8	10/22-11/1(88)	pH	<u>Scenedesmus</u>
9	11/1-11/11(88)	waste loading	<u>Scenedesmus</u>
10	12/3-12/17(88)	activated sludge seeding	<u>Chlorella</u> / <u>Scenedesmus</u>
11	12/3-12/13(88)	waste concentration	<u>Chlorella</u> / <u>Scenedesmus</u>

Table 3-1. Summary of field experiments

Exp. no.	Dates	Experimental variation	Algal genera dominant initially
1	Nov-Dec 83	mixing	<i>Synedra</i> / <i>Cryptos</i>
2	Jan-Feb 84	mixing	<i>Synedra</i> / <i>Cryptos</i>
3	Mar-Apr 84	CH_3I^+ addition	<i>Chlorella</i> / <i>Scened</i>
4	May 84	mixing	<i>Scenedra</i> / <i>Chlorella</i>
5	Jun-Jul 84	waste loading	<i>Synedra</i> / <i>Cryptos</i>
6	Jul-Sep 84	waste loading rate	<i>Synedra</i> / <i>Cryptos</i>
7	Sep-Oct 84	biofilm (algae) seeding	<i>Scenedra</i> / <i>Chlorella</i>
8	Nov 84	pH	<i>Chlorella</i> / <i>Scenedra</i>
9	Dec 84	purple sulfur bacteria seeding	<i>Chlorella</i> / <i>Scenedra</i>
10	Jan/Feb 85	waste type	<i>Chlorella</i> / <i>Scenedra</i>
11	Mar-Apr 85	waste loading	<i>Chlorella</i> / <i>Scenedra</i>
12	May-Jun 85	mixing velocity	<i>Chlorella</i> / <i>Scenedra</i>
13	Oct-Nov 85	mixing velocity	<i>Synedra</i> / <i>Cryptos</i>
14	Dec 85	activated sludge seeding	<i>Chlorella</i> / <i>Scenedra</i>

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 anaschis lagoon effluent was prepared by centrifuging anaschis lagoon effluent at 2,500 $\times$ g for 10 min with a centrifuge (RC8-B, Ross Service, Inc., Norwalk, Conn.). Typical characteristics of alga flocculated and centrifuged anaschis lagoon effluents are given in Table 2-2.

When alga flocculated or centrifuged anaschis 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 2,500-3,000 $\times$ g for 10 min. The volume of inoculum added ranged from 10-20 mL/L of culture medium.

At times, the culture medium obtained from the high-rate pond contained significant zooplankton populations (e.g., *Brachionus calanoides*, *Cyclops bicus*). To experiment 7, 8, and 10, zooplankton in the laboratory cultures were controlled by addition of KMnO_4 . The target free ammonia concentration was reported as 10 μM (Lincoln et al., 1983), a value sufficient to kill virtually all zooplankton without adversely affecting the algal population. In order to reach this concentration, 180 mg KMnO_4 (40 mg as K) was added per liter of medium. Then the pH of the cultures was raised to 8.5 with 10 N NaOH. At this

Table 3-3 Typical characteristics of anaerobically and aerobically digested sewage sludge

Source ^a	Anaerobically digested sewage sludge	Aerobically digested sewage sludge
TSS	65	84
VSS	60	72
BOD	4	15
COD	86	143
TSR	93	81
SP	15	26

^aAll values in g/g³

pH, approximately one-half of the total ammonia present exists in the undissociated form (NH_3). The pH was held at this level for 4-6 hours, then readjusted to 8.8 with 10 N H_2SO_4 .

2.2.2 General Experimental Plan

In all experiments, except exp. 8 and 9, cultures were maintained on a variable speed shaker table (Model 1000, Lab-Line Instruments Inc., Milner Park, IL) which was operated at 145 oscillations/min. Illumination was provided a bank of five 180 cm long, 40 W, cool white fluorescent tubes spaced on 18 cm centers and suspended 18.8 cm above the top of the shaker table. The light intensity at the surface of the shaker table was measured as 7.12 W/m^2 , using a pyranometer/lightmeter (PILOT-7000, LI-COR, Inc., Lincoln, Nebraska/LI-100, Lioba Instruments Corp., Lincoln, Nebraska).

Cultures were grown in 250-ml Erlenmeyer flasks, each containing 100 ml medium volume. Flasks were capped with serum plastic plugs to allow air exchange. Trials were conducted in triplicate.

In experiments 8 and 9, a standard jar test apparatus (Higbie and Sinn, Richmond, VA) was used to provide various mixing intensities. Paddle-rotors were 3-bladed, each blade having a radius of 1.8 cm and length of 1.8 cm. The jars were made of acrylic plastic and had diameters of $12.8 \pm 11.8 \pm 10.8$ cm. The liquid volume used was 1.5 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 jar. Light intensity measured on the lateral surface of the jar was 11.6 W/m^2 . Trials were conducted in duplicate. Unless otherwise noted, cultures were adjusted to pH 8.0, on a daily basis.

3.2.2 Experimental Procedures

3.2.2.1 Effect of pH—Experiments 3, 4, 7 and 8

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

In exp. 3, three different pH levels (5.5, 7.0 and 8.5) were tested. Initial chl *a* concentration was 10 g/m^3 .

In exp. 4, trials at three different pH levels (5.5, 6.5 and 7.5) were repeated based on the results of exp. 3. The pH was not adjusted in the control. The culture medium was a mixture of 2 parts high-rate pond medium and 1 part slow flocculated wastewater lagoon effluent. Initial chl *a* concentration was 1.8 g/m^3 .

In exp. 7, culture media 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 1.8 g/m^3 .

In exp. 8, trials at 3 different pH levels (5.5, 6.5 and 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. 3 and 4

The effect of mixing on bioflocculation was evaluated in exp. 3 and 4. 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. Aired gases were 54% Chlorzilia and 50% Baccodur on a blowdown basis in both experiments. Initial chl *a* concentrations in exp. 3 and 4 were 12.6 g/m³ and 8.8 g/m³, respectively.

3.2.3.3 Effect of Waste Loading Rate--Exp. 4 and 5

The effect of waste loading rate on bioflocculation was investigated in exp. 4 and 5. Protocol in these experiments were identical, except that dominant aired gases were Chlorzilia and Baccodur in exp. 4 and Bromochlorzilia in exp. 5. Cultures were loaded with fixed bed reactor effluent at rates of 25, 45, and 65 of culture volume per week. The control was not loaded. Initial chl *a* concentrations in exp. 4 and 5 were 4.2 g/m³ and 4.4 g/m³, respectively.

3.2.3.4 Effect of Waste Pre-treatment--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 acetic acidium 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 45 of the culture volume (15 ml) per week. Aired gases were 50% Chlorzilia and 50%

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

Source ^a	Settled waste supernatant	Fixed bed reactor effluent
TS	7,465	8,180
VSS	5,800	3,580
COD	13,801	8,383
BOD	1,326	1,570
NO ₃ -N	201	1,864

^aAll values in g/m³

Synedra on a bicarbonate basis. Initial chl *a* concentration was 4.9 g/m^3 .

3.2.3.3 Effect of Activated Sludge Addition--Exp. 12

This experiment employed activated sludge as a source of bacterial seed. The amount of activated sludge added was 5%, 10%, and 15% of the sludge dry weight. Algal genera were 10% *Chlorocella* and 90% *Synedra* on a bicarbonate basis. Initial chl *a* concentration was 4.9 g/m^3 .

3.2.3.4 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 *Chlorocella*. Initial chl *a* concentration was 3.5 g/m^3 .

3.2.3.5 Preliminary experiment

The effect of algal density on bioflocculation was tested. Three levels of initial algal density (3.5, 4.5 and 5.5 g chl g/m^3) were applied to cultures initially dominated by *Chlorocella* and *Synedra*.

Another preliminary experiment examined the growth potential of *Synedra* under mixed and unmixing conditions at 21 °C and 18 °C. Mixing (48 rev/min) was provided by the Jar test apparatus. Temperature was maintained by placing the Jar test apparatus with an electronic heater in a closed foam box. Illumination was provided by a 30 W fluorescent light located 8 cm from the lateral face of jars. Initial chl *a* concentration was 4.9 g/m^3 .

3.2 Field Experiments

3.2.1 Description of Field System

Studies of field waste biotransformations were conducted at the University of Florida Swine Aqueous System. The principal components of this system were raw waste generation, anaerobic/facultative treatment, photolytic oxygenation and algae harvesting (Fig. 3-1). Finished wastes like an average population of 250 pigs housed over concrete slats or slatted floors were regularly discharged to anaerobic treatment (digester or digester) after temporary storage in underground pits. Average daily wastewater values ranged from 1 to 3 m^3/day depending on the number and age of pigs in residence at the farms, the frequency of finishing, and use of cooling sprays. Total solids (TS) concentrations averaged 1-2%.

The anaerobic digestion system consisted of a settling tank, a conventional, mixed digester and a fixed bed reactor. A portion (2.5-3.5 m^3/d) of the finished wastes from the waste confinement buildings was pumped to the 20 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 0.5-1.5 m^3/d . The digester was made from a 20 m^3 polyethylene tank and was mixed and unheated. The loading rate ranged from 0.14-1.20 kg volatile solids (VS)/ m^3/d and averaged 0.87 kg VS/ m^3/d . 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 ambient temperature. This unit was constructed from a 10 m³ polyvinyl tank and filled with cypress chips that acted as support media for anaerobic bacteria. Its loading rate ranged from 0.10-1.12 kg VS/m²d and averaged 1.08 kg VS/m²d, on an empty bed volume basis.

Excess effluent flowing from the digester system, as well as finished waste not pumped to the settling tank, was discharged to the anaerobic lagoon. The anaerobic lagoon had a surface area of 1,100 m² and depth of 1.4 m. It received an average loading of 13 g VS/m²d and had a detention time of 200 days. The anaerobic lagoon functioned as a continuous culture, predominantly of the purple sulfur bacteria, *Thiospirillum*. Its overflow was received by a facultative pond which averaged 1.4 m in depth and had a surface area of 800 m². Effluents from the settling tank, anaerobic digester, fixed bed reactor, anaerobic lagoon, and facultative pond were used as alternative feed sources for the photosynthetic oxygenation stage (high-rate pond) and algae harvesting stage (microencapsulation channels).

The high-rate pond (designated C-4) was situated in heavy clay soil and had a length of 18 m and surface area of 400 m². A race-track configuration was imparted by a central baffle which extended for 18 m. Flow-mixing at a velocity for 18 cm/s was achieved by means of an electric motor driven paddlewheel. Mixing was carried out for 18 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 18 m long and 170 m² in surface area, with center baffles arranged to give a doubletrack configuration. Mixing schedules and waste loading were varied according to the experiment in progress, operating depth ranged from 0.3-0.8 m.

1.3.2 Field Scale Experimental Procedures

The general experimental procedure was to fill the paired bioflocculation channels, C-1 and C-2, with mature culture taken 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.

1.3.2.1 Effect of Mixing on Bioflocculation, Ex. 1, 2, 4, 11, and 12

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 slow mixing velocities (14 rpm and 10 rpm) were examined in exp. 11 and 12.

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 14 rpm whereas C-1 was not mixed. Ammonia digester effluent was added to each channel two times weekly. Ammonia hydroxide was added to control grasses on two occasions.

In exp. 2, C-1 was completely drained and cleaned after termination of exp. 1. Then culture medium from C-1 was transferred to C-2 and C-2 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 14 cm/s and C-1 was not mixed. 100 L of renewable 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 9.3 g/m³. The dominant algal genus was *Synechococcus* initially.

In exp. 4, culture medium from the high-rate pond was transferred to the biofloculation channels, giving each an initial depth of 21 cm. C-1 was mixed at 14 cm/s and C-2, the control, was not mixed. Neither channel was loaded during the experiment. Initial chl *a* concentrations were 18.8 and 10.7 g/m³ in C-1 and C-2, respectively. Dominant algal genera were *Synechococcus* and *Chlorocella/Chlorocoides*. The relative proportions of these two populations were 48% and 52%, respectively.

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 biofloculation channels to an initial depth of 40 cm. C-1 was mixed at 14 cm/s and C-2 was mixed at 30 cm/s. Both channels were loaded with 1,700 L of screened mixed bed reactor effluent weekly. Dominant algal genera were *Chlorocella* (70%) and *Scenedesmus* (14%).

Initial chl *a* concentrations in C-1 and C-2 were 7.8 $\mu\text{g}/\text{m}^3$ and 4.8 $\mu\text{g}/\text{m}^3$, respectively.

In exp. 10, the biofouling channels were filled to a depth of 15 cm with medium from the high-rate pond. C-1 was mixed at 10 cm/s and C-2 was mixed at 14 cm/s. Both channels were loaded with 1,000 L of screened fixed bed reactor effluent weekly. Dominant algal genera were *Synedra* (93%) and *Chlorococcum* (17%) initially. Initial chl *a* concentrations in C-1 and C-2 were 7.8 $\mu\text{g}/\text{m}^3$ and 4.8 $\mu\text{g}/\text{m}^3$, respectively.

1.1.2.3 Effect of Waste Loading Rates: 5, 8, 10, and 11

The effect of waste loading was investigated in 4 experiments. Loaded cultures were compared to non-loaded cultures in exp. 8 and 11. Different waste loading rates were evaluated in exp. 9. The influence of waste pretreatment was studied in exp. 10.

In exp. 9, culture medium from C-1 was used to fill the biofouling channels to an initial depth of 17 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 10 cm/s. The major algal genus was *Synedra*. Initial chl *a* concentrations in C-1 and C-2 were 4.1 $\mu\text{g}/\text{m}^3$ and 4.8 $\mu\text{g}/\text{m}^3$, respectively.

In exp. 11, culture medium from the high-rate pond was used to fill the biofouling channels to an initial depth of 17 cm. C-2 was loaded with 1,000 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 85% *Chlorella*, 17% *Spirulina*, and 9% *Thiospirillum* sp. Initial chl *a* concentrations in C-1 and C-2 were 15.3 g/m³ and 17.8 g/m³, respectively.

In exp. 8, culture media obtained from the high-rate pond was transferred to C-1 and C-2, giving each an initial depth of 25 cm. C-1 and C-2 were loaded with 500 L and 1,000 L, respectively, of screened FBR effluent weekly. Both channels were mixed at 14 cm/s. The dominant algal species was *Synachloristia*. Initial chl *a* concentration was 5.8 g/m³.

In exp. 10, culture media from the facultative pond and high-rate pond were used in relative proportions of 70% (70 cm) and 30% (30 cm) to fill the bioflocculation channels to a depth of 25 cm. C-1 and C-2 were loaded three times weekly with 500 L of settled waste supernatant and with 500 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 42% *Chlorella*, 44% *Spirulina* and 14% *Thiospirillum* sp. Initial chl *a* concentrations in C-1 and C-2 were 8.3 g/m³ and 8.3 g/m³, respectively.

1.3.1.3 Effect of Algal Biofilm and Bacterial Seeding-- Exp. 7, 9 and 11

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

seeded culture in exp. 3, and activated sludge seeded culture and non-seeded culture in exp. 14 were compared.

After termination of exp. 4, 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-2 was allowed for 18 h. C-2 was then filled to a depth of 11 cm (20% of culture volume) with supernatant from C-1. Additional C-2 supernatant was discarded until a depth of 11 cm remained, containing most of the biofloculated sludge from experiment 4. Both biofloculation channels were then brought to a depth of 24 cm by adding culture medium from C-1. The channels were sized at 14 cm²/s. Each received 500 L of screened fixed bed reactor effluent. Dominant algal genera were *Chlorocella* and *Synechococcus* initially. The relative proportions of these two genera were 10% and 90%, respectively. Initial chl *a* concentrations in C-1 and C-2 were 1.2 g/m³ and 1.8 g/m³, respectively.

Experiment 5 was begun with culture medium carried over from exp. 4. No new medium from the facultative or high-rate ponds was added. *Anaerobis lignum* effluent, containing a dense population of the purple sulfur bacteria, *Thioploa* sp., was added to C-2 on two occasions during the experiment. Initial depths in C-1 and C-2 were 28 cm and 20 cm, respectively. Both channels were sized at 14 cm²/s. Each received 500 L of screened fixed bed reactor effluent weekly. Dominant algal genera were *Chlorocella* and *Synechococcus*. The relative proportions of these two genera were 10% and

oil, respectively. Initial oil g concentrations were 4.1 g/m³ in both channels.

Culture medium from the fermentative pond was used to fill the bioflocculation channels to a depth of 24 cm at the start of experiment 18. C-3 received 180 L of activated sludge from a municipal waste treatment plant (8.7% solids). Both channels were mixed at 10 cm/s and each received 1,000 L of fixed bed reactor effluent on two occasions. Dominant algal genera were *Chlorella* and *Monodina* throughout the experiment. Initial oil g concentration was 5.5 g/m³ in each channel.

3.3.3.4 Effect of *Carbonate-supplementation* on *Oil*

After termination of exp. 1, 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 fermentative pond, was split between the bioflocculation channels, giving initial depths of 24 cm and 17 cm for C-1 and C-2 respectively. The relative proportions of medium from C-2 and the fermentative pond were 83% and 17%, respectively. Fixed bed reactor effluent was added to the channels on two occasions. Oil was dosed with 21.5 kg sodium carbonate to give an added carbonate-carbon concentration of 10 g/m³. Both channels were mixed at 10 cm/s. Initial oil g concentrations were 1.3 g/m³ in both channels. Dominant algal genera were *Chlorella* and *Monodina*.

3.3-3.5 Effect of pH on algae

A mixture of 40% 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 4.5 or less, whereas the pH of C-1 was not controlled. Both channels were mixed at 14 cm/s. Each recovered 400 L of screened FBR effluent weekly. Major algal species were *Chlorella* (45%) and *Scenedesmus acuminatus* (24%). Initial chl *a* concentrations of C-1 and C-2 were 5.5 g/m³ and 3.4 g/m³, 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 0.25 ml was placed on a glass slide and flattened with a 32 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 counted. Counts were expressed as number of cells per Whipple Grid. Given an average liquid layer thickness of 180 μ m slip and slide and the 180 μ m x 180 μ m dimensions of the Whipple Grid (at 400x), a count of 1 cell per grid corresponds to 1.4×10^6 cells per L. Counts of algae other

then chlorophyll *a* were normalized to chlorophyll *a* 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 (1962). A 25 mL sample was centrifuged for 10 min at 2,400 x g and supernate poured off. Then, 10 mL of boiling 70% 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 10 °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:

$$\text{Chl } a = 11.8 \times (D_{665} - D_{750}) \times (v/V) \quad (3-10)$$

where chl *a* has units of g/L, 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 Viability and Estimation of Volatile Suspended Solids

Viability was measured using a 10 cm diameter petri

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.

Visible suspended solids concentration of the cultured algae and bacterial biomass was calculated from visibility measurements based on the empirical formula of Lincoln and Hill (1982):

$$\text{VSS } [g/m^3] = 180/d \quad (3-3)$$

where d = the depth of visibility expressed in inches.

3.4.4 Sedimentability

Sedimentability of algae was determined by retaining samples in a 1 L beaker over a 24 hr period in the absence of light. Adherence of algal cells to the sides was minimized by gently rubbing 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 Kasper et al. (1974). Sample pH was adjusted to 8.0 with 0.1 N NaOH. Triplicate 10 mL aliquots were assayed with 1.0 mL of 0.1% 2-[p-iodophenyl]-3-(p-nitrophenyl)-5- phenyltetrazolium chloride (IPT) (Eastman Kodak) and incubated at room temperature (18 ± 2 °C) for 1 hr in the dark. Fixing was

provided by a hematology mixer. Incubation was terminated by adding 1.8 ml 1% formaldehyde. IST-formazan (ISTF) formed during the incubation period was extracted according to the following procedure. A 10 ml IST-treated sample was placed in a centrifuge tube and centrifuged at 2,100 x g for 20 min. Supernate was decanted, leaving a pellet approximately 2.1 ml in volume, and replaced with 10 ml mixture of 4:6 tetrachloroethylene/acetone. The tube was capped and vortexed for 10 sec. Extraction was continued in the dark for 10 min. Extract was clarified by centrifugation at 2,100 x g for 20 min and optical density of the extract was determined at 570 nm. IST-dehydrogenase activity (IST-DHA) was calculated according to the following equation:

$$\text{IST-DHA} = 1000 \text{ } \bar{E}_{570} \text{ } V / V \times F \quad (1-2)$$

where IST-DHA is expressed in units of equivalent oxygen uptake ($\mu\text{g O}_2/\text{m}^3\cdot\text{h}$), $\bar{E}_{570}$ = absorbance at 570 nm, V = volume of supernate (ml), V = volume of sample (ml), t = incubation time (min), and F = factor to account for sample dilution by IST and formaldehyde (0.833).

3.4.4 Residue, Organic Matter and Nutrients

Analyses of residue and organic matter were carried out according to APHA (1995). These included total solids (TSS), volatile solids (VSS), total suspended solids (TSS), volatile suspended solids (VSS), biochemical oxygen

demanded (0012, 002), and chemical oxygen demanded (0044). Total Kjeldahl nitrogen and ammonia nitrogen were measured by the distillation (00412) and digestion (00415) procedures described in the EPA (1974). Total phosphorus was determined by the persulfate digestion procedure (00445) of EPA (1974).

3.4.7 pH, Dissolved Oxygen, and Optical Density

The pH was monitored by an electrode andpacer system (4412, 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, Bausch and Lomb, Rochester, NY).

CHAPTER 4 RESULTS OF LABORATORY EXPERIMENTS

4.1 Effect of pH

The effect of pH on bioflocculation was investigated in 4 experiments. Algal species tested were *Chlorella* in exp. 1 and 2 and *Spirulina* in exp. 3 and 4.

Under illuminated conditions of exp. 1, the stimulatory effect on the growth of *Chlorella* was observed in the culture adjusted to pH 7.0. Counts of these algae in the culture adjusted to pH 7.0 fell to less than 1st of the initial count within 4 days, whereas those in the culture adjusted to pH 8.0 declined very slowly over the period of experiment. Complete bioflocculation marked by cell aggregation and rapid settling of cells occurred within 4 days and 80% removal of algae was obtained in 8 days in the culture adjusted to pH 7.0. 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 4 days. A substantial fraction of the bacterial suspension was associated with these flocs. In contrast, the cultures adjusted to pH 7.0 and 8.0 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 3.5 and 4.5. In contrast, the cultures adjusted to pH 7.5 and control showed no sign of flocculation. Macroscopic flocs became visible after 7 days and these were more compact than those developed in exp. 1. They appeared as a predominantly tan-green to orange-brown matrix of *Chlorella* cells, not bleached noticeably with time. A substantial bacterial component was present in these flocs. Bacterial signal recoverable by 2 h sedimentation showed 81% on day 1 in the culture adjusted to pH 4.5 and 70% on day 11 in the culture adjusted to pH 3.5 (Fig. 4-4). The time when this phenomenon occurred was well correlated with the densifier 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. 1. The control grew well for first 12 days then declined rapidly. Declines in cell numbers in the cultures adjusted to pH 3.5 and 4.5 were most rapid between 8 and 12 days.

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

In exp. 3 and 4, 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. 4-5,

4-4). The extent of cell decline in the cultures 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, fine particles became visible after 4 days and appeared to have a tan-green to orange-brown matrix with *Synochrysis* cells as the major component. *Chlorella* and *Monodina* contributed approximately 5% of the total biomass. Maximum algal removal by 2 h sedimentation showed 48% and 18% in the cultures adjusted to pH 5.5 and 7.5, respectively, on day 8 (Fig. 4-4). OHA decreased as the algal growth decreased.

Effect of pH on maximum algal removal and corresponding OHA and chl *a* levels in cultures initially dominated by *Chlorella* (exp. 7, 8) and *Synochrysis* (exp. 7, 8) is summarized in Table 4-1.

4.3 Effect of Mixing

The effect of mixing on bioaccumulation was investigated in exp. 5 and exp. 6. Three levels of mixing intensities, 20 rev/min, 40 rev/min, and 60 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 *Monodina*. Then declines gradually after 8 days.

Figure 4-1 Effect of pH on temporal variation of antibiotic activity, DMS and chl a in a culture initially dominated by *Chlorococcum* sp. J.

Figure 4-2 Effect of pH on temporal variation of cell viability, ORA and chl a in a culture initially dominated by *Chlorella* sp. 1.

Figure 4-3 Effect of pH on temporal variation of cell density, cell volume and cell weight in a culture dominated by *Synchocystis*, exp. 7.

Figure 4.4 Effect of pH on temporal variation of cell viability, OMA and OMA-g in a culture initially dominated by *Synchytrium*, sp. 8.

Table 2.1 Effect of pH on maximum algae removal and corresponding levels of BSA and Chl. *a* levels in cultures initially dominated by *Chlorella* (exp. 2, 3) and *Synechococcus* (exp. 7, 8)

Exp.	pH	Max. algae removal, %	BSA, $\mu\text{g O}_2/\text{ml}\cdot\text{d}$	Chl. <i>a</i> , $\mu\text{g}/\text{ml}$
2	5.5	88.8	24.52	5.35
	7.5	7.4	8.82	17.85
	8.5	2.8	13.82	1.35
3	8.7 ^{***}	7.2	7.36	2.81
	8.5	38.8	18.56	1.85
	8.3	82.8	7.36	3.73
	7.5	3.2	6.56	7.55
7	5.5	7.2	3.17	3.88
	6.5	58.8	8.18	3.88
	7.5	12.2	7.58	3.88
	8.5	4.8	3.87	8.28
8	7.5 ^{***}	12.2	18.68	8.88
	8.5	13.2	3.38	3.48
	8.5	88.8	8.38	1.35
	7.5	27.5	7.42	8.88

^{***}pH of the control when maximum algae removal was obtained

The best growth rate with a maximum chl *a* concentration of 20 g/g^2 was observed in the culture mixed at 40 rev/min, whereas $15 \text{ g chl } a/\text{g}^2$ was the maximum in the control after 4 days.

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

The production of settleable solids in the mixed cultures increased progressively in the order of mixing intensities, 20 rev/min, 40 rev/min, and 60 rev/min, whereas this parameter in the control increased very slowly or remained stationary. Algal removal and settled solids values in the culture mixed at 40 rev/min were greater than those in the control (max. 10% vs 20% and 45 mg/L vs 14 mg/L) (Fig. 4-2).

The pH dropped gradually in all the cultures, initially, but declined to pH 4.8 rapidly in the cultures mixed at 40 rev/min and 60 rev/min after 4 days. The time when pH dropped coincided with the period of the extensive biofloculation (Fig. 4-2, 4-3).

Figure 4-5 Effect of mixing on temporal variation of cell number, pH and chl. a in cultures initially dominated by *Chlorella* and *Microcystis*, exp. 5.

In exp. 4, complete bioflocculation was repeated but changes in the characteristics of the mixed cultures were less pronounced, compared to those in exp. 3. The bright-green colonies of *Chlorella* and *Synechococcus* cells were less compact 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. 80% vs 24%). A higher growth rate was also observed in the culture mixed at 40 rev/min. Maximum chl *a* concentrations of 18 g/g² was obtained in the culture mixed at 40 rev/min, whereas the maximum chl *a* was 17 g/g² in the control (Fig. 4-4).

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 50% below saturation after 4 days. The pH in the mixed cultures showed a rapid decline from 8.5 to 6.8 over days 2-4 but they remained at a pH below 6.0 after 10 days (Fig. 4-5). It was observed that the extensive bioflocculation occurred when DO dropped below saturation and pH went down below 6.0.

Growth characteristics of *Synechococcus* 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 4-4 Effect of mixing on temporal variations of cell number, dry weight, pH and optical density in cultures initially dominated by *Chlorella* and *Pseudocapsa*, exp. 8.

In all cultures,

Effect of mixing intensity on maximum cell density and corresponding levels of pH and dO_2 in cultures initially dominated by *Chlorella* and *Spirulina* (exp. 8, 9) was summarized in Table 4-2.

4.2 Effect of Waste Loading

The effect of waste loading was investigated in 3 experiments. Different waste loading rates were evaluated in exp. 4 and 5. The influence of waste type was studied in exp. 11. Algal genera dominant during investigations were *Chlorella* and *Spirulina* in exp. 4, 11 and *Synechocystis* in exp. 5.

4.2.1 Waste loading rate

In exp. 4, no significant difference in growth rates among the cultures was observed. The cultures loaded with FWS effluent weekly at 25 and 45 of media volume grew better than the control. Waste loaded cultures generally formed flocs earlier than the control. Flocs less than 100 μm across in diameter were visible after 8 days in waste loaded cultures, but these showed poor settleability. A substantial bacterial component was identified in the floc structure. After 16 days, 40% algal removal was obtained in the culture loaded with FWS effluent weekly at 45 of the media volume, compared to 15% removal achieved in the control on day 16 (Fig. 4-7).

The pH dropped gradually in all the cultures initially, declined rapidly over days 4-8, then remained near pH 8.0

Table 4-2 Effect of mixing on maximum settleability and corresponding levels of pH and Ca^{++} in cultures initially dominated by *Chlorella* and *Monodhan*, exp. 3, 4

Exp.	Mixing speed, rpm/min	Res. settled volume, ml/L	Sec algae removed, %	pH	Ca^{++} $\mu\text{mole/l}$
3	0	24	86	8.91	12.76
	20	38	85	7.97	14.42
	40	45	82	8.36	18.02
	60	55	72	8.27	19.14
4	0	33	38	8.45	28.38
	20	39	57	8.45	17.82
	40	49	68	8.58	22.56
	60	52	47	8.55	16.77

Figure 4-7 Effect of waste loading on temporal variation of water quality, DO, pH and alkalinity to estuaries initially dominated by *Chlorella*, and *Monodon*, exp. 4.

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

In exp. 8, the effect of waste loading rate on biofloculation was investigated with *Synochloris*. Growth at this stage in all the cultures declined gradually during the experiment. The extent of decline in heavily waste loaded cultures (pH of culture values) was somewhat less than those in other cultures. Two-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 10% achieved in the control on day 8. DMA was also declined progressively (Fig. 4-8).

Effect of waste loading rate on maximum algal removal and corresponding levels of DMA, pH and chl *a* in cultures initially dominated by *Chlorella* and *Monodhan* (exp. 9) and *Synochloris* (exp. 7) was summarized in Table 4-1.

4.3.2 Waste Pre-treatment

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

Figure 4-6 Effect of waste loading on temporal variation of ammonia, ORN, pH and chl. a, in cultures initially dominated by *Synechococcus*, exp. 9.

Table 4-3 Effect of waste loading on maximum sludge removal and corresponding levels of BOD, pH and O₂ in cultures initially dominated by *Chlorella* and *Spirulina* (exp. 4) and *Synsphaerotilus* (exp. 5)

Exp.	Waste loading rate, g/l BOD/day	Max. sludge removal, %	max. O_2 , g/m ³ -d	pH	Chl. a, g/m ³
4	0	85	11.12	8.85	8.28
	4	20	12.78	8.87	10.23
	12	38	14.88	8.95	8.81
	24	42	17.22	8.98	9.12
5	0	15	8.71	7.92	1.08
	4	28	9.84	7.82	1.38
	12	21	14.12	7.85	1.48
	24	68	15.72	7.82	2.88

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 *Monodina* with a small bacterial component. *Saccharothrix* and *Spinidium* sometimes were associated with flocs formed. They were easily identified by 18S uptake. Fia flocs were developed but they did not grow further and showed poor settleability. Maximum algal removal of the cultures loaded with FFA effluent and settled waste supernatant were 48% and 41%, respectively on day 10. In contrast, 18% algal removal was obtained in the control (Fig. 4-6, Table 4-3).

WGA 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 Loading

In exp. 18, each culture received different levels of activated sludge as 0%, 12% and 20% of algal weight, whereas the control did not receive any. *Chlorella* and *Monodina* 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 3 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 4-2 EFFECT of waste pretreatment on temporal variation of settleability, O&A, pH and redox in waste loaded columns initially dominated by *Chlorella* and *Spirulina*, exp. 11.

Table 1-4 Effect of waste pretreatment on maximum algae growth and corresponding levels of bio, pH and Chl *a* in cultures initially dominated by *Chlorella* and *Scenedesmus*, exp. 11

Exp. ^{aa}	Waste pretreatment	Max. algae removal, %	Chl. <i>a</i> , $\mu\text{g}/\text{ml}$ -d	pH	Chl <i>a</i> , $\mu\text{g}/\text{ml}$
11	control	17	10.81	8.39	1.07
	SBR	45	20.84	8.61	1.53
	FBR	46	14.63	7.99	1.18

^{aa} Control: waste not loaded
 SBR: settled waste supernatant
 FBR: fixed bed reactor effluent

of *Chlorella* and *Spirulina* to *Flora* was noticeable. Though full bioflocculation was not achieved, 45% algal removal was obtained in the culture seeded at 10% of algal weight on day 1. In contrast, the control showed 25% algal removal (Fig. 4-18, Table 4-3).

The pH was gradually decreased in all the cultures. pH was proportionately higher in the heavily seeded cultures. Maximum DOB obtained was $17 \text{ g O}_2/\text{m}^3\text{-d}$.

4.3 Effect of Other Variables

4.3.1 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. Algal removals of 85% and 95% were obtained in cultures added to 50 g carbon/m^3 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.

4.3.2 Effect of Algal Density

Three different initial algal concentrations (rows 1-3

Figure 4-12 Effect of activated sludge seeding on temporal variation of settleability, O_2 , pH and chl. a in cultures initially dominated by *Chlorella* and *Clostridium*, exp. 18.

Table 4-5 Effect of activated sludge loading on various algae removal and corresponding levels of O_2 , pH and Ca^{++} in cultures initially dominated by *Chlorella* and *Synchloa*, exp. 11

Exp. no	Al loading, % of algae dry weight	Alg. algae removal, %	O_2 g O_2 /m ³ ·d	pH	Ca^{++} g /m ³
11	0	24	14.12	8.35	6.84
	5	25	15.82	8.45	7.52
	10	45	24.42	8.48	7.75
	20	45	30.88	8.43	7.47

^{no} = Activated sludge

7 chl a/b^2 , intermediate; 4.8 μ chl a/b^2 , and high 8.8 μ chl a/b^2) were employed in cultures dominated by *Chlorella* and *Scenedesmus*. The culture with high algal density showed very slow growth rate initially, then declined rapidly after 4 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 associated in the cultures with high and intermediate algal-density after 4 days. The film density was much greater in the high algal-density culture. No sign of filamentation 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 (mean 42% vs 14%).

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

CHAPTER 2 RESULTS OF FIELD EXPERIMENTS

2.1 Integrated System Operation

The quality characteristics of waste streams added to the aigai cultures and operational data for the field system are given in this section. Climatological data are given in section 2.2. Details of field-scale biofloculation experiments are described in section 2.3.

2.1.1 Settling Tank and Supernatant

Quality characteristics of settled waste solids and settled waste supernatant from the settling tank are given in Tables 2-1 and 2-2, respectively. The total solids (TS) content of settled waste solids was variable with season. Concentrations were highest in winter (e.g., 47,500 g/g³ in January 1988) and lowest in summer (e.g., 4,000 g/g³ in June 1989). This tendency was attributed to the use of cooling sprays for the animals with concomitant dilutions of the raw waste stream during the summer. The TS of settled waste solids averaged 17,500 g/g³. Volatile solids (VS) were approximately 74% of TS. Chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) averaged 14,740 g/g³ and 1,417 g/g³, respectively, giving a COD:TKN ratio of 10.4.

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

Table B-1. Monthly average characteristics of settled waste solids^a

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Oct. 83	29 800	14 800	95 400	1 800	1 200
Nov	24 800	14 100	82 800	3 800	3 300
Dec	---	---	---	---	---
Jan. 84	27 800	14 110	14 800	2 800	2 800
Feb	17 800	12 800	14 800	3 400	1 600
Mar	14 800	14 200	40 200	3 200	1 400
Apr	18 200	11 200	42 400	1 800	1 800
May	8 800	8 800	14 800	1 100	000
Jun	7 100	4 100	28 800	1 200	000
Jul	13 400	8 800	27 800	1 200	000
Aug	8 800	4 400	28 800	000	000
Sep	13 800	10 800	27 800	1 300	100
Oct	10 100	14 800	51 800	3 000	1 100
Nov	13 800	18 800	48 400	8 000	1 800
Dec	14 400	18 800	50 800	3 800	2 800
Jan. 85	12 400	14 800	40 800	3 800	3 800
Feb	14 800	13 800	48 800	3 200	1 700
Mar	14 400	14 800	30 200	3 800	2 200
Apr	15 200	18 800	48 800	1 800	1 800
May	13 800	18 200	34 200	1 400	000
Jun	4 800	8 800	8 800	000	000
Jul	8 800	8 800	14 200	000	000
Aug	8 800	3 800	7 800	000	000
Sep	10 800	7 800	14 400	1 200	000
Oct ^{bb}	7 800	4 800	8 800	1 200	1 200
Nov	13 800	12 200	38 200	1 800	3 800
S.O.	18 000	8 800	17 800	000	100

^aValues in g/m³^{bb}Aerobic digester stop running at Oct 27, 1984

SOURCE: E. Nordstedt, Personal communication, 1984, unpublished data.

Table 1-2. Monthly average characterization of settled waste supernatant^a

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb 84	18 480	8 838	28 888	1 876	1 480
Mar	18 820	7 888	33 842	2 288	1 480
Apr	20 810	7 132	37 888	2 882	1 880
May	22 860	8 128	38 488	3 488	2 220
Jun	22 810	8 882	38 588	2 482	1 880
Jul	8 780	4 188	8 472	1 120	882
Aug	7 810	1 882	5 438	887	888
Sep	7 810	4 888	18 842	988	742
Oct	11 810	7 718	28 742	1 482	1 188
Nov	12 218	11 888	37 788	1 818	1 188
Dec	18 818	28 828	48 818	2 478	1 888
Jan 85	12 858	11 118	42 128	2 478	2 288
Feb	18 428	12 878	32 888	2 888	2 828
Mar	18 478	11 428	41 888	2 488	1 888
Apr	17 878	12 488	38 888	2 488	2 888
May	4 728	4 482	18 188	1 128	888
Jun	2 488	2 128	4 288	478	282
Jul	8 788	2 428	8 188	828	842
Aug	2 822	2 128	2 422	888	872
Sep	4 827	2 828	8 778	1 888	888
Oct	4 888	2 787	5 281	1 884	888
Nov	8 112	2 828	8 888	788	888
Dec	7 482	8 888	12 821	1 128	881
Mean	8 782	6 712	21 828	1 882	1 188
S.D.	5 187	1 818	14 278	788	228

^aValues in g/m³

Source: R. Svardstedt, Personal communication, 1984, unpublished data.

approximately 348 and 326, respectively, of the settled solids fraction in terms of VS and VS₅. Its average concentrations of COD and VS were 434 and 315, respectively, of those in the solids fraction. The average COD:VS ratio of settled waste supernatant was 14.1.

The volumetric feed rate of settled waste solids to the conventional digester was initially 1.6 m³/d (Oct. 13-Dec. 84) and was raised subsequently to 2.2 m³/d (Dec. 14-Oct. 88), corresponding liquid detention times were 22 and 15 days, respectively. Mean organic loading was 0.81 kg VS added daily per m³ of liquid volume (kg VS₅/m³d), as shown in Table 3-2. Digester temperature ranged from 13.5-23.7 °C, averaging 22.6 °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 1.46 m³/d during Jan. 1984-Oct. 1985 and 1.6 m³/d during Nov.-Dec. 1985, giving corresponding liquid detention times of 16.5 days and 8.3 days, respectively. Organic loading rates ranged from 0.100-1.317 kg VS₅/m³d, averaging 0.56 kg VS₅/m³d (Table 3-3). The operating temperature ranged from 19.4-22.4 °C, averaging 20.5 °C.

Monthly average characteristics of effluents from the conventional digester and fixed bed reactor are given in Tables 3-4 and 3-5. Pollutant levels in both waste streams were lowest in the summer and greatest in winter, reflecting the

Table 1-1. Loading and temperature of anaerobic digester and fixed bed reactor.

Month	Anaerobic digester		Fixed bed reactor	
	Loading,	Temp.,	Loading, ^a	Temp.,
	kg VS ₂ /m ³ d	°C	kg VS ₂ /m ³ d	°C
Oct 83	0.381	24.4	---	---
Nov	0.870	22.4	---	---
Dec	(8)	13.4 ^b	---	---
Jan 84	1.840	22.5	---	---
Feb	0.837	24.5	1.344	19.4 ^c
Mar	0.714	22.8	1.212	17.2
Apr	0.542	21.4	1.342	24.2
May	0.542	26.7	1.262	27.2
Jun	0.544	26.5	0.272	26.5
Jul	0.488	26.8	0.708	28.8
Aug	0.582	26.5	0.508	26.4
Sep	0.887	27.4	0.480	28.2
Oct	1.134	28.4	1.124	27.2
Nov	1.078	18.8	1.888	22.1
Dec	0.578	12.8	1.772	18.2
Jan 85	0.864	18.4	2.182	16.2
Feb	0.427	18.2	1.817	12.4
Mar	0.882	22.2	0.860	22.4
Apr	0.870	24.2	1.458	25.2
May	0.804	27.8	0.818	26.8
Jun	0.178	28.7	0.278	26.8
Jul	0.278	27.2	0.628	27.2
Aug	0.282	27.4	0.274	27.5
Sep	0.428	28.8	0.388	28.8
Oct	0.328	28.4	0.322	24.2
Nov	(8)	---	0.882	22.4
Dec	(8)	---	1.268	12.2
Mean	0.412	22.8	0.832	22.8
S.D.	0.277	5.2	0.278	5.2

^aEmpty bed volume basis

Digester not operated during this period

^bAmbient temperature

Source: R. Goodland, Personal communication, 1988, unpublished data.

Table 3-4. Monthly average characteristics of municipal disposable effluent^a

Month	Total Sulphur	Volatile Solids	Chemical Oxygen Demand	Total Fixed-Solids Nitrogen	Ammonia Nitrogen
Oct. 83	32 068	25 778	34 430	2 468	2 422
Nov	31 038	25 088	34 348	2 338	2 380
Dec	30 008	24 008	34 008	2 208	2 208
Jan. 84	34 848	27 738	37 848	2 828	2 828
Feb	38 458	30 738	40 888	3 488	3 748
Mar	38 768	30 778	41 888	3 438	3 888
Apr	38 378	32 388	38 388	3 858	3 888
May	39 728	3 388	32 838	3 358	3 388
Jun	4 048	3 458	15 488	1 308	1 448
Jul	4 438	4 878	8 738	1 388	1 338
Aug	4 438	3 878	8 888	1 388	1 428
Sep	7 588	4 818	7 348	888	888
Oct	13 188	8 808	24 848	1 488	2 068
Nov	13 188	10 888	14 958	1 428	2 188
Dec	18 148	13 338	24 878	2 388	2 488
Jan. 85	14 338	11 878	14 888	2 448	2 888
Feb	18 038	14 578	20 338	2 468	2 888
Mar	20 388	14 788	21 538	2 888	2 328
Apr	18 858	13 448	22 488	2 848	2 828
May	13 388	8 888	14 488	1 888	1 788
Jun	3 888	3 438	4 438	8 28	378
Jul	3 888	3 428	8 878	821	718
Aug	4 817	3 838	8 438	888	748
Sep	7 463	4 388	8 388	1 818	1 448
Oct	4 884	4 428	8 888	1 828	2 878
Mean	22 872	21 348	28 352	1 968	1 482
S.D.	9 428	7 328	13 368	874	438

^aValues in g/m³

Source: R. McDuffett, Personal communication, 1984, unpublished data.

Table 2-6. Monthly average characteristics of fixed bed reactor effluent^a

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb	18 440	8 830	23 130	1 870	1 700
Mar	20 820	7 450	23 050	2 320	1 410
Apr	20 510	7 330	18 880	1 580	1 340
May	20 510	8 320	25 480	1 680	1 230
Jun	23 218	8 800	28 380	1 400	1 020
Jul	8 760	4 380	8 870	1 110	980
Aug	3 610	1 940	2 810	930	880
Sep	3 230	1 640	2 870	720	680
Oct	7 880	3 100	10 720	1 380	1 130
Nov	16 368	7 800	14 210	1 480	2 870
Dec	13 820	6 410	17 880	2 170	1 820
Jan 85	13 450	7 880	21 430	2 580	2 140
Feb	18 300	8 420	17 840	2 250	1 780
Mar	13 940	7 280	14 450	2 550	1 890
Apr	14 080	8 460	22 730	2 880	1 930
May	4 880	2 180	8 830	1 590	1 360
Jun	8 880	401	8 840	884	814
Jul	3 640	1 340	3 790	540	480
Aug	3 880	1 480	2 424	774	480
Sep	3 840	1 291	2 833	893	834
Oct	3 410	1 880	2 750	1 033	980
Nov	4 130	1 770	2 287	487	340
Dec	4 180	2 580	2 253	1 130	1 840
Mean	7 827	4 764	17 890	2 074	1 280
S. D.	4 978	2 088	18 521	857	486

^aValues in g/m³

Source: R. Hardstedt, personal communication, 1988, unpublished data.

variation of effluent streams. TO ranged from 5,500-25,400 g/m^3 in digester effluent and from 1,040-14,000 g/m^3 in FTS effluent, averaging 18,470 g/m^3 and 7,837 g/m^3 , respectively.

Respectively, COD and TSS in digester effluent averaged 18,813 g/m^3 and 1,343 g/m^3 , respectively. The corresponding two parameters in FTS effluent averaged 13,487 g/m^3 and 1,474 g/m^3 , respectively. Mean COD:TSR ratios were 18.5 in digester effluent and 5.3 in FTS effluent.

5.1.2 Immunology Lagoon and Facultative Pond

The anaerobic lagoon functioned as a continuous culture, predominantly of the purple sulfur bacterium (*Chromatium*). This media mass, 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 evidence is attributed to the oxidation of H_2S to elemental sulfur by this *C. roseum*.

As shown in Table 5-4, mean COD and TSS concentrations were reduced to 1,340 g/m^3 and 400 g/m^3 , respectively. Mean effluent phosphorus concentration was 120 g/m^3 .

The phototrophic 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 5-4).

5.1.3 Biofloculation Channel

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

Table 3-4 Age, sex, and characteristics of aspen, larch, and
fir forests (and efficiency)

Stage	Overlaid forest density (g/m^2)	Recolonized density (g/m^2)	Total sapling density (g/m^2)	Volume sapling density (g/m^3)	Total cylinder volume, (m^3/ha)	Total above- ground, (g/ha)	No. of samples
Aspen, 1	200 (100)	200 (100)	1 200 (120)	817 (204)	408 (475)	119 (100)	19
Fir, 1	140	—	—	—	111 (475)	9 (100)	27

*Normalized at invasion given in 4.1

most significant feed sources for biofilm/aerobic channels. Waste loading to the biofilm/aeration channels was varied according to the experiment in progress (Table 3-7). 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.

Acidification populations were controlled by addition of equal volumes of ammonium hydroxide to the channel on an as-needed basis (Table 3-8). In experiment 8, ammonium hydroxide was not added to the experimental culture (C-2) in order to avoid raising the 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 3-8, as well as variations in the initial total ammonia concentration, which were not assessed.

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 4.0 (Table 3-9). The target pH was sometimes undershot, however, dropping the pH below 4.5 in several instances.

3.1.4 High-rate Pond

Alternative feed sources for the high-rate pond (D-4) included effluents from the facultative pond, anaerobic digester, and fixed bed reactor (Table 3-10). Facultative pond (F-3) effluent (average 2.15 m^3/d) was the most

Table 3-7. Waste loading to biodegradation channels*

Exp.	C-1		C-2	
	Source	Rate, L/d	Source	Rate, L/d
1	STD	21	STD	21
2	STD	3	STD	3
	FSD	9	FSD	9
3	FSD	13	FSD	13
4	--	8	--	8
5	--	8	FSD	140
6	FSD	32	FSD	207
7	FSD	88	FSD	88
8	FSD	38	FSD	38
9	FSD	8	B-1	138
			FSD	25
10	FSD	188	FSD	188
11	FSD	180	--	0
12	FSD	180	FSD	180
13	FSD	83	FSD	83
14	FSD	67	FSD	67

*Abbreviations for waste sources:
 STD = settled waste supernatant
 STD = anaerobic digester
 FSD = fixed bed reactor
 B-1 = anaerobic lagoon
 B-2 = facultative lagoon

Table 3-8. Ammonium hydroxide additions to biofloculation channels for growth control

Exp.	Day	C-1			C-2		
		Added NH ₄ OH g N/L	pH	Total NH ₄ ⁺ g N/L	Added NH ₄ OH g N/L	pH	Total NH ₄ ⁺ g N/L
1	50	56	9.3	38	44	9.3	28
	88	37	8.6	7	37	8.4	28
2	51	25	9.7	18	24	9.9	19
	41	58	10.1	49	41	10.5	38
3	54	33	9.0	13	25	9.3	18
4	-1	20	9.4	7	20	9.0	7
	1	20	9.0	7	20	9.1	8
	18	28	9.3	18	25	9.8	30
5	1	35	8.9	11	37	8.5	11
	18	38	8.4	23	38	8.9	12
	28	54	9.4	25	57	9.0	20
6	5	43	8.9	9	44	8.3	7
8	-3	54	9.1	25	58	9.1	21
	12	138	9.3	78	---	---	---
11	4	45	8.8	18	48	9.1	18
12	-1	37	9.7	15	37	9.2	13
13	8	32	8.5	8	32	8.5	8
14	5	46	9.1	18	47	8.1	18

^aContributed directly by added NH₄OH

Table 3-9. Sulfuric acid additions to channel 0-2 during experiment 8

Day	Volume, l	Initial pH	Final pH
0	11.0	6.7	6.3
1	4.0	7.3	6.8
3	4.0	7.8	6.1
4	4.0	7.6	5.9
6	3.0	7.1	6.1
8	1.0	6.9	6.3
12	6.7	7.5	6.8
16	6.8	7.3	6.3
17	1.0	6.6	5.8
20	6.8	7.1	--
26	7.5	6.1	6.5

Table 2-12. Waste loading to high-rate pond^a

Month	Waste source			
	Final. pond	Appt. dig.	Fixed bed aeras.	Other
Dec. 83	---	0.13	---	---
Nov	6.57	0.27	---	---
Oct	---	---	---	---
Sept. 84	---	0.12	0.20	---
Feb	---	---	0.28	---
Mar	---	---	0.75	0.12 ^b
Apr	---	---	0.20	---
May	7.52	---	0.45	---
Jun	0.40	---	0.47	---
Jul	---	---	0.78	---
Aug	---	---	0.55	---
Sept	0.08	---	0.42	---
Oct	0.47	---	0.27	---
Nov	4.08	---	0.25	0.49 ^b
Dec	1.08	---	0.42	---
Jan. 85	0.97	---	0.58	0.97 ^b
Feb	0.31	---	0.74	---
Mar	0.97	---	0.80	---
Apr	0.28	---	0.08	---
May	---	0.74	1.06	1.06 ^b
Jun	---	---	0.93	---
Jul	0.81	---	0.64	---
Aug	---	0.09	0.72	---
Sept	---	0.20	0.57	---
Oct	0.17	0.83	0.78	---
Nov	1.80	---	1.43	---
Dec	---	---	2.02	---
Mean	2.23	0.08	0.95	0.12

^akg/d^bkg/yr^cWastewater lagoon^dWetland waste supernatant

important food source as a vegetative basis followed by FES effluent (8.33 g²/d). When organic loading rate in terms of COD is considered, FES effluent (average 7.4 kg COD/d) was the most significant food source, followed by facultative pond effluent (average 5.8 kg COD/d).

Infections of rotifers (*Brachionus calanoides*) and cladocerans (*Daphnia magna*) in the high-rate pond were controlled by temporarily raising the free ammonia concentration to a target value of 20 g/m³ (Lincoln et al., 1987) by addition of NH₄OH solution (Table 5-12). Algal growth is not inhibited by ammonia hydroxide dosages equal to or somewhat higher than those listed in acclimation.

Without mechanical mixing, the major algal genera dominating in high-rate pond medium were *Chlorella*, *Scenedesmus*, and *Synechocystis*. As shown in Table 5-13, *Chlorella* and *Scenedesmus* persisted but *Chlorella* was dominant during the winter and early spring. *Scenedesmus* 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 biweekly basis, *Chlorella* was dominant during 12 months of the 18 month period monitored, whereas *Synechocystis* was dominant in the other 7 months. *Scenedesmus*, although second in overall importance to *Chlorella*, did not predominate during any of months studied. Phototrophic bacteria (*Rhodospira rubra*) were commonly observed, but were not significant on a biweekly basis.

Table 3-17. Sodium hydroxide additions to
High-Delta pond for grazer control

Date	NaOH added g m ²	pH	Free NH ₃ ^a g m ²
8 Oct 83	50	8.6	6
14 Oct	30	8.1	16
25 Oct	30	7.6	17
10 Mar 84	20	8.5	6
18 Apr	20	8.7	6
3 Jul	40	8.5	5
24 Jul	40	8.6	27
7 Sep	100	8.5	89
3 Oct	37	8.3	66
15 Oct	41	8.7	9
20 Jan 85	51	8.6	5
22 Jul	184	8.3	60
8 Sep	31	8.4	18
22 Sep	55	8.3	7
10 Oct	24	8.2	6
14 Sep	25	8.8	6
4 Nov	24	8.3	11

^aContributed directly by added NaOH

Table 8-12. Pathogenetic Community Composition of high-rain pond^a

Month	Normalized cell count (10 ⁶ /g)			
	Chloro ₁	Microb ₁	Synco ₁	Chloro ₂
Oct. 83	1.3	0.3	47.5	5.3
Nov	47.4	0.5	7.7	3.4
Dec	25.7	1.4	0.5	8.5
Jan. 84	4.3	0.4	0.1	0.7
Feb	29.6	0.3	0.2	3.7
Mar	28.5	18.3	5.2	6.8
Apr	22.8	1.5	65.7	7.1
May	41.4	14.4	21.1	15.8
Jun	38.4	19.8	24.5	8.4
Jul	4.5	1.3	48.1	4.1
Aug	5.4	8.5	28.1	0.5
Sep	33.6	22.8	14.8	4.0
Oct	45.3	31.7	1.2	3.8
Nov	59.5	18.2	5.2	4.5
Dec	33.3	28.8	5.2	4.3
Jan. 85	183.0	54.8	0.7	7.3
Feb	183.7	73.2	5.2	3.7
Mar	147.5	81.5	5.2	18.6
Apr	128.7	45.7	0.7	3.0
May	44.6	29.2	3.3	45.5
Jun	133.3	14.0	3.2	5.4
Jul	80.2	21.1	0.5	5.3
Aug	46.0	23.5	0.4	5.4
Sep	8.3	4.4	77.5	0.2
Oct	19.7	4.8	127.0	0.5
Nov	45.7	20.7	14.7	1.1
Dec	25.5	45.3	0.1	5.6
Mean	54.2	28.5	17.3	6.2

^aAbbreviations:

Chloro₁ = *Chlorella* spp.
 Microb₁ = *Micrococcus* spp.
 Synco₁ = *Synthyris* spp.
 Chloro₂ = *Chlorella* sp.
 0.7 = trace

3.2 Climatological Information

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

Monthly average minimum and maximum temperatures are shown in Figure 3-1. The highest monthly average maximum temperature was 32.4°C in June 1983. The lowest monthly average minimum temperature was 8.2°C in January 1983. The variation of monthly average photosynthetically active radiation (PAR) was similar to that of temperature. The highest monthly average PAR was in May 1983 ($1445 \text{ Einstein/m}^2\text{-d}$) whereas the lowest was in December 1983 ($400 \text{ Einstein/m}^2\text{-d}$) (Fig. 3-2).

Wind movement was greatest in the spring and fall and least in the summer (Fig. 3-3).

Monthly rainfall was greater in the late spring and summer than in the late fall and winter. Highest monthly rainfall was in August 1983 (17.4 cm) and the lowest in December 1983 (1.2 cm) (Fig. 3-4).

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 1983 (30 cm) and the lowest in December 1983 (4.2 cm) (Fig. 3-5).

Figure 3-1 Monthly average maximum and minimum air temperature

Figure 5-2 Monthly average wind movement at 50 cm above ground level (a) and monthly average photosynthetically active radiation (b).

Figure 5-3 Monthly rainfall (a) and monthly pan evaporation (b).

3.3 Field Trials

3.3.1 Effect of Mixing

The effect of mixing on biofloculation was investigated in 3 experiments; mixed cultures and unmixd controls were compared in exp. 1, 2, and 3. The relative effects of two different mixing velocities (14 cm/s and 30 cm/s) were examined in exp. 12 and 13.

3.3.1.1 Mixing versus Settling

Algal cultures tested in exp. 1 and 2 was *Synachocystis* (Table 3-13, 3-14). Counts of this alga suspended in mixed cultures fell to less than 2% (exp. 1) and 20% (exp. 2) of those in unmixd 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. 3-5), characteristics of the mixed culture (C-2) changed dramatically during the first three weeks. Complete biofloculation marked by aggregation and rapid settling of cells occurred within 20 days. Settling tests showed 99% removal of the algae in 4 min with a settling rate of 8 cm/s and settled volume of 15 ml/L. No settling occurred in the samples from C-1. This phenomenon was preceded by a decline in CO₂ from an average concentration of 18 g/L³ on days 0-7 to 5 g/L³ on days 8-13. The pH in the mixed culture showed a progressive decline, from 9.0 to 8.8 over days 0-13. In contrast, the unmixd culture (C-1) showed no signs of flocculation.

Table 3-13. Effect of mixing on photosynthetic community composition in *Saxifragaceae* rhizosphere, exp. 1^a

Day	Treat	Normalized cell count (x10 ⁶ /g)			
		Chlor.	Syned.	Unid.	Thios.
0	C-1	1.8	0.4	43.0	0.8
	C-2	1.8	0.4	43.0	0.8
4	C-1	3.8	1.8	48.3	0.8
	C-2	3.8	1.8	44.7	0.8
10	C-1	3.4	0.8	32.8	1.2
	C-2	3.2	0.8	34.4	0.2
18	C-1	1.8	0.8	43.4	0.8
	C-2	1.8	0.8	44.0	0.8
26	C-1	1.8	0.8	41.4	0.8
	C-2	0.8	0.8	4.8	0.8
32	C-1	1.8	0.8	33.4	0.8
	C-2	0.8	0.2	8.8	0.8
38	C-1	1.2	0.4	48.2	0.8
	C-2	1.4	0.8	8.2	0.8
42	C-1	8.2	0.8	34.8	0.8
	C-2	8.8	0.8	8.8	0.8
48	C-1	1.8	1.1	48.4	0.8
	C-2	1.8	1.7	8.8	0.8
52	C-1	8.3	1.4	32.8	0.8
	C-2	0.8	1.7	8.8	0.8

^aC-1: Control (not mixed)

C-2: Mixed

Table 8-14. Effect of mixing on photosynthetic community composition in biofloculation chambers, exp. 2*

Day	Dord	Normalized cell count (1/grid)		
		Chloro.	Protozo.	Fungi.
0	C-1	8.3	0.7	24.2
	C-2	8.3	1.1	23.2
1	C-1	8.7	1.4	22.8
	C-2	8.8	1.4	22.3
4	C-1	8.8	0.8	14.8
	C-2	8.8	0.4	18.4
5	C-1	8.7	1.0	18.1
	C-2	8.8	1.3	17.4
7	C-1	8.4	1.1	21.4
	C-2	8.4	2.4	18.8
9	C-1	8.4	1.1	21.4
	C-2	8.4	2.3	18.5
10	C-1	1.4	1.8	24.2
	C-2	2.0	4.8	18.4
12	C-1	3.4	1.8	21.8
	C-2	2.5	2.4	17.7
18	C-1	1.8	2.8	28.9
	C-2	5.7	4.8	14.3
19	C-1	3.4	3.8	18.8
	C-2	7.8	12.1	14.1
20	C-1	8.3	7.0	19.8
	C-2	8.1	14.2	12.4
21	C-1	11.4	7.0	22.1
	C-2	8.8	18.1	11.5
23	C-1	12.8	8.4	22.3
	C-2	12.8	28.7	9.7
24	C-1	11.8	8.4	22.8
	C-2	12.3	18.7	4.2
28	C-1	11.8	8.8	21.4
	C-2	11.3	22.8	4.8
32	C-1	14.8	8.8	24.3
	C-2	13.1	27.1	3.4
35	C-1	14.8	9.1	23.1
	C-2	14.2	24.5	3.8
38	C-1	17.8	18.3	18.3
	C-2	23.8	25.4	1.3
42	C-1	15.8	18.3	18.8
	C-2	23.3	28.7	1.3

*C-1 Control (not mixed)

C-2 mixed

Figure 3-4 Temporal variation of *Hydrophormis*, DO, pH and visibility in mixed (C-1) and untreated (C-2) cultures. Exp. 1.

The pH rose to 7.4-8.8 and DO consistently reached peak values in excess of 15 g/m³. Averages for these two parameters were 18 g/m³ and 8.0, respectively.

Microscopic examination showed that flocs of *Synochrostris* were present after 4 days. Macroscopic flocs became visible after 12 days. Flocs in the mixed culture consisted of *Synochrostris* cells with no visible bacterial component. They ranged from 50 to 500 microns in diameter. The flocs at first retained the blue-green coloration typical of *Synochrostris*, but bleached noticeably with time.

In exp. 3, changes in the characteristics of the mixed (C-2) culture were less pronounced. Microscopic examination showed that *Synochrostris* cells became aggregated after 5 days. Flocs grew to diameter up to 50 microns diameter after 12 days. Floc particles of 100 to 200 microns diameter were abundant in 18 days (density of 1.2 flocs/field at 100 x). Settling ability of the culture was poor, showing 10% removal of chl *a* by sedimentation at day 18 (Fig. 3-8).

Chlorocella and *Bracteacoccus*, though initially negligible, developed significant populations by the end-point of the experiment (Table 3-24). These algae showed no tendency to flocculate. DO and pH levels in the mixed culture were somewhat less than those in the unseeded culture (Fig. 3-8).

Experiment 4 started with co-dominant cultures of *Synochrostris* and *Chlorocella* (Table 3-25, exp.). *Synochrostris* counts declined rapidly in both the mixed (C-1) and unseeded

Figure 5-5 Temporal variation of growth rate, mortality and chl. *a* in mixed (C-2) and unseeded (C-1) cultures, Exp. 2.

Figure 3-4 Temporal variation of DHA, CO and pH in mixed CO-11 and unixed CO-11 cultures initially dominated by *Brachyornis*, Exp. 1.

Table 5-15. Effect of aging on photosynthetic community composition in biofilm-associated channels, exp. 4, 12, 13

Exp. #	Day	Seed	Normalized cell count (AFU/cell)			
			Cyano.	Proteo.	Actino.	Unkn.
4	4	Q-1	38.8	4.2	28.8	4.8
		Q-2	28.8	8.8	40.8	0.4
	8	Q-1	33.8	3.7	28.4	3.8
		Q-2	28.8	7.2	40.8	3.0
	12	Q-1	40.4	20.8	23.4	3.0
		Q-2	44.4	4.8	28.8	3.8
	16	Q-1	35.7	7.7	3.4	3.8
		Q-2	24.8	3.1	6.4	3.1
	20	Q-1	82.8	43.4	0.3	3.3
		Q-2	82.4	43.4	0.3	0.7
12	4	Q-1	127.8	78.8	0.8	3.0
		Q-2	127.8	78.7	3.4	3.8
	12	Q-1	186.6	45.8	0.8	3.3
		Q-2	186.2	38.8	0.1	3.8
	24	Q-1	75.2	38.8	0.4	3.3
		Q-2	48.8	18.8	0.0	3.8
	32	Q-1	77.2	3.8	112.8	0.3
		Q-2	16.8	3.8	112.0	0.8
	40	Q-1	13.4	0.8	77.8	0.0
		Q-2	3.8	0.8	104.8	0.0
13	12	Q-1	38.4	5.8	14.3	0.4
		Q-2	18.1	1.8	7.7	0.0

*Exp. 4: Q-1 Fast
Q-2 Control (not mixed)

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

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

(C-1) culture after two weeks, whereas *Chlorocella* and *Synochloris* cell counts increased. Bifilose of 10 to 20 microns in diameter were noticed in the mixed, C-1 medium within one week and progressively increased in number and size, whereas filae 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 associated at 12 days. They were composed predominantly of *Synochloris* (40-70%) with some *Chlorocella* and *Synochloris* (10-20%) interspersed. 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 methanolic solids contents of the two cultures (Fig. 3-7). A minimum settled solids volume of 17 mL/L was measured in the mixed culture, whereas this parameter remained near zero in the unseeded culture. Despite the flocculation of C-1 medium, algae removal obtained by 14-h sedimentation declined from 12% to 1% during the course of the experiment. In contrast, up to 10% algae removal was obtained with the non-flocculant C-2 medium.

The growth pattern and DO of the mixed culture were similar to those of the unseeded culture. The mixed culture grew faster initially, but was overtaken after 12 days by the unseeded culture (Fig. 3-8). Sedimentation activity was generally much greater in the mixed culture (avg. 15 vs. 17 g O_2 /m²/d) whereas pH was less (avg. 8.0 vs. 10.2).

Figure 3-7 Temporal variation of cell viability and cell size in sized (C-1) and unsorted (C-2) cultures initially dominated by *Hydrocolella* and *Chlorocella*. Exp. 4.

Figure 5-8 Temporal variation of pH, NO and NH₄ in mixed (C-1) and unseeded cultures (C-2) initially dominated by *Amorpha* and *Chlorella*. Rep. 4.

3.1.1.3 Floc Mixing Velocity

Two different mixing intensities, 14 rpm and 30 rpm, were employed during exp. 10 and 11.

In exp. 10, *Chlorocella* and *Synochloa* were co-cultures initially and remained so throughout the experiment (Table 3-13, middle). Flocs became visible after 13 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 20 to 40 microns with a density of 1.1 flocs/field. A maximum settled solids volume of 33 mL/L was measured in C-2 compared to 22 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 (see, for ex., 24h) (Fig. 3-5).

pH and pH were generally greater in the slow-mixed culture, whereas ORP and algae concentration were greater in the fast-mixed culture (Fig. 3-6, 3-10).

In exp. 11, *Synochloa* was dominant initially but declined rapidly in both cultures after the first week. Counts of this alga in both cultures fell to less than 1% of the initial counts over two weeks in each trial (Table 3-14, bottom). Besides the inhibitory effect of mixing on *Synochloa*, significant populations of the protozoan, *Flammonium*, also affected this rapid decay.

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

Figure 3-3 Temporal variation of cell number and cell mass in slow mixed (174 cm/s, C-1) and fast mixed (338 cm/s, C-2) cultures initially dominated by *Chlorella* and *Spirulina*. Exp. 12.

Figure 5-10 Temporal variation of pH, NO₃-N and NH₄-N on slow mixed (O-1) and fast mixed (O-2) vultures initially dominated *Chlorella* and *Spirulina*. Exp. 12.

tan-green to orange-brown solids, *Sphaerocypris* solids being the major component. A substantial bacterial component was also present in this matrix. *Chlorella* and *Spirulina* composed approximately 10% of the total biomass of sludge.

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. 8-13). Algae removed by 24-h sedimentation was significantly greater in C-1 (mean 448 vs. 20%). Algae concentrations and DBA were similar in both cultures (Fig. 8-11, 8-12). DO and pH were generally greater in C-2.

8.2.2 Effect of Waste Loading

The effect of waste loading was investigated in 4 experiments. Loaded cultures were compared to cultures not loaded in exp. 8 and 11. Different waste loading rates were evaluated in exp. 4. The influence of waste type was studied in exp. 10. Algal genera dominant initially were *Sphaerocypris* in exp. 8 and 4 and *Chlorella* and *Spirulina* in exp. 10 and 11.

8.2.2.1 Loading versus Solids

In exp. 8, *Sphaerocypris* 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 82 vs. 73 cells/ μ l) (Table 2-18, top). *Sphaerocypris* was still the dominant genus in both cultures at the end of the experiment.

Figure 3-11 Temporal variation of settleability and chl. *a*, in fast mixed (1% chl. *a*, C-1) and slow mixed (1% chl. *a*, C-2) cultures initially dominated by *Amphicorypha*, Exp. 12.

Figure 3-12 Temporal variation of DM, DO and pH in fast mixed (C-1) and slow mixed (C-2) cultures initially dominated by *Synedra*. Exp. 73

Table 3-15. Effect of waste loading on photosynthetic community composition in biofilm reactor channels, exps. 9, 11

Exp. ^a	Day	Load	Normalized cell count (1/μg chl)				
			Chla ₁	Chla ₂	Chla ₃	Chla ₄	Chla ₅ ^{**}
9	-1	C-1	11.3	1.4	85.8	1.3	0.0
		C-2	10.4	0.0	88.4	1.3	0.0
	3	C-1	9.8	3.8	109.4	0.0	0.0
		C-2	18.4	3.7	109.0	0.0	0.0
	10	C-1	8.3	3.2	110.2	0.0	0.0
		C-2	8.2	3.4	110.6	0.0	0.0
	18	C-1	7.4	4.1	88.4	0.0	0.0
		C-2	4.8	3.4	110.6	0.0	0.0
	25	C-1	3.8	1.3	81.2	0.0	0.0
		C-2	9.3	1.8	77.4	0.0	0.0
	30	C-1	1.4	0.7	73.0	0.0	0.0
		C-2	19.2	4.4	57.0	0.0	0.0
11	0	C-1	128.4	27.8	0.0	0.0	0.0
		C-2	128.8	29.0	0.0	0.0	0.0
	8	C-1	149.6	48.2	0.0	0.0	0.0
		C-2	140.4	71.4	0.0	0.0	0.0
	18	C-1	184.0	63.2	0.0	0.0	0.0
		C-2	171.8	88.8	0.0	1.4	0.0
	23	C-1	218.0	99.2	0.0	0.0	0.0
		C-2	208.0	84.8	0.0	0.0	0.0
	30	C-1	172.8	70.0	0.0	0.0	0.0
		C-2	142.8	88.0	0.0	0.0	0.0

^aExps. 9: C-2 Waste loaded
C-1 Control (not loaded)

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

^{**}Chlorophyll *a*

The waste-loaded C-2 medium became visibly filamentous after 5 days, whereas filaments were not observed in C-1 medium. Microscopic examination showed that filaments in C-2 medium ranged from hundreds to thousands of microns in diameter. They consisted almost exclusively of bright green *Sphaerocyclus cells* (224). Smaller filaments less than 100 microns in diameter were observed in C-1 medium. Additional observations made using a stereoscopic dissecting microscope showed the C-2 filaments to be irregularly or stellate shaped, with distinct lobes. C-1 filaments were roughly spherical in shape. Filamentous bacteria with a density of approximately 1 filament/grid contributed to C-2 filaments. Filaments at a density of 2 filaments/grid contributed to C-1 filaments. Figure 3-13 indicates production of settleable solids was greater in the waste-loaded (C-2) culture. A constant settleable solids volume of 15 mL/L was measured in C-2 compared to 4.5 mL/L in C-1. Algae removed by 24-h sedimentation was initially similar in both cultures, but became significantly greater in the waste-loaded culture after 10 days. Maximum removals in C-2 and C-1 media were 41% and 16%, respectively. Growth patterns of the two cultures were similar. DO and BOD were greater in the loaded culture (avg. 0.1 vs. 28 g O_2^4 /m³d), whereas pH was less (avg. 5.3 vs. 4.4) (Fig. 3-14).

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

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

Figure 3-14 Temporal variation of TMA, CO and pH in multi-loaded (O-21) and solvent (O-1) cultures initially dominated by *Synochococcus*-Exp. 1.

almost entirely of *Chlorella* and to a lesser extent *Synechococcus* throughout the experiment (Table 3-14, bottom). Macroscopic flocs appeared after approximately one week in both cultures. Floc size was greater than 400 microns in both cultures by day 12. They were composed of *Chlorella* and *Synechococcus* 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 (ana. 34 vs. 46 mg/l/d (Fig. 3-18). Algal removals by 24-h sedimentation in both cultures were nearly equal (ana. 338 vs. 318) throughout the experiment. DO and pH were generally greater than in the non-loaded (0-2) culture. Algae grew better in the waste-loaded culture. DMA was similar in both cultures (Fig. 3-14).

3.3-3.3 Waste Loading Rate

Two different waste loading rates of FBA effluent, 72 L/d in C-1 and 317 L/d in C-2, were employed during exp. 3 with *Synechococcus* 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-flourescent *Chlorella* in both cultures (Table 3-14). Flocs became visible after 4 days in both cultures. They were similar in size but more numerous in C-2 than in C-1 (avg. 3.8 vs. 2.8 flocs/flocid, 100x). The flocs were composed predominantly of *Synechococcus* (80%), with *Chlorella* and bacteria making up the balance. Flocs

Figure 3-18 Temporal variation of antithrombin and chl. a in white-loaded (C-1) and control (C-2) cultures initially dominated by *Chlorella* and *Scenedesmus*. Exp. VI.

Figure 3-18 Temporal variation of CO_2 , SO_4 and pH in waste-loaded 1C-19 and control 1C-20 cultures initially dominated by *Chlorococcum* and *Scenedesmus*. Exp. 19.

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

Production of settleable solids was almost identical in the two cultures. Algae removals were similar throughout the first 18 days, then became greater in C-1 medium (Fig. 3-17). Maximum algae removals were 80% in C-1 and 10% in C-2. Algae concentration and BOD were greater in the more heavily loaded culture. DO and pH were similar in both cultures (Fig. 3-18).

3.1.3.3 Waste Pretreatment

Two different waste types, settled waste supernatant in C-1 and FIB effluent in C-2, were employed at the same loading rate of 500 L/d during exp. 18. *Chlorella* and *Monas* co-dominated 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 contained a relatively high population of *Thraupella cornu* in C-1. The abundance of *Chlorella* and *Thraupella cornu* decreased in C-2 during the experiment (Table 3-17).

Flocs became visible after 18 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 300 microns in diameter with a density of 10 flocs/ μm^2 at 400x. In C-1 medium flocs were 30 to 40 microns in diameter and were present at a density of 0.5 flocs/ μm^2 . C-2 flocs were

Figure 5-17 Temporal variation of settleability and pH in lightly loaded (73 L/d, Q-1) and heavily loaded (217 L/d, Q-2) cultures initially dominated by *Thalassiosira* sp. 1.

Figure 3-18 Temporal variation of ORP, DO and pH in lightly loaded 00-01 and heavily loaded 00-21 cultures initially dominated by *Synochloris*, Sep. 4.

Table 1-17. Effect of waste loading on photosynthetic community magnitude in bioflocculation channels, exp. 1, 10

Exp. #	Day	Feed	Normalized cell count (10 ⁶ /ml)				
			Chlor.	Green.	Brown.	Blue.	Sludge
8	4	C-1	3.5	8.1	70.2	1.5	8.0
		C-2	3.4	8.8	70.2	8.1	8.8
	7	C-1	4.1	0.8	57.2	8.8	8.8
		C-2	3.6	0.8	58.4	0.8	8.8
	11	C-1	3.8	0.8	44.4	8.1	8.8
		C-2	4.8	0.8	71.4	8.4	8.8
	16	C-1	8.3	0.7	46.0	0.8	0.8
		C-2	13.8	0.1	47.8	8.8	0.8
	18	C-1	7.7	0.5	32.4	4x	0.8
		C-2	10.7	0.3	89.8	12	0.8
	21	C-1	3.4	8.3	48.8	0.8	0.8
		C-2	3.5	8.0	43.8	0.8	8.0
	28	C-1	11.8	8x	16.8	0.8	3.8
		C-2	21.8	8x	12.4	8.0	8.8
	33	C-1	5.5	8.3	8.8	8x	8.8
		C-2	12.4	8.3	0.5.	8.0	7.5
10	3	C-1	44.8	43.8	8.8	11.8	8.8
		C-2	43.8	43.8	8.8	13.8	8.8
	6	C-1	43.8	43.8	8.8	13.8	8.8
		C-2	88.8	47.8	8.8	17.8	8.8
	13	C-1	88.8	38.8	8.8	17.8	8.8
		C-2	84.8	47.8	8.8	7.5	8.8
	19	C-1	84.8	44.8	8.8	11.8	8.8
		C-2	88.8	44.8	8.8	4.8	8.8
	26	C-1	88.8	48.8	8.8	11.8	8.8
		C-2	27.8	38.8	8.8	8.8	8.8
	34	C-1	108.8	58.8	8.8	8.8	8.8
		C-2	17.8	38.8	8.8	0.5	8.8
	48	C-1	128.8	84.8	8.8	4.8	8.8
		C-2	38.8	13.8	8.8	8.8	8.8

*Exp. 8: C-1 lightly loaded
C-2 heavily loaded

Exp. 10: C-1 settled waste supernatant
C-2 FBR effluent

composed entirely of *Chlorella* and *Monodina* (95%)—C-2 floccs consisted of *Chlorella* and *Monodina* (44%) and *Thalassiosira weissflogii* (44%).

Maximum settleable solids volume and algae removed in C-2 media were 30 mL/L and 43%, respectively. Maxima for these parameters in C-1 media were 18 mL/L and 14%, respectively (Fig. 3-10). DMA was consistently greater in C-1 than in C-2 (Fig. 3-9a). Algae concentrations, DO and pH were initially somewhat greater than those of C-2, but were similar initially. Values of these parameters eventually became greater in C-1 media.

3.3.2 EFFECT OF ALGAL SOURCE AND BACTERIAL SOURCE

3.3.2.1 Algal Source Series

In exp. 7, the culture (C-1) seeded with *Synedra* media developed in the previous experiment was compared with a control culture (C-2). *Chlorella* and *Monodina* were dominant initially. Counts of these algae declined progressively in both cultures throughout the experiment, ending near zero (Table 3-22). Settleable solids in C-1 increased for the first 3 days, leveled out, then eventually diminished (Fig. 3-11). The quantity of settleable matter remained greater in C-1. Algae removals in C-1 media were greater than in C-2 initially, but were eventually exceeded by those in C-2 media. Floc size and density were similar in both cultures. Maximum algae removals of 56% in C-2 and 61% in C-1 were obtained. Algae concentrations, DO and pH were similar in the two cultures (Fig. 3-12).

Figure 3-12 Temporal variation of sedimentability and mg/g in columns loaded with settled waste supernatant (C-1) and PES effluent (C-2). Sediment loss and sediment removal were constant. Rep. 18.

Figure B-20 Temporal variation of TSS, DO and pH in cultures loaded with agitated waste supernatant (O-I) and Fib. effluent (O-II). Chloridia and Brevibacterium were dominant initially. Sep. 75.

Table 3-18. Effect of algal biofilm and bacterial seedling on photosynthetic community composition in biofilmulation channels, exp. 7, 9

Exp. ^a	Day	Food	Normalized cell count (4/μl/dl)				
			Chlor. ₁	Chlor. ₂	Cyanob. ₁	Chlor. ₂	Algae
7	0	C-1	14.1	8.2	11.8	0.7	0.0
		C-2	10.2	8.2	17.8	0.5	0.0
	8	C-1	31.8	8.1	18.2	0.0	0.5
		C-2	28.7	8.1	24.8	0.8	0.1
	11	C-1	13.5	8.0	7.5	0.0	0.4
		C-2	18.7	8.5	5.1	0.0	0.0
	13	C-1	8.7	8.8	0.2	0.0	0.0
		C-2	7.0	8.8	0.0	0.0	0.4
	23	C-1	8.0	0.8	0.0	0.0	1.3
		C-2	8.8	0.8	0.0	0.0	0.8
9	1	C-1	11.8	23.0	0.0	0.1	0.4
		C-2	15.8	14.0	0.0	27.8	0.2
	8	C-1	18.8	14.0	0.0	0.8	40
		C-2	18.8	21.0	0.0	0.8	40
	11	C-2	22.8	24.0	0.0	28.8	0.0
	13	C-2	28.8	0.0	0.0	24.1	0.0
	23	C-2	24.8	28.0	0.0	13.5	0.0
	26	C-2	20.8	48.0	0.0	25.0	0.0

^aExp. 7: C-1 Algal biofilm seeded
C-2 Control (not seeded)

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

Figure 3-21 Temporal variations of sedimentability and chl. *a* in algal biofilms seeded (C-1) and control (C-2); cultures initially dominated by *Shlotheimia* and *Haemulon*, Exp. 5.

Figure 3-22 Temporal variations of pH, DO and ORP in algal biofilm-seeded (C-1) and control (C-2) cultures initially dominated by *Chlorella* and *Spirulina*. Exp. 7.

SSA was consistently greater in C-1 medium.

5.1.1.3 *Photosynthetic Bacteria Seeding*

In exp. 9, culture (C-2) seeded with anaerobic laggon effluent containing purple sulfur bacteria, *Thiomargarita* rosea was compared to a culture which was not seeded (C-1). *Chlorocella* and *Bracteolax* which were co-dominant initially, remained at significant levels throughout the experiment (Table 5-15, bottom). After seeding, *Thiomargarita* rosea declined progressively but still remained abundant. Microscopic examination of C-2 revealed the presence of flocs consisting largely of *Thiomargarita* rosea cells. Approximately 20% of the flocs consisted of *Chlorocella* and *Bracteolax* cells. The culture seeded with photosynthetic bacteria exhibited a progressive increase in cell counts rather but algae numbers decreased (Fig. 5-15). SSA, DO and pH dropped off towards the end of the experiment (Fig. 5-16).

5.1.1.4 *Activated Sludge Seeding*

In exp. 14, *Chlorocella* and *Bracteolax* were co-dominant initially. Counts of these algae increased progressively in both cultures throughout the experiment (Table 5-17). Microscopic examination of C-2 medium showed flocs consisting of a bacterial matrix surrounded by algal cells in C-2 within 3 days. Small flocs became visible in C-1 after 3 days. There were small, loose aggregates consisting of *Chlorocella* and *Bracteolax*. Complete biofloculation had occurred in both cultures by day 15. This phenomenon was

Figure 1-23 Temporal variation of cell count and substrate concentration in a culture (C-2) seeded with photosynthetic bacteria. Chloride and sulfate were dosed initially. Exp. 1.

Figure 3-24 Temporal variations of ORP, DO and pH in a culture (O-2) seeded with photosynthetic bacteria. *Chlorococci* and *Micrococcus* were dominant initially. Exp. 8.

Table 3-19 Effect of activated sludge addition on
photosynthetic capacity in biofloculation
channels, exp 14^a

Day	Channel	Normalized cell count (10 ⁶ /grid)				
		Chloro _a	Chloro _b	Prots.	Chloro _a	Other
-3	C-1	17.6	53.6	6.0	6.0	6.0
	C-2	17.6	54.0	6.0	6.0	6.0
3	C-1	155.6	63.6	6.6	6.6	6.6
	C-2	162.6	66.6	6.6	6.6	6.6
8	C-1	155.2	67.2	6.1	6.2	6.3
	C-2	168.6	70.6	6.1	6.6	6.1
12	C-1	171.2	66.6	6.2	6.2	6.6
	C-2	172.6	66.4	6.6	6.2	6.6
25	C-1	166.6	76.2	6.0	6.2	6.0
	C-2	172.4	66.6	6.0	6.2	6.0

^aC-1 Control

C-2 Seeded with activated sludge

provided by an increase in the settled volume of C-1 and C-2 to 23 mL and 22 mL, respectively (on day 20) and 22 mL and 20 mL, respectively, on day 24 (Fig. 3-13). Maximum algae removals were relatively low; 40% in C-2 and 25% in C-1. Algae concentrations were similar in the two ponds. DO consistently was higher in C-2. The pH and OD were similar in both cultures (Fig. 3-14).

3.3.4 Effect of carbonate-supplementation

In exp. 3, *Chlorocella* and *Scenedesmus* were co-dominant initially and remained so throughout the experiment (Table 3-24). The carbonate supplemented culture, C-3, began blooming after 22 days and the cultures were olive brown from day 14 on. The first distinct signs of flocculation was seen in both cultures on day 18. Floccs consisted of an olive green matrix of *Chlorocella*, *Scenedesmus*, and *Thalassiosira weissflogii*. Extensive flocculation was evident in both cultures by day 22, but settling of the cultures did not leave clear supernatants. Settled solids values in both cultures decreased gradually for the first 12 days, then increased up to day 20 and then steadily 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 15 mL/L and 14 mL/L, respectively (Fig. 3-17).

Carbonate addition did not affect the photosynthetic community in terms of biomass or bioflocculation. The DO was greater in the carbonate supplemented culture (Fig. 3-18). The pH, OD and algae concentrations were virtually

Figure 3-25 Temporal variation of methanability and CH₄ g in activated sludge seeded (C-2) and deseeded (C-1) cultures initially dominated by *Chlorella* and *Monodora*. Exp. 14.

Figure 5-16 Temporal variation of ORA, DO and pH in activated sludge seeded IC-21 and control IC-13 cultures initially dominated by *Chlorocella* and *Synedra*. Exp. 14.

Table 3-28. Effect of carbonate supplementation and pH reduction on photoautotrophic community composition in biofloculation channels, exp. 3, 8

Exp. ^a	Day	Food	Normalized cell count (8/grid)				
			Chlor.	Rhod.	Synech.	Thios.	Misc.
3	2	C-1	24.5	15.4	4.0	15.8	0.8
		C-2	27.4	15.4	3.8	15.4	0.8
	3	C-1	25.1	13.8	3.8	13.7	0.8
		C-2	20.4	13.4	0.5	14.4	0.8
	5	C-1	20.0	13.8	0.2	11.8	0.0
		C-2	21.4	18.7	0.8	12.1	0.0
	9	C-1	20.4	20.4	0.2	8.2	0.0
		C-2	28.7	18.4	0.8	3.4	0.0
	14	C-1	25.0	20.8	0.0	2.8	0.0
		C-2	28.0	20.8	0.0	3.0	0.0
	20	C-1	15.5	17.3	0.0	1.4	0.0
		C-2	28.1	20.8	0.0	3.2	0.0
	24	C-1	5.8	14.5	0.0	0.0	0.0
		C-2	15.8	48.3	0.0	0.0	0.0
	30	C-1	8.4	21.4	12	0.8	0.0
		C-2	21.8	43.8	12	0.7	0.0
	34	C-1	0.3	0.4	0	0.0	1.0
		C-2	2.0	7.4	0.0	0.0	0.0
	40	C-1	1.4	0.5	0	0.0	0.0
		C-2	1.4	3.0	0.1	0.0	0.2
	45	C-1	7.8	4.8	0.0	0.0	0.2
		C-2	0.8	0.5	0.0	0.0	0.0
	49	C-1	5.5	21.4	0.0	0.0	1.1
		C-2	3.4	13.1	0.0	0.0	2.3
	54	C-1	1.7	18.9	0.0	0.0	1.2
		C-2	1.8	2.4	0.0	0.0	1.0
8	2	C-1	55.0	34.0	0.0	2.5	0.0
		C-2	63.0	23.0	0.0	2.2	1.1
	5	C-1	73.0	13.0	0	2.1	0.0
		C-2	40.0	22.8	12	1.8	2.1
	15	C-1	48.0	28.0	0.0	0.0	0.0
		C-2	68.0	44.8	12	0.0	4.0
	22	C-1	30.8	25.8	0.0	0.0	0.0
		C-2	88.8	17.8	0.0	0.0	8.7
	28	C-1	43.8	19.0	0.0	0.7	5.0

^aExp. 3: C-1 Control (not supplemented)
C-2 Carbonate supplemented

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

Figure 3-27 Temporal variation of sedimentability and Chl. a in wastewater supplemented (C-2) and control (C-1) cultures initially dominated by *Chlorocella* and *Synechococcus*, exp. 1.

Figure 5-28 Temporal variation of OSA, DO and pH in carbonate supplemented OC-21 and unbuffered OC-19 cultures initially dominated by *Chlorella* and *Synechococcus*, Exp. 1.

identical in the cultures throughout the experiment.

3.3.3 Effect of pH Reduction

In exp. 8, the effect of intermittently acidifying a culture inoculated initially by *Chlorella* and *Monodina* was evaluated. Concentrated sulfuric acid was used to reduce pH of the experimental culture (C-2) to the target value of 4.0. Sometimes pH values significantly lower than this were experienced (Table 3-8). Despite decrease in pH, algal density in C-2 was greater than the control (C-1) (Table 3-8B, bottom). Settlingable solids and algae removals were similar in the cultures until day 19, just after C-1 was treated heavily with ammonium hydroxide (Fig. 3-29). 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 *Monodina* (approx. 45-50%) and *Thiospirilla* sp. and other bacterial components (approx. 50%). Maximum algae removals in C-1 and C-2 were 74% and 54% respectively. Algae concentration and DO were consistently greater in C-2 throughout the experiment (Fig. 3-30). No significant differences 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 22 which resulted in extensive algal cell death.

Figure 8-29 Temporal variation of seed viability and Chl. a in modified (C-2) and control (C-1) cultures initially dominated by *Chlorella* and *Scenedesmus*. Exp. 4.

Figure 3-30 Temporal variation of pH, DO and Ia acidified (C-1) and control (C-2) cultures initially dominated by *Shigella* and *Parabacter*- Sep. 8.

CHAPTER 4 DISCUSSION

4.1 Effect of Mixing

4.1.1 Algal Species Control to Favorable Bioflocculation

The dominant algal genera encountered initially in field experiments included *Synochloris*, *Chlorocella*, and *Scenedesmus*. 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, *Chlorocella* and *Scenedesmus* coexisted in roughly equal numbers. *Scenedesmus* gradually became dominant in late spring and declined in early summer. The minute unicellular cyanobacterium, *Synochloris*, received dominance 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 Hoopes, 1981, 1983).

Complete bioflocculation marked by cell aggregation was achieved in the field experiment 1. Microscopic examination revealed that *Synochloris* 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 extracellular polymers by the algae themselves in contrast to the bioflocculation

associated with *Synechococcus* which is dependent largely on its genetic tendency to form coccidia, along with a substantial bacterial contribution (Eisenberg et al., 1981; Lincoln and Hoopes, 1982). Absence of bacterial interaction in *Synechococcus* 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 unicellular paired 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 *Synechococcus* by continuous flow mixing was observed in the field exp. 1, 2, 4 and 15 (Fig. 3-4, 3-5, 3-7, 3-11) and laboratory exp. 7, 8 and 9 (Fig. 4-3, 4-4, 4-6). This response contrasts with that of bioflocculating *Microcystis* cultures, which retained high photosynthetic capacities under continuously mixed conditions. *Synechococcus* counts also declined rapidly in field exp. 4 with an initially co-dominant culture of *Synechococcus* and *Chlorella* while *Chlorella* and *Scenedesmus* cell counts kept increasing in the mixed culture. The stimulatory effect of mixing on the growth of *Chlorella* and *Scenedesmus* was also confirmed in the laboratory experiments (Fig 4-3, 4-6).

4.1.3 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 unmixd cultures (Fig. 3-3, 3-7). Generally, bioflocs become viscous after an initial 4-8 days in the mixed cultures, but no sign of bioflocculation was evident in unmixd cultures, therefore it is concluded that mixing is required for induction of the bioflocculation process.

Settleable matter production and algae removal were found to be proportional to mixing intensities in field experiments 12 and 13 (Fig. 3-9, 3-11). 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 4-1). This phenomena was confirmed by the results of laboratory experiments 7 and 8. As G value increased, floc density increased with the occurrence of much smaller flocs. It is obvious that as velocity gradient increased, shear stress increases; thus floc particles become weaker and are subject to being torn apart, reducing the extended length and number of attached polymers. Eventually, the flocs showed poorer settleability (Fig. 4-3). Therefore, there is a maximum size of floc particles associated with each mean velocity gradient.

Table 1-3. Mass velocity gradient (G) applied in sludge experiments

Experiment No.	Channel	Operating depth (cm)	Flow velocity (cm/min)	G (sec ⁻²)	Days required for initial floc formed
1	C-1	11	0	0.0	18
	C-2	11	18	8.8	4
2	C-1	20	0	0.0	13
	C-2	20	18	8.8	5
4	C-2	10	15	5.4	7
	C-2	10	0	0.0	13
12	C-1	48	14	4.3	7
	C-2	48	14	14.4	7
13	C-1	38	14	14.4	7
	C-2	38	14	4.3	7
7 & 8**			0	0.0	20
			20 ^a	10	4
			40 ^a	20	4
			60 ^a	30	4

^asec/min.^{**}Laboratory experiments with Jar test apparatus

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

Bacterial activities were much higher in the slow cultures particularly in fast-mixed cultures than in unmixed and slow-mixed cultures (Fig. 3-8, 3-9, 3-10). 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, film particles normally became visible within 7 days and non-flocculent cultures developed on pin films less than 100 microns in diameter. Usually these pin films 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. Extensive activity of extracellular enzymes occurs during the early phase of logarithmic growth, releasing low molecular weight polymers into the media. These polymers show little ability to promote aggregation of microorganisms (Scales et al., 1970). Consequently, partial immobilization or coagulation 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 immobilization should be employed.

It was generally observed that at high specific growth rates, microfouling growth is usually present, less biofouling tendency, whereas low specific growth rates tend to create conditions which promote biofouling (Fig. 4-5, 4-7, 5-15, 5-17, 5-18, 5-21, 5-23, 5-25).

The fact that most of biofouling in the field experiments occurred during the declining or the endogenous phase of algal growth (Fig. 5-21, 5-23, 5-25, 5-27, 5-29, 5-31) supports earlier findings that the extracellular biopolymers synthesized during the logarithmic growth phase are always modified to more insoluble forms so that they remain in the vicinity of the cells and can entangle cells and suspended solids.

4.3 Effect of Waste Loading

One of the principal reasons 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 raw carbon skeletons for both algae and bacteria. Since waste water has a very low carbon to nitrogen ratio, 4:1 or less, the development of raw carbon sources is essential if the nitrogen is to be converted into biomass and converted as organic nitrogen (Pye and Sivaria-Segura, 1980).

Algal growth rates of both waste loaded and unloaded cultures were found to be similar in field experiment 2

(Fig. 3-12). However in field experiment 11, the waste-loaded culture exhibited a large increase in algal growth potential (Fig. 3-13). The more heavily loaded culture showed a moderate increase in algal growth activity but the difference was not noticeable in field experiment 8 (Fig. 3-17). This result might be due to difference in initial nutrient conditions in each experiment. With enriched nutrient levels initially (333 kg COD load/ha-d) (Table 3-2) as in field experiment 8, loading of the culture had no appreciable effect on growth while a big difference in algal growth potential was shown in culture media which was lightly loaded with nutrients initially (104 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. 4-5, 3-18). Average substrate characteristics of wastes loaded to biodegradation systems during these experiments were similar, representing COD values of 14,840 g/m³ and 20,710 g/m³ and TSS values of 1,800 g/m³ and 2,340 g/m³ for anaerobic digester effluent and fixed bed reactor effluent, respectively.

ODR was substantially greater in the waste-loaded than the non-loaded culture, as expected, in field experiment 8 (Fig. 3-14), while ODR was not stimulated by the waste addition in field experiment 11 (Fig. 3-18). The heavily loaded cultures exhibited only a small increase in ODR in both field and laboratory experiments (Fig. 4-7, 3-14).

Table 4-2. Average DOB loading to high-rate pond prior to addition of media to bioflocculation channels

Experiment No.	Experiment Variables	Average DOB loading (kg DOO ₅ /ha-d)
1	± mixing	75
2	± mixing	14
3	± CO ₂ **	*
4	± mixing	104
5	± waste loading	210
6	waste loading rate	100
7	± addition (algae) seeding	80
8	pH	104
9	± purple sulfur bacteria seeding	**
10	waste pre-treatment	205
11	± waste loading	104
12	flow mixing velocity	28
13	flow mixing velocity	71
14	± attached algae addition	*

*Residualative pond effluent was used

**Cultured media 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 3, conducted with *Synsphaerella* heavily dominant, FMS effluent-loading had a pronounced, positive effect on both settleable solids production and algal removal (Fig. 3-11). However, with *Chlorocella* and *Scenedesmus* co-dominant, FMS effluent-loading showed a slightly negative effect on settleable solids production, while not affecting algal removal (Fig. 3-10). Also, there was little difference between high and low loading with FMS effluent in terms of settleable matter production or algal removal (Fig. 3-12). On the other hand, in laboratory experiments 4 and 10, algal removal was found to be proportional to waste loading rates with *Chlorocella* and *Scenedesmus* (Figs. 4-5) and with *Synsphaerella* (Fig. 4-6) dominant initially.

The mixed results of the FMS effluent loading experiments would perhaps have been due to several reasons. First, nature culture media of bioflocculation channels,

C-1 and C-2, had received enriched substrates before transfer from the growth pond (C-4) which received 0.54 g^3 of FMS effluent on an average daily basis (Table 4-10).

No correlation was shown between bioaccumulation potential and average COD loading to the high-rate pond prior to transfer to bioaccumulation channels (Table 4-3). There was a slight tendency to observe a better bioaccumulation potential with low COD loading to the high-rate pond. It was also found that the amount of loading in excess of 18 per day (as KH_2PO_4) was generally not critical to bioaccumulation (Minicich et al., 1984).

If floc forming bacteria are significantly contributory to algal bioaccumulation, 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 anoxic 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 (20-30 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 sludged well and would develop anoxia. This might explain the results that the fast mixed culture (flow velocity 18 cm/sec) provided substantially higher bacteroid activity,

presumably producing more extracellular biopolymers by elevated bacterial metabolism, thus accelerating biofloculation.

Second, flocs turning waste-like bacteria used biodegradable organics as their substrates but much of the biodegradable matter is FBR effluent is reserved 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 in. Contrary to the hypothesis, it was found that the culture loaded with FBR effluent has a much higher biofloculation potential than that loaded with settled waste supernatant. As expected, bacterial activity was greater in the culture loaded with settled waste supernatant. However, much 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 silver-white, primarily *Chlorococcoides* and *Hydrogenis*, while in the culture loaded with settled waste supernatant, flocs consisted almost entirely of purple sulfur bacteria, *Chlorococcoides* sp. In contrast to the *Chlorococcoides* predominance resulting from continuous mixing and settled raw waste loading (Lawson and Hooper, 1981), there was no silver-white change.

As with the biofloculation experiments conducted at Suisun, California, by Rosenberg et al. (1982), 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 waste water medium needs a faster mixing velocity to reach the same degree of bioflocculation through the action of the flow forming bacterial population than did the average effluent medium of the Richmond experiment. Filtration of culture medium medium of 0-4 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.

4.3 Effect of Algal Biofilm 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 flocu developed in the previous experiment still would have vacant sites so that algae and bacteria could attach onto them. Field experiment 2, conducted with *Synechococcus* 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 18 days (Fig. 5-21). Extended seeding might have reduced the length and number of adhered polymers on the seeded culture, preventing reestablishment. On the other hand, it was

inferred that 10% of the non-needed 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 (*Thiobacillus rosea*) introduced into either the high-rate pond or bioflocculation channels with anaerobic laguna 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 3, conducted with *Chlorella* and *Spirulina* as-dominant initially showed that photosynthetic bacteria bioflocculated as predicted from previous observations, but there was only moderate interaction with algal cells. Flume consisted of about 80% *Thiobacillus* and 20% *Chlorella* and *Spirulina* on a biomass basis. Biopolymer concentration increased in parallel with settlement solids values (Fig. 3-15). This result indicates that bacterial exopolymers can be quite specific to bacteria, limiting the degree of algal-bacterial interaction in flow formation.

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

Knox, 1971; McDriff and McKinney, 1972; John and Bokil, 1978). 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 7:1 (McDriff and McKinney, 1972) and 3:1 (John and Bokil, 1978). A 10:1 ratio was employed in field experiment 14, but no effect on microflocculation was shown. However, in laboratory experiment 15, a culture loaded at the algae/bacteria ratio of 20:1 exhibited substantially greater algal removal than those cultures loaded at ratios of 1:1 and 10:1 (Fig. 4-30). Bacterial activity was found to be proportional to the amount of activated sludge seeding in both laboratory and field experiments (Fig. 4-30, 5-24).

4.4 Effect of pH

A critical factor in pH in artificial flocculation, sometimes occurred in high-saline ponds at elevated pH (10-12) as a result of the consumption of CO_2 by algal photosynthesis. Levy et al. (1980) found that *Coelastrum* and *Scenedesmus* are capable of microflocculation, forming aggregates which settle down when not agitated.

Several chemical reactions such as phosphate precipitation, calcium and aqueous hydroxide formation, and complexation were probably responsible for spontaneous algal microflocculation at pH values greater than 8.8, the minimum pH value at which phosphate precipitation will occur (Kolins and David, 1969; Garden and Chapman, 1978; Schenck et al., 1980). However, the efficiency of algal settling was

also found to be independent of pH at levels as low as 7.0 (McGruff and McKinney, 1971). Their observation of spontaneous algal flocculation in activated sludge systems was more probably due to macropolymer interaction.

Several findings support the assumption that biofloculation achieved in the present experiments did not involve autoflocculation. First, complete biofloculation was shown in the culture having the lower pH in field experiment 1, 4, 10 and 14 and laboratory experiments 1, 2, 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 constituted largely of *Synochloris* or *Chlorocella*-*Monodina* 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 *Chlorocella* inoculum initially, complete biofloculation was observed within 3 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 polymerization 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 conditions, grazers, etc. Fogg (1965) and Fox and Dennis (1968) 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 experiment 5. 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 4 within 3-7 days. This occurred even after adjusting culture to pH 8.0 every day (laboratory experiments 4, 5 and 6; Fig. 4-3, 4-8, 4-11). The declining pH effect might be explained by the influence of nitrification in which 3.26 mg of bicarbonate alkalinity (as CaCO_3) was removed for each mg of nitrate produced. Further research is recommended to determine whether or not this nitrification would affect algal bioflocculation.

4.3 Effect of Algal Species

The bioflocculation tendency of different algal species has not yet been fully studied. However, their morphological characteristics can significantly affect the extent of bioflocculation because their shape and size or total available surface area can determine the optimal polymer concentration for bridging. For example, *Microcystis* and *Sphaeromonas* 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 *Scenedesmus* make them less prone to attach to other cell surfaces or polymeric chains.

Microalgal genera previously identified in the bioflocculation experiments include *Schistocladum* (Koopman et al., 1978), *Microcystis* (Gilesbury et al., 1981), *Chlorella* (McKewey et al., 1971), *Sphaeromonas* (Gould et al., 1978), and *Synochocystis* (Lincoln et al., 1984). The principal algal genera encountered in the present research were *Synochocystis*, *Chlorella* and *Scenedesmus*. 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 *Synochocystis* through continuous flow mixing was highly effective. Complete bioflocculation marked by red cell aggregation was achieved in the field experiment 1. Settling tests showed 99% removal of the algae in 4 min with a settling rate of 4 cm/sec.

Maximum algal removals achieved in all field experiments in which *Synechococcus* was dominant or co-dominant averaged 99%. The biofloculating tendency when *Chlorella* and *Scenedesmus* were co-dominant was less, but still led to significant algal removal. Maximum algal removals obtained in all field experiments in which *Chlorella* and *Scenedesmus* were co-dominant averaged 44%. This may be much better demonstrated in terms of biomass recovery. For example, 18 kg and 48 kg dry algal solids were harvested after experiments 10 and 11, respectively.

4.4 Effect of Other Variables

Extracellular polymer production has been shown to be influenced by the relative concentrations of carbon and nitrogen in the growth medium (Jørgensen and Kristiansen, 1982). A similar result was reported by Sand et al. (1984). A high C:N ratio could enhance the polymer production of species rather than valuable materials.

Addition of 80 g carbonate³ to the culture medium induced biofloculation in laboratory experiment 1, inoculated with *Chlorella* from the high-rate pond (C-4) system at Odenseville. 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 1) with *Chlorella* and *Scenedesmus* co-dominant initially, no

increase in carbon/nitrogen ratio by the addition of substrate supplement (500 g/m³ 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).

Ferns et al. (1981) have shown that the production of extracellular biopolymer of unicellular algae increased during carbon limiting conditions. During late summer and early fall 1984, algal flocculated, anoxic/lagoon effluent of the University of Florida Seawater Algae Research System was found to be carbon limiting (Yang, 1985), representing long lag period and finally death phase. The observed high pH caused by CO₂ extraction from carbonate-bicarbonate system could be an indication of carbon limitation; carbon limitation of the anoxic/lagoon effluent was probably due to the excess carbon utilization by the higher activity of purple sulfur bacteria (*Thiopedia cava*) during the warmest seasonal period. It was also reported that carbon losses incurred in methane production could lead to subsequent carbon limitation (Hedder 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/m³). Settling rate increases 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 nucleus film formation associated with the optimum algae and bacteria concentration, an algae concentration should be selected with this in mind before transferring mature algae to biofloculation channels.

4.7 Preliminary Cost Evaluation

The energy cost of waste ponds system are low since the stabilization of waste organics is supported by photosynthetically derived oxygen, thereby obviating the need for mechanical aeration which is one of the principal energy requirement source in a conventional activated sludge system. Gulp et al. (1974) estimated the direct energy requirement for aerating waste to be 321 kWh (\$12.42) per million gallons treated in a diffused aeration system. The energy cost of trickling filter plants has also been estimated to be 158 kWh (\$6.42) per one million gallons treated. It can be seen that energy costs for high-rate activated sludge systems estimated by McKinney et al. (1971) and conventional activated sludge system are competitive. The energy cost of the photosynthetic oxygenation system utilized in the present research is estimated to be 138 kWh per million gallons of high-rate pond effluent treated by the biofloculation process.

An evaluation of the energy cost of the biofloculation process can be compared to the cost of chemicals for lime flocculation. It was found that the AC water driving the precipitation of the high-rate pond utilized 185 units of a

Flow mixing velocity of 20 cm/s. With continuous mixing, energy consumption was 5.12 kWh/d. at \$0.06/kWh, the energy cost would be \$0.31/d. At an average flocculation time of 20 days, the cost of mixing would thus be \$61.20 for 300 m³ of culture volume. Experimental harvests of the bioflocculating cultures yielded approximately 8.5 kg/m³ air-dry solids on three occasions. With 300 m³ of culture volume, a total of 255 kg signal solids could be produced. The corresponding mixing energy cost would be \$0.06 per kg of signal solids. Using slum priced at \$200/tonne, and a typical slum/TSS ratio of 1.2 (Freeman et al., 1980), the slum cost would be \$0.14 per kg of signal solids. When using chitosan, a synthetic biodegradable organic polymer, priced at \$15.00 per kg, with a typical chitosan/TSS ratio of 8.00 (Lee, 1987), chitosan cost would be \$0.20 per kg of signal solids. Thus, the energy cost for the bioflocculation process actually obtained under field conditions would be one-third of that for the slum 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 48 rev/min was optimum for the maximum yield of settleable matter in laboratory cultured continuous flow mixing was inhibitory to the photosynthetic capacity of *Synechococcus*, leading to bioflocculation.
2. Spontaneous algal bioflocculation observed in the present research was due to extracellular polymer mediated flocculation, not attachment to chemical precipitation (auto-flocculation).
3. Algal species composition is the strongest parameter in bioflocculation of microalgae grown in aerobically treated waste water. S.B., *Synechococcus* showed the greatest bioflocculation tendency.
4. Bacterial composition in wastewater influences the types of microalgae and bacteria which grow in the

unique media. Concentrated wastewater 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 cellistic number was accompanied by pH decline and by the declining or stationary 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 Q VALUE

7

The velocity gradient, $G = (R/\mu)^{1/2} = (\text{Force}/\text{Volume} \times \text{Viscosity})^{1/2}$. $R/\mu = \text{Flow} \times \text{Frequency} / (A \times L) = QW/AL = \text{ft}^3/\text{s}$. Therefore, $G = (QW/\mu)^{1/2}$. Where, A = cross sectional area, L = length in ft, Q = flow in cfs, W = specific weight in 62.4 lb/cu-ft, L = head loss in ft, v = velocity in fps and μ = absolute viscosity is 3.104×10^{-5} lb-sec/cu². 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, $G = (v \times n/1.49 \times R^{2/3})^{1/2}$. The width of the bifurculation channel is 7.51 ft; when the depth is 1.55 ft (21 cfs), $R^{2/3} = 0.18$, $v = 0.515$ fps then $G^{1/2} = 1.7 \times 10^{-3}$. Accordingly $G = (QW/\mu)^{1/2} = (1.555 \times 62.4 \times 2.7 \times 10^{-3}/3.104 \times 10^{-5})^{1/2} = 4.5$ for a value of channel (C-2) when Exp. 1 was conducted.

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He is married to Hsiao Lee and has two children, Hys 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.

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