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BIOLOGICAL FLOCCULATION OF WASTE GROWN ALGAL CULTURES

Final Report

by

Ben Koopman, Edward P. Lincoln,
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ABSTRACT

Bioflocculation, the formation of cellular aggregates by means of exocellular polymers, is a potentially economical process for harvesting microalgae from oxidation pond effluent. Field scale application of this process was investigated over a two year period. Algae were grown in an outdoor treatment system processing anerobically digested swine waste. Management variables tested were algal species composition, flow mixing, waste loading, waste type, algal biofloc seeding, bacterial seeding, carbonate supplementation, and pH reduction. Flocculation parameters included algal removal, settled volume, biopolymer concentration, dehydrogenase activity, and optical density. Chlorophyl *a*, pH and dissolved oxygen were also monitored.

The most significant variable affecting bioflocculation was algal species composition. Of the remaining management variables, mixing was most important. Waste loading had a slight, positive effect. There was some indication that the type of pretreatment applied to the waste could determine whether or not waste loading was beneficial. Carbonate supplementation, pH reduction, algal biofloc seeding, and bacterial seeding were either neutral or inhibitory towards bioflocculation.

The greatest immediate potential for utilization of the bioflocculation phenonmenon in algae harvesting lies in combining this process with natural or synthetic polymer addition as a means of reducing the dosage of chemical flocculant which is required to effect separation. Initial aggregation of algae (to 50-100 μm diameter flocs) in the bioflocculation ponds occurred rapidly. At this point, flocculant could be introduced. Because flocculant requirements are a strong function of particle size,

the initial aggregation achieved by mixing could reduce the overall cost of harvesting.

1. INTRODUCTION

1.1. Problem Statement

The high productivity of the microalgae, potentially in excess of 50 tonnes dry weight/ha a in favorable climates (Goldman 1979), makes them a leading candidate for the production of biomass for fuel, feed, chemical feed stocks, and other commercially valuable products. For the majority of such uses, considerable economy can be attained through the use of organic wastes as a nutrient source. A particularly abundant source is the manure produced at a rate of more than 10^8 tonnes (dry weight) annually in the confined livestock operations of the U.S. (Van Dyne and Gilbertson 1978). Of special importance in this regard is the effluent from anaerobic digesters used for the generation of methane from manure slurries. Algae grown on these effluents can be brought to maturity in dense cultures with little or no chemical supplementation at virtually no cost. In the coming decades, it is probable that methane fermentations on various substrates will engender widespread development of algal culture technology, as this is an effective means of utilizing solar energy not only for biomass production, but also for the renovation and reclamation of wastewater, particulary digester effluents.

In order to make use of the high productivities of the microalgae for biomass production, methods for initial concentration of algal cells which are effective yet low in cost are needed. Bioflocculation, the formation of cellular aggregates by means of exocellular polymers, is seen as a promising harvesting technique in this respect. Isolation of waste pond effluents in batch, secondary ponds was found to induce bioflocculation and subsequent settling of algal populations in both

pilot and field scale systems (Koopman et al. 1981, Eisenberg et al. 1981). Application of continuous flow-mixing was shown to promote microbial aggregation within high-rate pond cultures in systems at Manila, the Philippines (Oswald et al. 1978) and Richmond, California (Eisenberg et al. 1981). A common characteristic of these systems was that they treated domestic sewage. It was also noted that the algal floc developed contained a significant bacterial component (Eisenberg et al. 1981).

1.2. Research Goals and Rationale

The goals of this research were to gain a better understanding of the mechanism of bioflocculation in waste grown algal cultures, with emphasis on elucidating the contribution of bacteria and their exopolymers to the aggregation process, and to devise and test techniques for inducing and promoting algal bioflocculation in field scale pond systems. The waste chosen for use as algal culture medium was swine manure. This choice was made partly because previous algal bioflocculation studies had involved domestic sewage exclusively, and it was felt that use of a different medium would enable valuable comparisons to be made. An additional reason for the choice was the availability at the University of Florida of a field scale pond system treating swine waste.

The initial research plan was divided into consecutive laboratory and field phases. In the laboratory phase, it was planned to characterize the exocellular polymer production kinetics and flocculation behavior of representative algal and bacterial populations. Based on the laboratory results, techniques for inducing and promoting bioflocculation were to be devised and tested in in the field scale pond system. The planned

order of experimentation was reversed, however, after difficulty was experienced in obtaining suitable flocculating algal species for the laboratory studies, whereas preliminary field experiments gave very promising results. Described in this report are the results of field scale studies carried out over a period of 19 months. Results of laboratory studies which are now in progress will be given in the Ph.D. dissertations of the student investigators co-authoring the present report.

1.3. Project Objective

The project objective was to test the effect of selected management variables on the bioflocculation of algal mass cultures. Variables investigated were algal species composition, flow mixing, waste loading, waste type, algal biofloc seeding, bacterial seeding, carbonate supplementation, and pH reduction. Flocculation parameters included algal removal, settled volume, biopolymer concentration, dehydrogenase activity, and optical density. Chlorophyll *a*, pH and dissolved oxygen were also monitored.

2. LITERATURE REVIEW

2.1. Polyelectrolytes and Bridging Theory

Polyelectrolytes are linear or branched chains of small subunits (monomers), which contain ionizable groups (e.g., -COOH, -OH, $-NH_2^+$, $-R_1NR_2^+$). They are water soluble and are affected by electrostatic forces between their active (ionizable) sites. Natural polyelectrolytes include proteins, polyamines, nucleic, pectic, and alginic acids, polysaccharides, and numerous polyacids. Synthetic polyelectrolytes are formed by polymerization of simple monomers. Polyelectrolytes can be classified as cationic, anionic, or amphoteric. Uncharged (nonionic) polymers are also classed as polyelectrolytes if their neutral character is due to equal summations of cationic and anionic groups.

Aggregation of colloidal microorganisms with polymers is achieved by a stepwise process involving adsorption of the polymer onto the solid, cross-linkage of the segments of the polymer to form bridges between the dispersed microorganisms, and formation of a loose, three-dimensional structure. Further interaction of adsorbed and extended lengths of polyelectrolytes with vacant bonding sites of adjacent microrganisms can lead to additional bridging and induce growth of the aggregate (LaMer and Healy 1963, Harris and Mitchell 1973). The bridging model is shown schematically in Figure 2-1.

The following physicochemical variables are important in polymer-induced coagulation:

(a) Polymer concentration - bridging between particles is optimal when the particles are 50% covered with adsorbed segments of the polymer. More extensive coverage can lead to redispersion of the

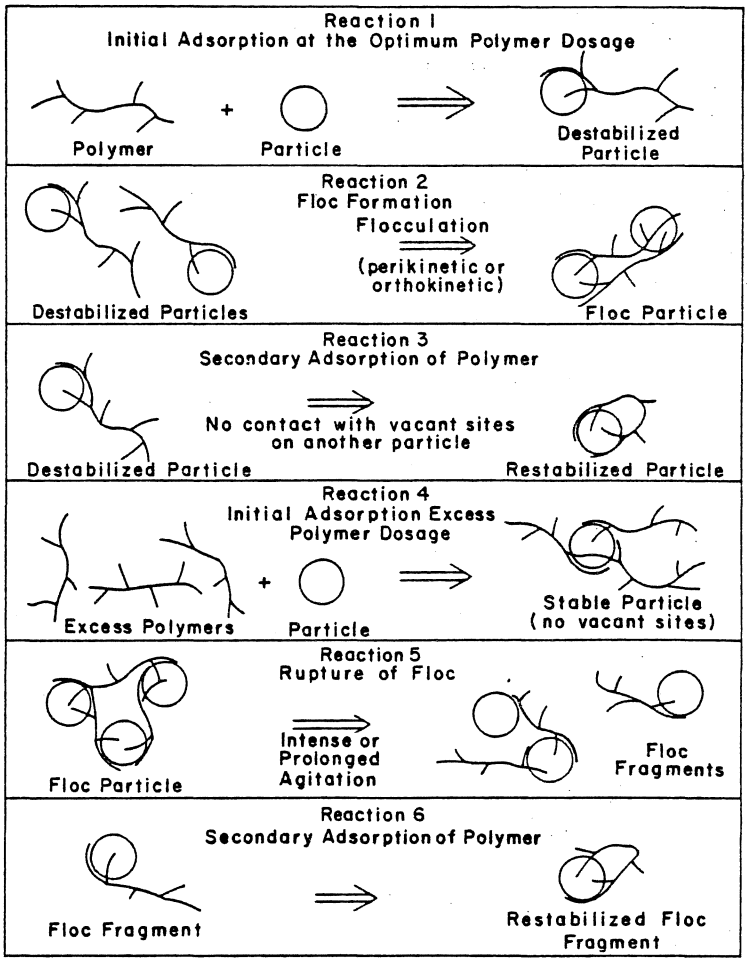

Figure 2-1. Schematic representation of the bridging model for the destabilization of algal cells by polymers. (After O'Melia 1972)

suspension.

(b) Intensity and time of agitation - optimal time and intensity of agitation exist for maximum aggregation. Excessive mixing intensity can decrease the amount of polymer adsorbed, whereas extended agitation periods can decrease the degree of aggregation by reducing the extended length and number of adsorbed polymers.

(c) Qualitative properties of polymer - generally, elongation of the polymer chain enhances the probability of bridge formation. For significant aggregation of colloidal particles with nonionic polymers carrying the same charge as the particles, polymer molecular weights in excess of several hundred thousand are required, whereas the counter ionic charged polymers are usually effective at lower molecular weights.

(d) pH - pH has a pronounced effect on polymer induced aggregation by its influence on electrical double layer thickness and on elongation and ionization of polymers.

(e) Electrolyte concentration - polyvalent electrolytes are particularly significant in the aggregation of nonionic or like charged polymers and particles. A significant increase in ionic strength will normally decrease the extent of polymer adsorption on solids owing to competition for adsorption sites by inorganic ions that carry the same charge as the polymer.

2.2. Characteristics and Production of Exocellular Polymers

2.2.1. Characteristics

Exopolymers of floc forming bacteria can differ both structurally and chemically. Characteristics of exopolymers from even a single species can differ with carbon source, nutrients and environmental conditions (Costerton et al. 1978). The more soluble extracellular

biopolymers are easily sloughed off cells, increasing the viscosity of surrounding medium. The synthesis of relatively insoluble extracellular biopolymers, which can entangle cells and other suspended solids, is a key feature in bioflocculation. The extracellular biopolymers synthesized during the logarithmic growth phase are slowly modified to more insoluble forms. This might explain why bioflocculation is usually incomplete during rapid growth (Dugan and Pickrum 1972). Gulas et al. (1979) reported that even though large quantities of extracellular biopolymer were detected at the lower cell ages, pinpoint flocs were observed. They concluded that exopolymers released by autolysis of cells in the logarithmic growth phase consisted of low molecular weight molecules which had little ability to promote aggregation of microorganisms.

Zoogloea ramigera, which may be an important species in the activated sludge process, produces a gelatinous matrix that contains only glucose. It resembles cellulose, having ß-1,4 linkages. This material is capable of entrapping other cells and particulate materials, forming flocs (Friedman et al. 1968, 1969). Tezuka (1973) reported, however, that extracellular biopolymers produced by the genus Zoogloea were quite different in their chemical composition from those reported by Friedman et al. (1968, 1969). According to Tezuka (1973), these biopolymers were mucopolysaccharides composed of two aminosugars (n-acetylgluco-samine and n-acetylfucosamine).

Table 2-1 shows the binding materials (cellulose, mucopolysaccharide, proteins, nucleic acids) causing the aggregation of various microorganisms. These binding materials were identified with enzyme treatments which completely destroyed the aggregates and appeared to remove much of adhesive material surrounding cells (Harris and Mitchell 1973).

Table 2-1. Binding materials of flocculated microorganisms

Species	Binding material	Deflocculation enzyme	Reference
Corynebacterium xerosis	protein	papain (proteolytic enzyme)	Stanley & Rose (1967)
Pseudomonas aeruginosa	polysaccharide	hyaluronidase	Warren & Gray (1955)
Acetobacter xylinium, Rhizobium sp., Agrobacterium sp., Azotobacter sp.	polysaccharide (cellulose)	cellulase	Deinema & Zevenhuizen (1971)
Pasteurella pestis	DNA	DNase	Wessman & Miller (1966)
Micrococcus halodenitrificans, Vibrio costicolus	DNA	DNase	Smithies & Gibbons (1955)
Flavobacterium	protein	pronase, trypsin chymotrypsin	Endo et al. (1976) Endo & Takahashi (1980)
Pseudomonas (phenol adapted activated sludge)	muco-polysaccharide	deflocculation enzyme produced by floc former	Tago & Aida (1977)
Pseudomonas (glycerol adapted activated sludge)	DNA	DNase I, II	Sakka et al. (1981), Sakka & Takashashi (1981)

Endo et al. (1976) and Endo and Takahashi (1980, 1981) found that the binding material of Flavobacterium sp. included protein as well as divalent cations which can form ionic bonds with negatively charged exobiopolymers. They showed that extracted cells from various growth phases were reconstituted with the purified binding material which represented only a certain portion of the extracted proteins (the "aggregation factor") and was thought to be under genetic control.

Mucopolysaccharide was considered to be the main binding material causing the aggregation of Pseudomonas which were isolated from phenol-adapted activated sludge. However, this material constituted only 10% of the total polysaccharides from Pseudomonas, whereas the other 90% of the polysaccharides could not be shown to contribute to floc formation (Tago and Aida 1975, 1977).

A relatively small amount of double stranded DNA was one of the factors involved in floc formation by Pseudomonas isolated from glycerol-adapted activated sludge. Denatured DNA and DNA having molecular weights of less than 6×10^6 were shown to be incapable of reflocculating the extracted cells, whereas double stranded DNA having a molecular weight of more than 6×10^6 sucessfully flocculated extracted cells (Sakka et al. 1981, Sakka and Takahashi 1981). A similar result was reported for activated sludge (Vallom and McLoughlin 1984). DNA released on lysis of cells can act as an aggregation factor and can also survive breakdown or depolymerization.

The key feature of bioflocculation could thus be in the aggregation factors which can cause microorganisms to flocculate, once sufficient quantities of extracellular biopolymer have accumulated at the microbial surfaces.

Besides floc formers and their extracellular polymers, filamentous bacteria are essential to the integrity of the macrostructure of the activated sludge floc. Filaments form a rigid backbone for the floc, to which flocculent zoogloeal microorganisms attach like flesh on a bone (Parker et al. 1971, 1972; Sezgin et al. 1978, Palm et al. 1980). If there are insufficient filaments, the floc will be weak and subject to breakup into smaller aggregates in the turbulent environment of the mixed reactor.

2.2.2. Production

Extracellular biopolymers of microorganisms originate from cell lysis, biological excretion, and extracellular synthesis. Generally, the amount of extracellular polymer produced is related to the growth phase of the microorganisms. Several investigators have observed that production of extracellular polymers, notably polysaccharides, by unicellular algae significantly increases during the late logarithmic phase of growth and continues until slightly after cells reach the stationary phase (Lewin 1956, Moore and Tischer 1964). Pavoni et al. (1972) reported that the total amount of biopolymers accumulated increased sharply after bacteria entered the endogenous growth phase. This behavior was most pronounced under carbon limiting conditions. Pavoni et al. (1972) suggested that the majority of biopolymer was from autolysis. This would explain why large quantities of protein, RNA and DNA were detected in microbial polymer extracted during later stages of growth (Dunican and Seeley 1965, Pavoni et al. 1972, Sakka et al. 1981). Conversely, Gulas et al. (1979) observed that the exopolymer content per unit biomass decreased at increasing sludge ages in continuous activated sludge reactors. They concluded that the maximum activity of autolytic enzymes

occurs during the early phase of logarithmic growth, and, as sludge age increases, autolysis of cells slows. A similar result was reported by Rudd et al. (1984). The total polysaccharide content of activated sludge (expressed on a per gram suspended solids basis) varied proportionally with the food-to-microorganism ratio and inversely with sludge age. The concentration of soluble polymer also decreased with increased sludge age.

Extracellular polysaccharides are believed to be synthesized from monosaccharides and simple substrates through phosphate ester intermediates. Enzymes involved in exopolysaccharide synthesis are associated with the membrane underlying the cell wall and are usually observed to be in the form of particulate complexes. Lipoidal transport components, notably polyphenols, may transport polar monosaccharide and oligosaccharide repeating unit precursors through the nonpolar cell membranes to the exterior where polymerization can occur (Slodki and Cadmus 1978). In Streptococcus spp., three enzymes were reported to be involved in the production of glucan exopolymer from sucrose: invertase, glucocyltransferase, and fructosyltransferase (Costerton et al. 1978). Dunican and Seeley (1965) attributed the variability of glucan synthesis by Lactobacillus spp. to enzyme deficiencies of spontaneously occurring mutants.

When growth is limited by certain nutrients (e.g., nitrogen, phosphorus, sulfur) in the presence of excess carbon, microorganisms generally produce less protein, more lipids and more polysaccharides than when the same cells are grown in carbon limited media. Duguid and Wilkinson (1953) observed that with decreasing concentration of the nitrogen, sulfur, or phosphorus source until it became limiting, the

amount of polysaccharide produced per cell of K. aerogenes rose to a maximal level. Maximal polysaccharide production, as measured by the polysaccharide: nitrogen ratio of cells, was 32 for nitrogen deficient cultures, 40 for phosphorus deficient cultures, and 17 for sulfur deficient cultures. The rate of polysaccharide synthesis was highest during the logarithmic growth phase and diminished progressively thereafter. In batch cultures of Aureobasidium pullulans, polysaccharide production commenced when nitrogen (ammonia) limiting conditions were reached, i.e., the presence of free ammonium ion in the medium appeared to suppress polysaccharide elaboration (Seviour and Kristiansen 1983). A similar result was observed in the activated sludge process, where sludge adapted to low ammonium concentration produced higher amounts of extractable polysaccharides than non-adapted sludge (Salanitro et al. 1983). Rudd et al. (1984) suggested that a higher C:N ratio could enhance the polymer production of capsular rather than soluble material. This could result in a more dispersed floc formation and a concomitantly higher sludge volume index (SVI).

Gauthier (1981) reported that oxygen concentration was significant to formation of flocs. Under oxygen limiting conditions, a higher percentage of dispersed growth was observed in continuous cultures of Zoogloea ramigera. A similar result was observed at full-scale plants operated with low DO concentrations in the aeration basins (Starkey and Karr 1984). At low DO concentration, exocellular polymer production was inhibited, resulting in increased effluent turbidity.

The presence of lytic products of other microorganisms may promote bioflocculation by stimulating growth of zoogloeal bacteria. The floc forming bacteria, Z. ramigera and Z. filipendula, do not utilize carbohy-

drate compounds (Dugan and Lundgren 1960, Tezuka 1973). Low molecular weight carbon sources (e.g., alcohol, acetate, glycerol) were metabolized by zoogloeal strains which led to small growth increases for acetate and glycerol and a sizable increase for ethanol. Growth of these bacteria was greatly stimulated by nucleotides and combinations of purine and pyrimidine, or biotine, folic acid and vitamin B_{12} (Dugan and Lundgren 1960). Zoogloea spp. preferentially utilized amino acids as a carbon and energy source when grown in the presence of glucose and amino acids (Gauthier 1981). Zoogloeal strains were reported to have varying abilities to produce the enzymes necessary for degradation of polymeric substrates, whereas non-floc forming bacteria, such as Cytophaga strains, grew very well in carbohydrate medium. The Cytophaga strains produced gelatinase and dextranase, whereas Zoogloea strains produced no gelatinase and only weak dextranase. Zoogloea strains grew very well with Cytophaga strains in this medium (Güde 1982). Gauthier (1981) observed that even Zoogloea strains lost their floc forming trait in pure culture. He suggested that the study of floc formation should be conducted with mixed populations, under conditions where there is a selective pressure to maintain floc forming ability.

Extracellular polymer synthesis was observed by Parsons and Dugan (1971) to increase as a function of the concentration of carbon source in the medium. The accumulation of poly-beta-hydroxybutyrate (PHB) in the capsule of Zoogloea was observed to follow the rapid initial uptake of carbohydrate (Crabtree et al. 1965, Parson and Dugan 1971). The supernatant viscosity of whole cultures increased as PHB in the cells decreased.

2.3. Measurement of Exocellular Polymers

The forms of extracellular polymer produced may be classified as loose slime, capsule and microcapsule. Slime polymers remain in the dissolved form and increase the viscosity of surrounding medium, whereas capsular and microcapsular extracellular polymers are attached to the microorganism. Wilkinson (1958) defined arbitrarily that the capsule is visible by light microscopy and its thickness is greater than 200 nm, while the microcapsule is sub-light microscopic size and is under 200 nm in thickness.

Some strains of bacteria (e.g., Aerobacter cloacae) produce a structure of extracellular polymer intermediate between a capsule and slime (Wilkinson et al. 1954). The genus Zoogloea produces a gelatineous matrix in the form of packets surrounding cells, which are analogous to capsules (Friedman and Dugan 1968). Also, cells producing capsules were reported to produce slime material which had very similar compositions to capsular material (Wilkinson 1958).

A variety of extraction techniques have been developed (Table 2-2). These may be classified into two main groups, physical and chemical. The former includes high speed centrifugation, sonication, shear press and homogenization, while the later includes hydrolysis by acid, alkali or heat, and extraction by organic solvents. Extracted biopolymer is generally harvested by chemical precipitation and centrifugation. Methanol, ethanol or acetone is used for purification of extracted biopolymer. Novak and Haugan (1981) found that the concentration of biopolymer achieved with acetone precipitation was more than that of ethanol precipitation.

Centrifugation methods have commonly been used to strip out extra-

Table 2-2. Exobiopolymer yields from activated sludge reported for different extraction techniques

Extraction method	Modification	Yield, % TSS	Reference
Centrifugation	Sharples high-speed centrifuge	5.8	Wase & Balasundaram (1980)
Centrifugation	15 min at 36 500 x g	2.6-5.4	Kiff & Thompson (1979)
Centrifugation	15 min at 13 200 x g	2.17-4.17[a] 10.97-18.01[b]	Gehr and Henry (1983)
Boiling	successive 10-min extractions	1.70 ± 1.05[c] 4.25 ± 0.31[d]	WPRL (1971)
Heat	1 hour at 80°C, centrifuged 15 min at 10 000 x g	3.4-3.6	Kiff & Thompson (1979)
Heat	1 hour at 80°C	1.13-6.47	Kiff (1978)
Heat	1 hour at 100°C	4.6 (0.9-9.1)	Clarke and Forster (1982)
Steaming	ultrasonication pretreatment, 10 min steaming in autoclave	4.7 (4.2-5.2)	Brown & Lester (1982)
Alkaline extraction	0.1 N NaOH 2.0 N NaOH	1.12 4.55	Sato and Ose (1975)
Boiling and alkaline extraction	ethanol precipitation	2.7	Wallen & Davis (1972)

[a] sludge samples from plant treating domestic wastewater

[b] sludge samples from pilot plant treating synthetic wastewater with glucose as primary energy source

[c] samples from "conventional activated sludge treatment plant"

[d] samples from three different activated sludge plants

cellular biopolymer by shear forces generated during high speed centrifugation (e.g., 33 000 x g) (Lewin 1956, Wilkinson 1958, Pavoni et al. 1972, 1974, Kiff and Thompson 1979, Brown and Lester 1980, Novak and Haugan 1981). Centrifugation was recommended as the most reliable technique for the extraction of exobiopolymer of free bacterial cells in suspension (Brown and Lester 1980). However, this technique was able to extract only a small portion of the exobiopolymer of activated sludge because the vast majority of flocs settled before maximal shear force was attained (Brown and Lester 1980, Novak and Haugan 1981).

Heat extraction (steaming and boiling) has also been widely used for the extraction of microbial biopolymers (Wallen and Davis 1972, Kiff and Thompson 1979, Brown and Lester 1980, Clarke and Forster 1982, Rudd et al. 1983). The main mechanism involved in this method is the hydrolysis of exobiopolymers by elevated heat. The boiling technique was reported to be capable of achieving a reproducible, quantitative extraction of the exopolymers of activated sludge (Clarke and Forster 1982). The amount of exopolymer extracted by this technique was shown to be related to the settling properties of the sludge (Clarke and Forster 1982). However, Kiff and Thompson (1979) reported that the polymer obtained by this method contained significant amounts of intracellular materials, and was actually denatured. The steaming technique was developed as an alternative to boiling because of the destructive effect of boiling on cells, and was recommended for the extraction of the exopolymers of activated sludge (Brown and Lester 1980). Recently, Rudd et al. (1983) demonstrated the effectiveness of various extraction methods by comparing the released carbohydrate concentration with the estimated total carbohydrate present in the exopolymer, and also by comparing the ratio of

protein to carbohydrate in extracted exopolymer. They concluded that the steaming technique was more reliable than the boiling technique.

The solubility of fibrillar biopolymers is increased in alkaline solution. Thus, many researchers have used sodium hydroxide solution for the hydrolysis of exobiopolymers (Takiguchi 1968, Takiguchi 1972, Sato and Ose 1975, Sato and Ose 1980, Brown and Lester 1980, Rudd et al. 1983). Although sodium hydroxide treatments released significant quantities of hexose sugar from activated sludge, this method was thought to cause a vast amount of cellular disruption (Farrah and Unz 1976, Brown and Lester 1980). Sato and Ose (1980) demonstrated that the chemical composition of extracted exobiopolymers varied in relation to the applied concentration of sodium hydroxide. It was noted by Brown and Lester (1980) that alkaline hydrolysis gave relatively high yields of various polymers (e.g., carbohydrates, proteins, nucleic acids), whereas its average relative standard deviation was similar to that of the steaming extraction and ethylenediaminetetraacetic acid (EDTA) extraction methods for activated sludge, synthetic activated sludge and K. aerogenes culture (Brown and Lester 1980). Rudd et al. (1983) suggested that rapid hydrolysis methods, such as alkaline treatment or heat treatment, are useful for estimating the relative production of carbohydrates in different sludges.

EDTA solution was successfully used to deflocculate yeast (Stahl et al. 1983) and Flavobacterium spp. (Endo et al. 1976, Endo and Takahashi 1980, 1981). Divalent cations (e.g., Ca^{2+}), which are an important factor in the bioflocculation of microorganisms, can be removed by chelating agents, such as EDTA. However, Brown and Lester (1980) showed that extracted biomass was severely damaged by this method, releasing

vast amounts of intracellular biopolymers.

Physical turbulence generated by ultrasonication or homogenization has been used to destabilize aggregated microorganisms prior to enumeration of activated sludge bacteria (Pike et al. 1972). Kiff and Thompson (1979) suggested that sonication was a very effective method of deflocculation, and recommended that a combination of low level sonication with high speed centrifugation would possibly be the ideal method for extracting exobiopolymers. However, Brown and Lester (1980) observed that only a part of the total exopolymer was extracted by this method. They suggested that this combined method would be useful as a preliminary treatment, as it avoided significant cellular destruction. Homogenization was tried as a technique for stripping biopolymer from biomass (Kiff and Thompson 1979, Rudd et al. 1983). This method would probably cause less damage to microorganisms than most alternative techniques, but the yield of extracted biopolymer was low.

Kiff and Thompson (1979) obtained significant amounts of extracellular biopolymer with a "bel" cream-maker, by which the sample was extruded twice under pressure through a narrow orifice. They reported that polymer yield by this shear press method was comparable to heat extraction and possessed reasonable reproducibility.

Novak and Haugan (1981) found that qualitatively different biopolymers could be obtained by repeated elution of activated sludge with distilled water which removed divalent cations. This elution method was not considered to give quantitative results. Rudd et al. (1983) examined ion-exchange with Dowex resin as a method to obtain exobiopolymers quantitatively. The principle of this technique is that divalent cations are exchanged for sodium ions of Dowex chemical (Dowex 50-X8, Na^+ form).

This chemical does not cause denaturation of proteins by heat or excess pH, removes the various divalent cations, and reduces excessively disruptive effects. They observed that the addition of Dowex chemical to any extraction method could increase the polymer yield.

2.4. Algal Bioflocculation Experience

2.4.1. Laboratory scale systems

The harvesting of algae from water and wastewater was investigated with synthetic organic polyelectrolytes, e.g., cationic, anionic, or nonionic polymers (Cohen et al. 1958, Golueke et al. 1964, Tenney et al. 1969). Their results indicated that good flocculation was achieved using cationic polyelectrolytes, whereas no significant flocculation was observed with anionic or nonionic polyelectrolyte at neutral pH. Tenney et al. (1969) reported that the concentration of a cationic polyelectrolyte required for 50% flocculation of mixed algal culture reached a minimum during the declining growth phase of cultures. They thought that the initial exopolymer accumulated on the surface of the algae could promote floc formation, while excess exopolymer accumulated in later growth phases could act as a protective colloid. Pavoni et al. (1971, 1974) observed that algal flocculation directly coincided with algal exopolymer production. They found that floc formation was restricted to the declining growth phase of cultures and that the surface charge of algae remained negative throughout all growth phases. They concluded that the surface coverage relationship was the main mechanism of the algal bioflocculation and that reduction of surface potential was not prerequisite to bioflocculation. Avnimelech et al. (1982) reported that the extracellular polymers of Anabaena spp. could enhance floc formation and sedimentation in natural environments by forming a network structure

and adsorbing clay particles.

Rao et al. (1974) suggested that the intracellular polymers released by algal cells may have an important role in algal bioflocculation. They showed that over 80% removal of dispersed algae was achieved by bioflocculation which was induced by blending.

Chlorella spp. were observed to occur in clusters in an axenic culture which was maintained under alkaline conditions (pH 9.5). In this case, the key feature of aggregated cells was an incompletely ruptured mother cell membrane which was observed to be multi-layered (Malis-Arad et al. 1980, Malis-Arad and McGowan 1982).

Another type of algal bioflocculation was successfully achieved in activated algae systems (activated sludge and flocculating algae-bacteria system) using laboratory scale reactors (McKinney et al. 1971, Humenik and Hanna 1971, McGriff and McKinney 1972, John and Bokil 1979, Gupta and Rao 1980, Nambiar and Bokil 1981, Bokil and John 1981). With the continuous feeding of raw or synthetic sewage, flocculating algal-bacterial systems were developed by addition of activated sludge to algal culture medium (e.g., 490 mg/l of activated sludge and 260 mg/l of algae). John and Bokil (1979) reported that the optimum ratio of algae to bacteria for flocculation to take place was 60:40 (w/w). However, this system was not completely successful in field operations because of difficulties in balancing the quantities of algal and bacterial biomass. The mechanism of flocculation in this system is thought to be bridging and entrapment of algae by activated sludge.

2.4.2. Pilot and field scale systems

Oswald et al. (1978) reported that Scenedesmus spp. grown in a continuously mixed, high-rate pond system treating domestic wastewater

in Manila, the Philippines, settled quickly when transferred to quiescent conditions. Subsequently, Eisenberg et al. (1981) observed that bioflocculating Micractinium spp. predominated in continuously mixed, high-rate ponds treating domestic sewage at Richmond, California. Development of flocculent, readily settleable algal cultures in both of these systems was attributed to gentle flow-mixing imparted by paddlewheels. Mixing energy requirements were low (approximately 15 kWh/ha·d) because flow velocities of only 10-15 cm/s were needed. Removals of algal suspended solids by sedimentation ranged from 69-82% in the Manila study (Oswald et al. 1978). Eisenberg et al. (1981) reported that biomass removals obtained by settling the flocculent Richmond cultures in quiescent ponds averaged 79-80% during a 16-month period.

3. MATERIALS AND METHODS

3.1. Description of Field System

Principal components of the field system included raw waste generation, anaerobic/facultative treatment, photosynthetic oxygenation, and algae harvesting (Fig. 3-1). Flushed wastes from pigs housed over concrete slab or slatted floors were regularly discharged to anaerobic treatment (digesters or lagoon) after temporary storage in underground pits. The wastewater discharge rate was variable depending on the number and age of pigs in residence at the barns. Another factor affecting the discharge rate was ambient temperature. Cooling sprays employed during hot weather greatly increased wastewater flows. Based on an average population of 260 animals at mid-cycle and an average wastewater solids concentration of 1%, the estimated flow was 8.7 m^3/d.

The anaerobic digestion system consisted of a settling tank, conventional digester and fixed bed reactor. A portion (2.8-3.2 m^3/d) of the flushed wastes from the swine confinement buildings was pumped to the settling tank, which was 15 m^3 in volume and constructed of steel. Settled solids from this tank were fed to the anaerobic digester at a rate of 0.9-1.3 m^3/d. The digester was made from a 20 m^3 polyolefin tank and was unmixed and unheated. Its loading rate ranged from 0.14-1.98 kg volatile solids (VS)/$m^3 \cdot d$ and averaged 0.67 kg VS/$m^3 \cdot d$. Supernatant from the settling tank (1.9 m^3/d) was fed to the fixed bed reactor, which was operated at ambient temperature. This unit was constructed from a 20 m^3 polyolefin tank and was filled with cypress wood chips which acted as support media for anaerobic bacteria. Its loading rate (empty bed volume basis) ranged from 0.30-1.92 kg VS/$m^3 \cdot d$ and averaged 1.08 kg VS/$m^3 \cdot d$.

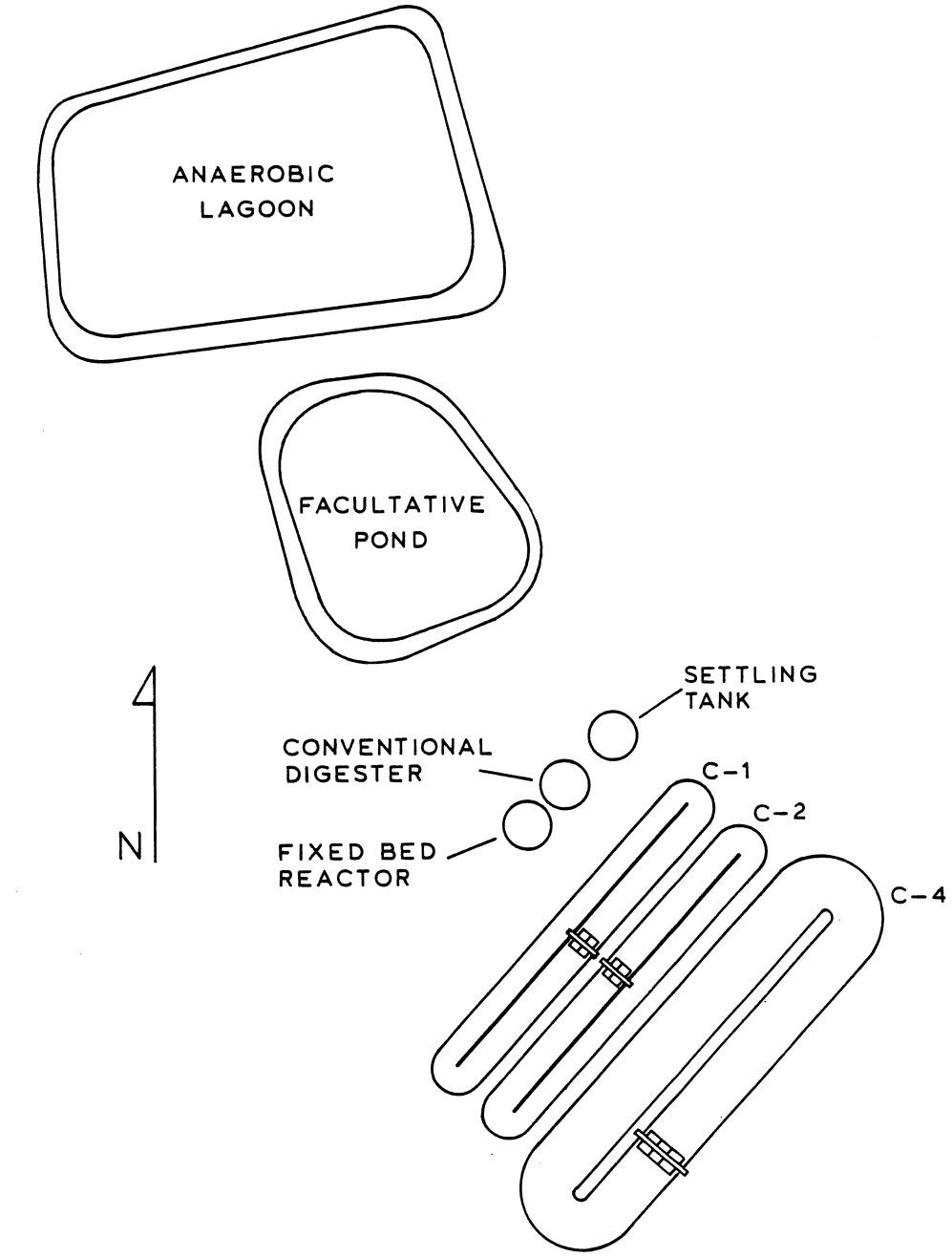

Figure 3-1. Principal components of field system

Excess effluent flows from the digestion system, as well as flushed wastes not pumped to the settling tank, were discharged to the anaerobic lagoon. This impoundment had a depth of 4.9 m and surface area of 1 200 m^2. Overflow from the anaerobic lagoon was received by a facultative pond which averaged 1.0 m in depth and had a surface area of 800 m^2. Effluents from the anaerobic digestion system, anaerobic lagoon and facultative pond were used as alternative feed sources for the photosynthetic oxygenation stage (high-rate pond) and algae harvesting stage (bioflocculation ponds).

The high-rate pond (designated C-4) was excavated in heavy clay soil and had a length of 46 m and surface area of 600 m^2. A racetrack configuration was imparted by a center baffle which extended for most of the pond's length (40 m). Flow-mixing at a velocity of 20 cm/s was achieved by means of an electric motor driven paddlewheel. Mixing was carried out for 30 min three times weekly, coinciding with periods of waste loading. Operating depth of the high-rate pond varied from 0.2-0.4 m. The bioflocculation ponds (designated C-1 and C-2) were identical concrete lined channels, each 38 m long and 170 m^2 in surface area, with center baffles arranged to give a racetrack configuration. Mixing schedules and waste loading were varied according to the experiment in progress. Operating depth ranged from 0.2-0.5 m.

Infestations of zooplankton (e.g., Brachionus rubens, Diaphanosoma brachyurum) were controlled in the high-rate and bioflocculation ponds by addition of concentrated (23% as N) ammonium hydroxide solution. The effect of these additions was to raise the free ammonia concentration. The target free ammonia concentration was 21 g/m^3, a value sufficient to kill virtually all zooplankton without adversely affecting the algal

population (Lincoln et al. 1983). NH_4OH solution was dosed over a period of approx. 10 min while the pond being treated was mixed.

3.2. Summary of Experiments

A total of 12 field experiments were conducted between November 1983 and June 1985. Experimental variables and conditions are summarized in Table 3-1.

3.3. Experimental Procedures

The general experimental procedure was to fill the bioflocculation ponds with mature culture medium, monitor the channels until bioflocculation took place, and then drain and clean the ponds thoroughly in preparation for the next experiment. Specific procedures for each experiment are given below.

3.3.1. Experiment 1

Culture medium from the high-rate pond was transferred to the bioflocculation ponds, giving each an initial depth of 32 cm. C-2 was mixed at 16 cm/s whereas C-1 was not mixed. Anaerobic digester effluent was added to each pond on two occasions. After termination of this experiment, C-2 only was drained and cleaned. The depth of C-1 at termination was 41 cm, with the increase over initial depth caused by interception of rainwater.

3.3.2. Experiment 2

Culture medium from C-1 was divided equally between the bioflocculation ponds, giving each an initial depth of 20 cm. As before, C-2 was mixed at 16 cm/s and C-1 was not mixed. One dose of anaerobic digester effluent and two doses of fixed bed reactor effluent were applied to each

Table 3-1. Summary of experiments

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

pond through the course of the experiment. At the termination date, C-1 only was drained and cleaned.

3.3.3. Experiment 3

Culture medium from C-2, supplemented with additional medium from the facultative pond, was used to fill the bioflocculation ponds to an initial depth of 27 cm. The relative proportions of medium from these two sources were 83% and 17%, respectively. Fixed bed reactor effluent was added to each pond on two occasions. C-2 was initially dosed with 22.5 kg sodium carbonate to give an added carbonate-carbon concentration of 50 g/m^3. C-1 and C-2 were each mixed at 19 cm/s, the flow velocity employed also in subsequent experiments.

3.3.4. Experiment 4

The bioflocculation ponds were filled to an initial depth of 31 cm with culture medium from the high-rate pond. C-1 was mixed and C-2 was not mixed.

3.3.5. Experiment 5

Culture medium was obtained from the high-rate pond. Initial depth in the bioflocculation ponds was 37 cm. C-2 was loaded with 0.5 m^3 fixed bed reactor effluent twice weekly, whereas C-1 was not loaded. Both ponds were mixed.

3.3.6. Experiment 6

Culture medium was obtained from the high-rate pond. Initial depth in the bioflocculation ponds was 35 cm. C-1 and C-2 were loaded with 0.2 m^3 and 0.6 m^3 fixed bed reactor effluent, respectively, three times weekly. Both ponds were mixed.

3.3.7. Experiment 7

Following termination of experiment 6, mixing was turned off in C-1 and sedimentation allowed for 24 h. Simultaneously, C-2 was drained and cleaned. C-2 was filled to a depth of 11 cm with supernatant from C-1. Additional supernatant was discarded until 11 cm remained in C-1, containing most of the bioflocculated algae from the previous trial. The bioflocculation ponds were brought up to a depth of 44 cm with culture medium from C-4. Both ponds were mixed. Each received a volume of 0.2 m^3 fixed bed reactor effluent three times weekly.

3.3.8. Experiment 8

A mixture of 80% culture medium from the facultative pond and 20% medium from the high-rate pond was used to fill the bioflocculation ponds to an initial depth of 30 cm. Sulfuric acid was added periodically to C-2 to reduce its pH to 6.0 or less. Both ponds were mixed and each received 0.2 m^3 fixed bed reactor effluent three times weekly.

3.3.9. Experiment 9

This trial was begun with culture medium carried over from experiment 8. The bioflocculation ponds were not intermixed, and no new medium from the facultative or high-rate ponds was added. Instead, anaerobic lagoon effluent, containing a dense population of the purple sulfur bacteria Thiopedia rosea, was added to C-2 on two occasions. Fixed bed reactor effluent was also added to the bioflocculation ponds. Both ponds were mixed. Initial depth in C-1 and C-2 was 28 cm and 30 cm, respectively.

3.3.10. Experiment 10

Culture medium from the facultative pond and high-rate pond were used in relative proportions of 69% and 31% to fill the bioflocculation

ponds to a depth of 36 cm. Each pond was loaded three times weekly: C-1 with 0.3 m^3 of settled waste supernatant and C-2 with 0.3 m^3 of fixed bed reactor effluent. Both ponds were mixed.

3.3.11. Experiment 11

The bioflocculation ponds were filled to a depth of 37 cm with medium from the high-rate pond. C-1 was loaded with 0.45 m^3 fixed bed reactor effluent three times weekly, whereas C-2 was not loaded. Both ponds were mixed.

3.3.12. Experiment 12

Culture medium from the facultative and high-rate ponds was used in relative proportions of 58% and 42%, respectively, to fill the bioflocculation ponds to an initial depth of 46 cm. C-1 was mixed at 14 cm/s and C-2 was mixed at 30 cm/s. Both ponds received 0.45 m^3 fixed bed reactor effluent three times weekly.

3.4. Analytical Methods

3.4.1. Algal counts

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

per grid corresponds to 2.9×10^8 cells per 1. Counts of algae other than Chlorella were normalized to Chlorella equivalents by multiplying them by the appropriate biovolume ratios.

3.4.2. Residue, organic matter and nutrients

Analyses of residue and organic matter were carried out according to APHA (1980). These included total solids (209A), volatile solids (209E), total suspended solids (209D), volatile suspended solids (209G), and chemical oxygen demand (508A). Filtrations were on Whatman GF/C glass fibre filters which have an average pore opening of 1.2 µm. Ammonia and total Kjeldahl nitrogen were determined according to the distillation (00610) and digestion (00625) procedures described in EPA (1974). Total phosphorus was determined by the persulfate digestion procedure (00665) of EPA (1974).

3.4.3. Chlorophyll a, visibility, exopolymers, settleable matter and settling efficiency

Chlorophyll a was determined by a modification of the method of Talling and Driver (1963). 10 ml of sample was centrifuged for 10 min at 2 400 x g and the centrate poured off. 10 ml of boiling 90% methanol was then added, the tube capped, and the pellet disrupted by vortexing for 15 s. Extraction of chlorophyll a was continued by placing the centrifuge tube in a 70°C water bath for 45 s. Then, the sample was 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 the following equation:

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

where Chl *a* has units of mg/l, D_{665} = optical density at the absorption maximum for Chl *a* (665 nm), D_{750} = optical density at 750 nm, to correct for turbidity, l = path length (cm), v = volume of extract (ml), and V = volume of sample (ml).

Visibility was measured using a 15-cm diameter secchi disc. The depth at which the disc disappeared from sight as it was lowered in the culture was taken as the visibility.

Exopolymers were determined according to the technique of Ueda (1963). A 45 ml sample was shaken vigorously for 20 s and centrifuged at 10 000 x g for 20 min. 30 ml centrate was combined with 60 ml ethanol in a vial and stored at 4°C for 24-48 h. The resulting precipitate (whitish or yellowish material) was separated by centrifugation at 10 000 x g for 10 min. Following centrifugation, centrate was discarded and the pellet transferred with several washings of distilled water to a pre-weighed crucible. Additional weighings were taken after 24 h drying at 103°C and 15 min ashing at 550°C. Biopolymer was expressed in terms of ash-free dry weight per unit volume (g/m^3).

Settleability of algae was determined by retaining a sample of culture medium in a 1 litre Imhoff cone over a 24 h period in the absence of light. Accumulation of algae on the sides of the cone was minimized by gently swabbing the inside of the cone with a rod after an initial settling period of 2 h. Volume of settled matter at the bottom of the cone was measured after an additional 22 h of retention. Decrease of algal biomass in the medium was calculated by comparing the initial Chl *a* concentration of the sample to the Chl *a* concentration of a 150 ml supernatant sample taken at the end of the 24 h settling period.

3.4.4. Dehydrogenase activity, pH and dissolved oxygen

Dehydrogenase activity was measured by a modification of the technique of Koopman et al. (1984). Sample pH was adjusted to 8.7 with 0.1N sodium hydroxide. Triplicate 10 ml aliquots were amended with 1.0 ml 0.2% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5- phenyltetrazolium chloride (INT) (Eastman Kodak) and incubated at room temperature (22 ± 2°C) in the dark. Incubation was terminated by adding 1 ml 37% formaldehyde. INT-formazan (INTF) formed during the incubation period was extracted according to the following procedure. 10 ml INT-treated sample was placed in a centrifuge tube and centrifuged at 1500 x g for 20 min. Centrate was decanted, leaving a pellet approximately 0.1 ml in volume, and replaced with 10 ml 4+6 tetrachloroethylene/acetone. The tube was capped, and vortexed for 15 s. Extraction was continued in the dark for 30 min. Extract was clarified by centrifugation at 1500 x g for 20 min and optical density of the extract determined at 490 nm. INT-dehydrogenase activity (INT-DHA) was calculated according to the following equation (Lopez et al. 1986):

$$\text{INT-DHA} = \frac{1024\ D_{490}\ v}{V\ t\ F} \qquad (3\text{-}2)$$

where INT-DHA is expressed in units of equivalent oxygen uptake ($g\ O_2^*/m^3 \cdot d$), v = volume of extract (ml), V = volume of sample (ml), t = incubation time (min), and F = factor to account for sample dilution by INT and formaldehye (0.833).

pH measurements were made with an Orion Model 601A electrode/ analyzer system. Dissolved oxygen (DO) was determined with a YSI Model 54A oxygen meter and polarographic electrode. The DO and pH of the

bioflocculation ponds were measured at a depth of 0.05 m between 2 pm and 5 pm local time.

4. RESULTS

4.1 Pond System Operation

Detailed information about the quality characteristics of various waste streams used to feed or supplement the algal cultures, as well as specific operational data, are given in this section. Results of the bioflocculation experiments are described beginning in section 4.2.

4.1.1. Settling tank and digesters

The quality characteristics of the underflow (settled waste solids) and overflow (settled waste supernatant) from the settling tank are given in Tables 4-1 and 4-2, respectively. Underflow total solids (TS) were variable with season. Highest concentrations were experienced in the winter (e.g., 32 480 g/m^3 in January 1985) whereas lowest concentrations occurred during the summer (e.g., 4 000 g/m^3 in June 1985). The tendency of the underflow TS to decrease during the summer was attributed to the use of cooling sprays for the animals with concomitant dilution of the raw waste stream. The TS of settled waste solids averaged 19 430 g/m^3.

Volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and ammonia N in settled waste solids varied approximately in proportion to TS. Volatile solids were approximately 75% of total solids. COD and TKN averaged 37 020 g/m^3 and 2 030 g/m^3, respectively, yielding a COD:TKN ratio of 18.2.

TS of settled waste supernatant also varied on a seasonal basis. On the average, the supernatant fraction was roughly one-half as concentrated as the settled solids fraction in terms of TS and VS. Its average concentrations of COD and TKN were 73% and 85%, respectively, of those in

Table 4-1. Monthly average characteristics of settled waste solids[a]

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Oct 83	19 830	14 940	27 650	1 840	1 200
Nov	24 050	16 730	52 880	3 580	3 220
Dec	--	--	--	--	--
Jan 84	47 500	38 110	36 900	2 880	2 520
Feb	17 030	12 650	38 980	2 470	1 640
Mar	18 550	14 200	42 380	2 170	1 650
Apr	15 290	11 570	42 650	1 810	1 570
May	8 080	5 350	14 850	1 100	913
Jun	7 140	4 110	15 840	1 250	983
Jul	13 460	9 590	27 070	1 390	980
Aug	9 680	6 640	19 000	924	699
Sep	13 500	10 010	27 180	1 225	723
Oct	25 140	19 850	51 670	2 000	1 350
Nov	23 800	18 980	48 470	2 030	1 520
Dec	24 640	18 520	50 040	2 755	2 100
Jan 85	32 480	24 810	66 810	3 330	2 780
Feb	18 870	13 750	43 250	2 370	1 750
Mar	26 420	20 090	50 290	2 900	2 130
Apr	25 320	18 870	49 350	2 625	1 760
May	13 820	10 160	26 150	1 470	940
Jun	4 000	2 600	8 910	479	339
MEAN	19 430	14 580	37 020	2 030	1 540
S.D.	9 950	8 130	15 460	838	741

[a]Values in g/m^3

Table 4-2. Monthly average characteristics of settled waste supernatant[a]

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb 84	10 670	6 530	22 680	1 970	1 600
Mar	10 620	7 010	32 840	2 220	1 440
Apr	10 510	7 230	37 980	1 560	1 340
May	10 940	8 120	25 480	1 460	1 210
Jun	12 210	8 600	20 350	1 400	1 000
Jul	6 760	4 100	8 670	1 110	963
Aug	3 910	1 940	5 610	927	808
Sep	7 010	4 605	20 940	945	749
Oct	11 010	7 715	28 740	1 480	1 180
Nov	15 210	11 600	37 740	1 610	1 180
Dec	18 030	12 820	40 510	2 470	1 900
Jan 85	15 850	11 110	43 120	2 470	2 300
Feb	18 420	13 070	33 550	2 920	1 925
Mar	16 470	11 420	41 880	2 660	1 995
Apr	17 670	12 480	39 050	2 490	1 880
May	4 750	4 460	15 100	1 130	850
Jun	3 400	2 170	4 355	479	353
MEAN	11 380	7 940	26 980	1 720	1 340
S.D.	5 000	3 690	12 930	712	533

[a]Values in g/m^3

the solids fraction. The average COD:TKN ratio of settled waste supernatant was 15.7.

The volumetric feed rate of settled waste solids to the conventional digester was initially 1.0 m^3/d (Oct. 83-Jun. 84) and was raised subsequently to 1.3 m^3/d (Jul. 84-Jun. 85). Corresponding liquid detention times were 20 and 15 days, respectively. Mean organic loading was 0.670 kg VS added daily per m^3 of liquid volume (kg $VS_a/m^3 \cdot d$), as shown in Table 4-3. The range of organic loadings (0.144-1.980 kg $VS_a/m^3 \cdot d$) was large because of the variablity in feed concentration. Digester temperature ranged from 13.5-29.7°C, averaging 22.6°C.

The fixed bed reactor (FBR) was fed settled waste supernatant at a constant volumetric rate of 1.90 m^3/d, giving an empty bed detention time of 10.5 days. Organic loading rates on an empty bed volume basis ranged from 0.300-1.917 kg $VS_a/m^3 \cdot d$, averaging 1.085 kg $VS_a/m^3 \cdot d$ (Table 4-3). The fixed bed reactor was started later in spring 1983 than the conventional digester, hence its average temperature (24.2°C) was somewhat greater. The range of operating temperatures was 15.6-30.4°C.

Monthly average characteristics of effluents from the conventional digester and fixed bed reactor are given in Tables 4-4 and 4-5. Pollutant levels in both waste streams were least in summer months and greatest during winter months. TS ranged from 5 550-38 410 g/m^3 in digester effluent and from 2 060-13 620 g/m^3 in FBR effluent. Overall average TS in the two flows were 17 540 g/m^3 and 9 050 g/m^3, respectively. COD and TKN concentrations in digester effluent averaged 23 390 g/m^3 and 2 100 g/m^3, respectively. Concentrations of these two parameters in FBR effluent averaged 17 260 g/m^3 and 1 680 g/m^3, respectively. Mean COD:TKN ratios were 11.0 in digester effluent and 10.3 in FBR effluent.

Table 4-3. Loading and temperature of anaerobic digester and fixed bed reactor

Month	Anaerobic digester		Fixed bed reactor	
	Loading, kg VS_a/m^3 d	Temp., °C	Loading,[a] kg VS_a/m^3 d	Temp., °C
Oct 83	0.751	24.6	--	--
Nov	0.870	19.4	--	--
Dec	(b)	13.6[c]	--	--
Jan 84	1.980	13.5	--	--
Feb	0.657	16.9	1.166	15.6[c]
Mar	0.714	19.0	1.212	17.2
Apr	0.541	21.4	1.162	24.2
May	0.242	26.7	1.263	27.1
Jun	0.144	28.5	0.973	29.5
Jul	0.658	28.8	0.709	29.8
Aug	0.395	29.5	0.300	30.4
Sep	0.567	27.4	0.658	28.3
Oct	1.134	25.4	1.156	27.0
Nov	1.076	19.8	1.588	22.1
Dec	0.578	17.8	1.772	19.0
Jan 85	0.544	15.0	1.152	16.3
Feb	0.427	15.2	1.917	17.4
Mar	0.661	21.1	0.940	22.4
Apr	0.670	24.1	1.438	25.5
May	0.604	27.8	0.668	28.8
Jun	0.178	29.7	0.375	30.0
MEAN	0.670	22.6	1.085	24.2
S.D.	0.400	5.3	0.452	5.3

[a] Empty bed volume basis

[b] Digester not operated during December 1983

[c] Ambient temperature

Table 4-4. Monthly average characteristics of anaerobic digester effluent[a]

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Oct 83	32 060	23 770	34 620	2 450	1 620
Nov	31 030	22 090	38 580	3 220	2 380
Dec	--	--	--	--	--
Jan 84	24 960	17 720	27 240	3 520	2 550
Feb	38 410	29 730	50 990	3 690	2 740
Mar	25 990	19 770	41 920	2 430	1 950
Apr	16 390	12 360	38 390	1 915	1 290
May	10 720	8 195	21 920	1 350	1 180
Jun	8 960	5 495	11 680	1 305	1 040
Jul	8 030	4 870	9 730	1 180	930
Aug	6 630	3 970	8 080	1 180	962
Sep	7 550	4 810	7 360	987	686
Oct	12 180	8 600	14 540	1 690	1 095
Nov	13 160	10 480	18 950	1 620	1 160
Dec	18 120	13 330	26 670	2 200	1 490
Jan 85	16 320	11 570	19 880	2 440	1 820
Feb	20 010	14 370	30 350	2 660	1 980
Mar	21 320	14 780	23 510	2 980	2 120
Apr	19 910	13 460	22 660	2 890	2 030
May	13 590	8 980	16 080	1 995	1 390
Jun	5 550	3 430	4 630	835	570
MEAN	17 540	12 590	23 390	2 130	1 550
S.D.	9 240	7 180	12 760	859	623

[a]Values in g/m^3

Table 4-5. Monthly average characteristics of fixed bed reactor effluent[a]

Month	Total Solids	Volatile Solids	Chemical Oxygen Demand	Total Kjeldahl Nitrogen	Ammonia Nitrogen
Feb	10 660	5 830	23 110	1 970	1 700
Mar 54	10 620	7 010	33 090	2 220	1 440
Apr	10 510	7 230	35 980	1 560	1 340
May	10 940	8 120	25 480	1 460	1 210
Jun	12 210	8 600	20 350	1 400	1 000
Jul	6 760	4 100	8 670	1 110	963
Aug	3 910	1 940	5 610	927	808
Sep	3 220	1 645	2 870	731	668
Oct	7 580	5 100	10 720	1 380	1 130
Nov	10 300	7 500	14 210	1 490	1 070
Dec	13 620	9 810	17 990	2 170	1 620
Jan 85	11 450	7 855	23 420	2 580	2 340
Feb	10 370	6 825	17 840	2 250	1 750
Mar	11 540	7 565	16 050	2 550	1 890
Apr	14 090	9 400	22 730	2 550	1 925
May	4 080	2 160	9 510	1 590	1 260
Jun	2 060	981	5 840	584	514
MEAN	9 050	5 980	17 260	1 680	1 330
S.D.	3 740	2 830	9 490	639	490

[a]Values in g/m^3

4.1.2. Anaerobic lagoon and facultative pond

The anaerobic lagoon supported a dense population of the purple sulfur bacteria (Chromatiacea), Thiopedia rosea. These bacteria commonly appeared as 1-2 μm diameter, spherical cells imbedded in flat sheets of capsular material, often containing intracellular sulfur deposits. For much of the project period, the lagoon medium was distinctly pink in appearance. At other times, it had a brownish-pink color. Thiopedia rosea were the most abundant microorganisms present regardless of the coloration of the medium.

Despite the fact that the lagoon received highly odorous raw and digested wastes, the lagoon medium itself was almost odorless. The efficient removal of odors was paralled by significant reductions in oxygen demand, total solids, and nitrogen. As shown in Table 4-6, mean COD and TKN concentrations were reduced to 1 300 g/m^3 and 450 g/m^3, respectively. Mean effluent phosphorus concentration was 120 g/m^3.

The photosynthetic microbial population of the facultative pond was dominated by microalge during summer and fall and by purple sulfur bacteria during winter and early spring. Further reductions in oxygen demand, nitrogen and phosphorus were achieved in the facultative pond (Table 4-6).

4.1.3. High-rate pond

Alternative feed sources for the high-rate pond (C-4) included effluents from the facultative pond, anaerobic digester, and fixed bed reactor effluent. As shown in Table 4-7, facultative pond effluent was the most significant source on a volumetric basis. However, when mass loading rates of organic matter and nutrients are considered, FBR effluent was most significant. For example, average COD loading from FBR

Table 4-6. Mean annual characteristics of anaerobic lagoon and facultative pond effluents[a]

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

[a]Coefficient of variation given in ( )

Table 4-7. Waste loading to high-rate pond[a]

Month	Waste source			
	Facul. pond	Anaer. dig.	Fixed bed react.	Other
Oct 83	--	0.13	--	--
Nov	6.10	0.27	--	--
Dec	--	--	--	--
Jan 84	--	0.12	0.31	--
Feb	--	--	0.88	--
Mar	--	--	0.64	0.10[b]
Apr	--	--	0.33	--
May	7.55	--	0.45	--
Jun	5.60	--	0.47	--
Jul	--	--	0.74	--
Aug	--	--	0.53	--
Sep	9.00	--	0.43	--
Oct	9.35	--	0.26	--
Nov	4.00	--	0.25	0.40[c]
Dec	1.06	--	0.37	--
Jan 85	0.97	--	0.55	0.01[d]
Feb	0.21	--	0.54	--
Mar	0.34	--	0.39	--
Apr	1.20	--	0.05	--
May	--	0.73	1.02	--
Jun	--	--	0.03	--
MEAN	2.16	0.06	0.39	0.02

[a] m^3/d

[b] C-1

[c] anaerobic lagoon

[d] settled waste supernatant

effluent was 6.7 kg/d, compared to a loading of 0.8 kg/d from facultative pond effluent.

Ammonium hydroxide (NH_4OH) was added to the high-rate pond on an intermittent basis to control grazing zooplankton (Table 4-8). The purpose of this chemical was to raise the free ammonia (NH_3) concentration to the target value of 21 g/m^3 established by Lincoln et al. (1983). NH_4OH had a dual action in this regard. First, by raising culture pH, it increased the relative proportion of free ammonia in the culture. Because total ammonia concentrations in the high-rate pond before NH_4OH addition were commonly on the order of 50-80 g/m^3 (Lincoln and Hill 1980), a pH of approximately 9.25 (i.e., the pK_a for NH_4) was sufficient to reach the target free ammonia concentration. The added chemical also contributed directly to the free ammonia concentration of the culture (Table 4-8), providing additional assurance that the target level was reached.

The major algal genera populating the high-rate pond medium were Chlorella, Monodus, and Synechocystis (Table 4-9). On a biovolume basis, Chlorella was dominant during 16 months of the 21 month period monitored, whereas Synechocystis was dominant in the other 5 months. Monodus, although second in overall importance to Chlorella, did not predominate during any of the months studied. Phototrophic bacteria (Thiopedia rosea) were commonly observed, but were of minor importance on a biovolume basis. Peak populations of these bacteria were recorded in May 1984 and again in May 1985. During the latter month the high-rate pond received a very heavy loading of FBR effluent.

4.1.4. Bioflocculation ponds

Waste loading to the bioflocculation ponds was varied according to

Table 4-8. Ammonium hydroxide additions to high-rate pond for grazer control

Date	NH_4OH added g N/m^3	pH	Free NH_3[a] g N/m^3
5 Oct 83	32	8.6	6
14 Oct	39	9.1	16
25 Oct	29	9.4	17
16 Mar 84	29	8.5	4
18 Apr	37	8.7	8
3 Jul	62	8.5	9
26 Jul	43	9.5	27
7 Sep	103	9.5	66
3 Oct	97	9.2	45
15 Oct	41	8.7	9
21 Jun 85	51	8.6	9

[a]Contributed directly by added NH_4OH

Table 4-9. Photosynthetic community composition of high-rate pond[a]

Month	Normalized cell count (#/grid)			
	Chlor.	Monod.	Synec.	Thiop.
Oct 83	1.7	0.2	47.9	1.3
Nov	47.4	0.9	7.1	3.4
Dec	25.7	1.4	0.5	0.1
Jan 84	4.3	0.6	0.1	tr
Feb	29.8	0.3	0.2	3.7
Mar	58.9	10.3	tr	0.2
Apr	20.9	1.5	45.7	7.1
May	41.4	14.6	21.1	15.8
Jun	30.4	19.6	44.5	4.8
Jul	4.5	1.3	68.1	6.1
Aug	0.8	0.1	46.1	0.9
Sep	73.6	22.5	19.9	1.0
Oct	45.3	31.7	1.2	3.6
Nov	59.3	10.2	tr	4.3
Dec	73.2	29.0	tr	4.3
Jan 85	103.0	58.0	tr	7.3
Feb	102.7	73.0	tr	7.7
Mar	161.5	81.5	tr	10.0
Apr	106.7	46.7	tr	3.0
May	66.0	29.3	2.3	41.1
Jun	133.5	16.0	2.2	1.4
MEAN	56.7	21.4	14.6	6.0

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

the experiment in progress (Table 4-10). In some cases the rate of loading or the type of waste added was the experimental variable studied. The feed type most commonly utilized was FBR effluent.

Grazing zooplankton populations were controlled by addition of ammonium hydroxide on an as-needed basis (Table 4-11). Equal volumes of NH_4OH solution were added to each pond except on day 12 of exp. 8. In this case, the experimental variable was reduced pH, and ammonium hydroxide was not added to the experimental culture (C-2) in order to avoid raising its pH.

Although added NH_4OH volumes were generally identical, slight variations in pond depth (and hence volume) caused the added concentrations to differ somewhat. Larger differences in the free ammonia concentration actually attained were caused by variations in initial culture pH, as indicated in Table 4-11, as well as variations in the initial total ammonia concentration, which were not measured.

During exp. 8, concentrated sulfuric acid was added to pond C-2 on an intermittent basis in order to reduce the culture pH to a target value of 6.0 (Table 4-12). The target pH was sometimes undershot, however, dropping to $\leq$ 4.5 in several instances. As discussed later, such low pH values did not inhibit the culture (at least, not permanently), but rather had an overall stimulatory effect.

4.2. Effect of Mixing

The effect of mixing on bioflocculation was investigated in 4 experiments. In three of these (exps. 1, 2, and 4), mixed cultures were compared to unmixed controls. In the fourth (exp. 12), the relative effect of two different mixing velocities was evaluated.

Table 4-10. Waste loading to bioflocculation ponds[a]

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

[a]Abbreviations for waste source:

SWS = settled waste supernatant
STR = anaerobic digester
FBR = fixed bed reactor
P-1 = anaerobic lagoon

Table 4-11. Ammonium hydroxide additions to bioflocculation ponds for grazer control

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

[a]Contributed directly by added NH_4OH

Table 4-12. Sulfuric acid additions to pond C-2 during experiment 8

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

4.2.1. Mixing versus non-mixing

An inhibitory effect of mixing on Synechocystis was observed in both exp. 1 (Fig. 4-1, Table 4-13) and exp. 2 (Fig. 4-2, Table 4-14). Counts of this alga in mixed cultures fell to less than 20% of those in unmixed controls over periods of one month in each trial. Declines in cell numbers were most rapid between the third and fourth weeks.

Characteristics of the mixed culture in exp. 1 changed dramatically during the first three weeks. Macroscopic flocs became visible after 13 days, and complete flocculation marked by aggregation and rapid settling of the cells occurred within 22 days (Fig. 4-3). This phenomenon was preceded by a decline in DO, from an average concentration 18 g/m^3 on days 0-7 to 5 g/m^3 on days 8-22. pH in the mixed culture showed a progressive decline, from 9.0 to 7.0 over days 0-22. In contrast, the unmixed culture showed no signs of flocculation. DO increased while pH remained relatively constant. Averages for these two parameters were 18 g/m^3 and 9.0, respectively.

Microscopic examination showed that flocs in the mixed culture consisted entirely of Synechocystis cells and ranged from 50 to several hundred microns in diameter (Fig. 4-4). The flocs at first retained the blue-green coloration typical of Synechocystis, but bleached noticeably with time.

In exp. 2, changes in the charactistics of the mixed culture were less pronounced. As before, macroscopic flocs appeared after approximately 13 days. However, they were fewer in number, and the high degree of culture settleabilty evidenced in the first trial was not repeated. Chlorella and Monodus, though initially negligible, developed

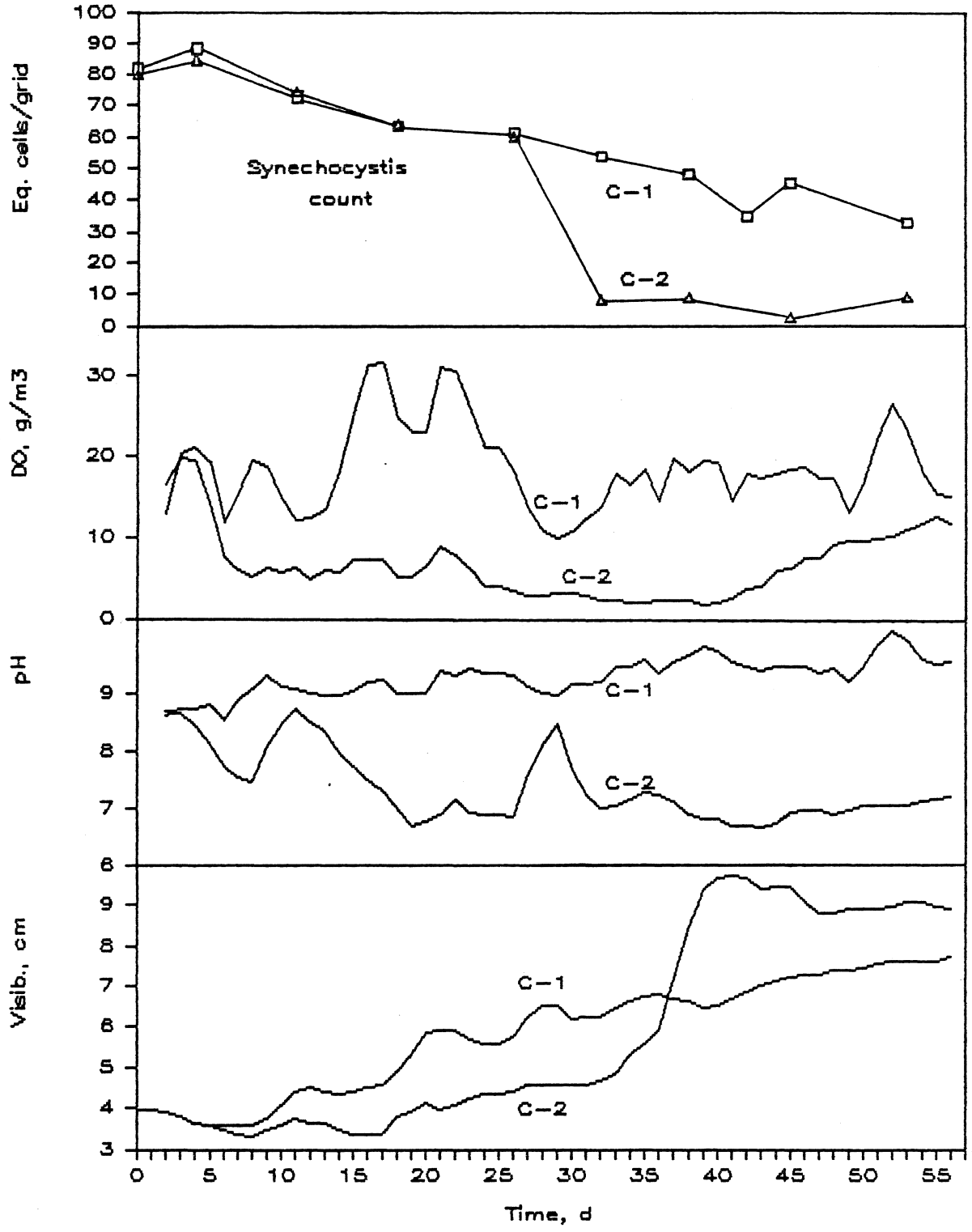

Figure 4-1. Temporal variation of Synechocystis, DO, pH and visibility in mixed (C-2) and unmixed (C-1) cultures. Exp. 1.

Table 4-13. Effect of mixing on photosynthetic community composition in bioflocculation ponds, exp. 1

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

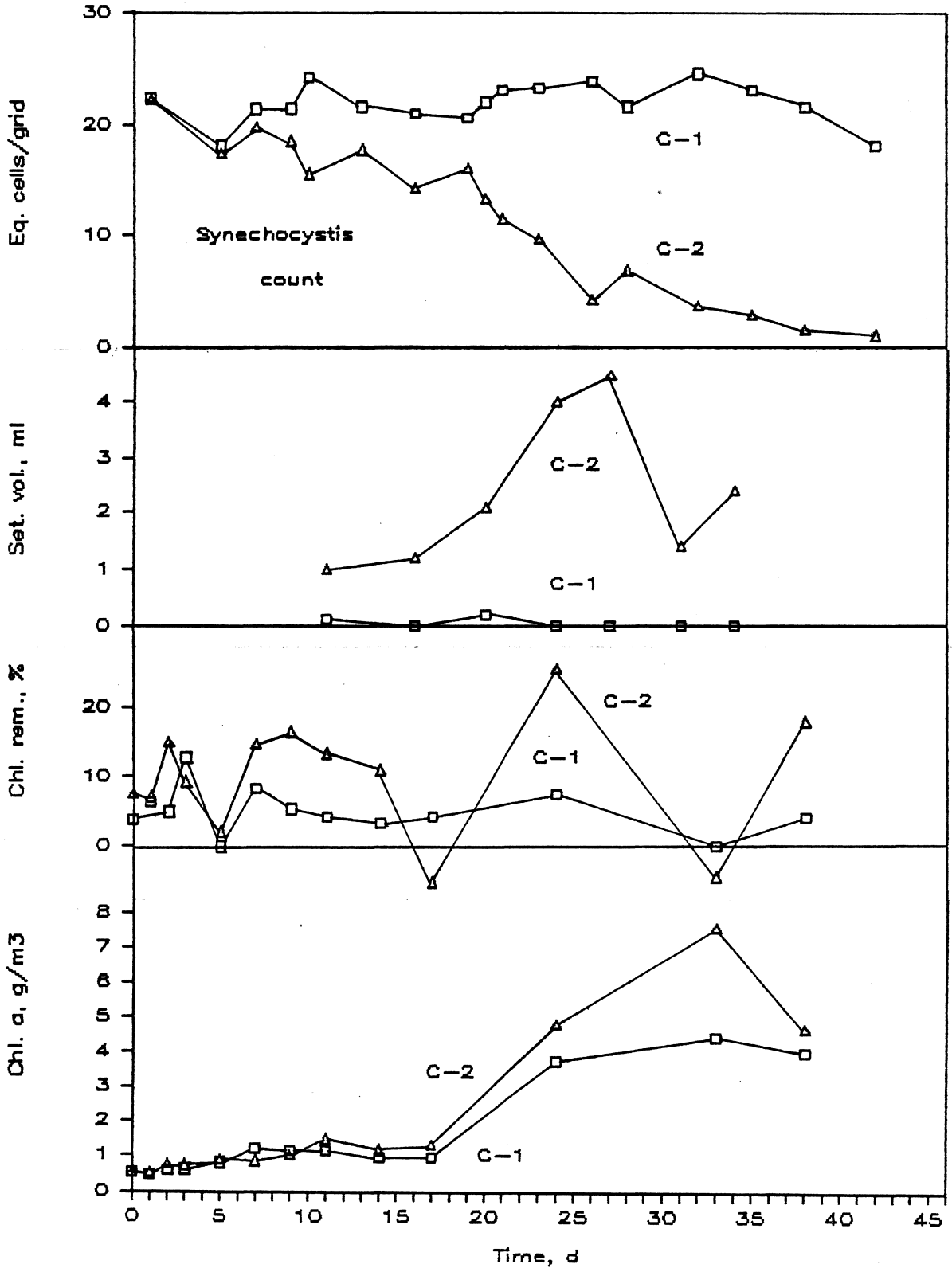

Figure 4-2. Temporal variation of Synechocystis, settleability, and chl. a in mixed (C-2) and unmixed (C-1) cultures. Exp. 2.

Table 4-14. Effect of mixing on photosynthetic community composition in bioflocculation ponds, exp. 2

Day	Pond	Normalized cell count (#/grid)		
		Chlor.	Monod.	Synec.
0	C-1	0.3	0.7	24.2
	C-2	0.3	1.1	23.2
1	C-1	0.7	1.4	22.5
	C-2	0.8	1.4	22.3
4	C-1	0.0	0.4	14.6
	C-2	0.0	0.6	14.6
5	C-1	0.7	1.0	18.1
	C-2	0.9	1.3	17.4
7	C-1	0.6	1.1	21.4
	C-2	0.8	2.4	19.8
9	C-1	0.4	1.1	21.4
	C-2	0.6	2.5	18.5
10	C-1	1.4	1.6	24.2
	C-2	2.0	4.8	15.6
13	C-1	1.4	1.6	21.6
	C-2	2.5	3.4	17.7
16	C-1	1.0	2.4	20.9
	C-2	5.7	8.4	14.3
19	C-1	5.4	3.9	20.5
	C-2	7.5	12.1	16.1
20	C-1	8.9	7.9	22.0
	C-2	8.1	16.2	13.3
21	C-1	11.4	7.5	23.1
	C-2	3.9	16.1	11.5
23	C-1	13.0	8.6	23.3
	C-2	13.8	24.7	9.7
26	C-1	11.9	8.6	23.8
	C-2	12.5	24.7	4.2
28	C-1	11.5	9.1	21.6
	C-2	11.3	20.0	6.8
32	C-1	14.8	9.5	24.5
	C-2	15.1	27.1	3.6
35	C-1	14.9	9.1	23.1
	C-2	14.2	24.5	2.9
38	C-1	17.0	10.2	21.5
	C-2	23.5	35.6	1.5
42	C-1	15.8	10.2	18.0
	C-2	23.0	29.7	1.0

Figure 4-3. Comparison of settling rate in mixed (C-2) and unmixed (C-1) Synechocystis cultures. Settling times: left, 0 min; center, 4 min; right, 12 min. Exp. 1

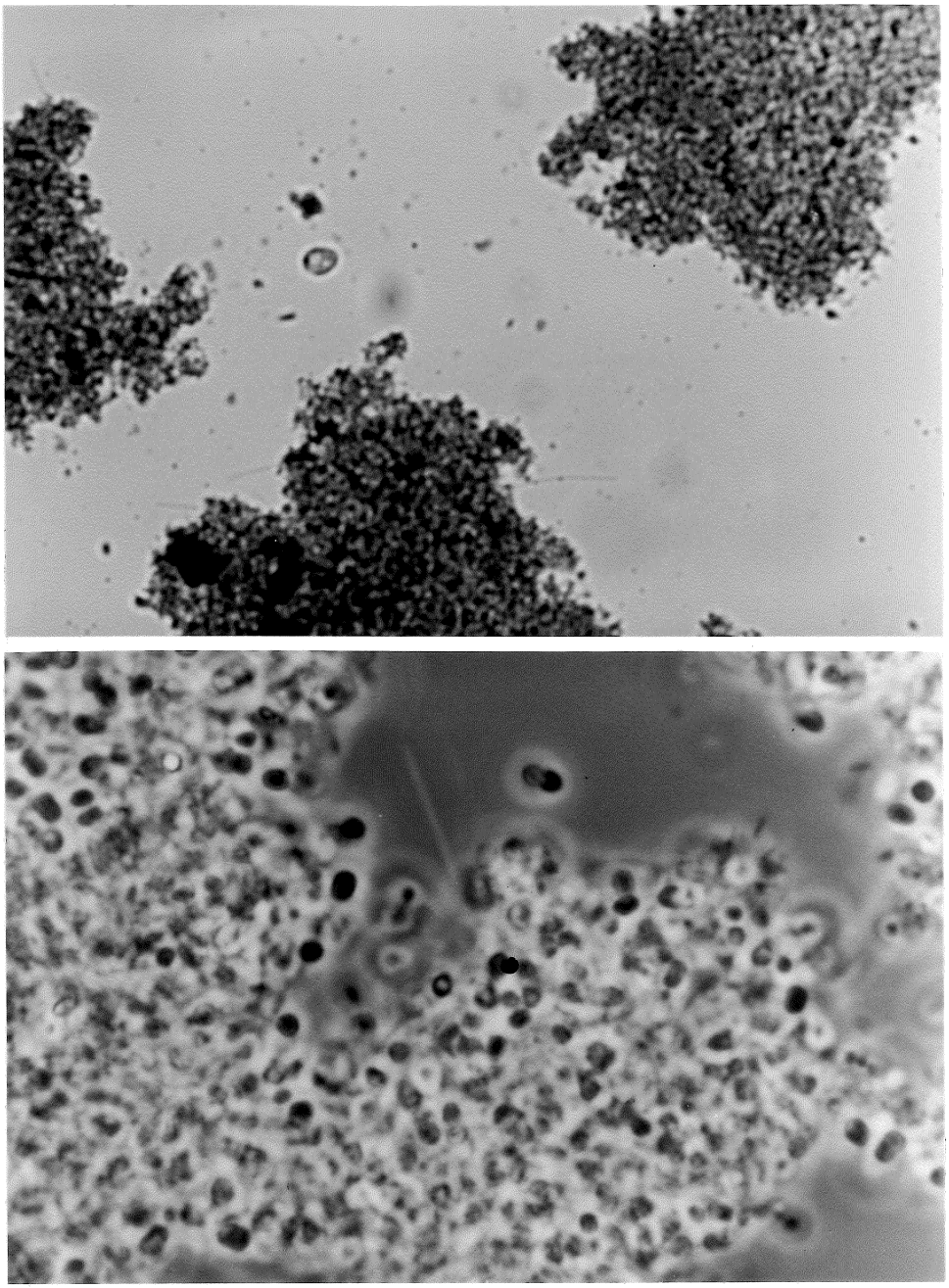

Figure 4-4. Bioflocs of Synechocystis formed in the mixed culture during exp. 1. Top, 100X; bottom, 1000X

significant populations by the mid-point of the experiment (Table 4-14). These algae showed no tendency to flocculate. DO and pH levels in the mixed culture closely paralled those in the unmixed culture (Fig. 4-5).

Mixed and unmixed cultures were compared for a third time in exp. 4. In this case, Synechocystis and Chlorella were co-dominant initially (Table 4-15). Synechocystis counts declined rapidly in both the mixed (C-1) and unmixed (C-2) cultures. Bioflocs were noticed in the C-1 medium within one week and progressively increased in number and size, whereas no flocs appeared in the C-2 medium. Microscopic examination showed the flocs from C-1 to be composed mostly of Synechocystis cells (approx. 75%) with the balance consisting of Chlorella cells and, to a lesser extent, Monodus and detritus.

The difference in visible flocculation characteristics was reflected in the respective settleable solids contents of the two cultures (Fig. 4-6). A maximum settleable solids volume of 17 ml/l was measured in the C-1 medium, whereas this parameter remained near zero in the C-2 medium. Despite the flocculation of C-1 medium, algal removals obtained by 24-h sedimentation declined from 12% to 1% during the course of the experiment. In contrast, up to 25% algal removal was attained with the non-flocculent C-2 medium.

Dehydrogenase activity was generally greater in the mixed culture (avg. 25 vs. 17 mg O_2*/m^3·d) whereas pH was less (avg. 9.0 vs. 10.3) (Fig. 4-7). DO and visibility were similar.

4.2.2. Flow mix velocity

Flow mixing velocities of 14 cm/s in C-1 and 30 cm/s in C-2 were employed during exp. 12. Chlorella and Monodus were co-dominant initially and remained so throughout the experiment (Table 4-15). As Figure 4-8

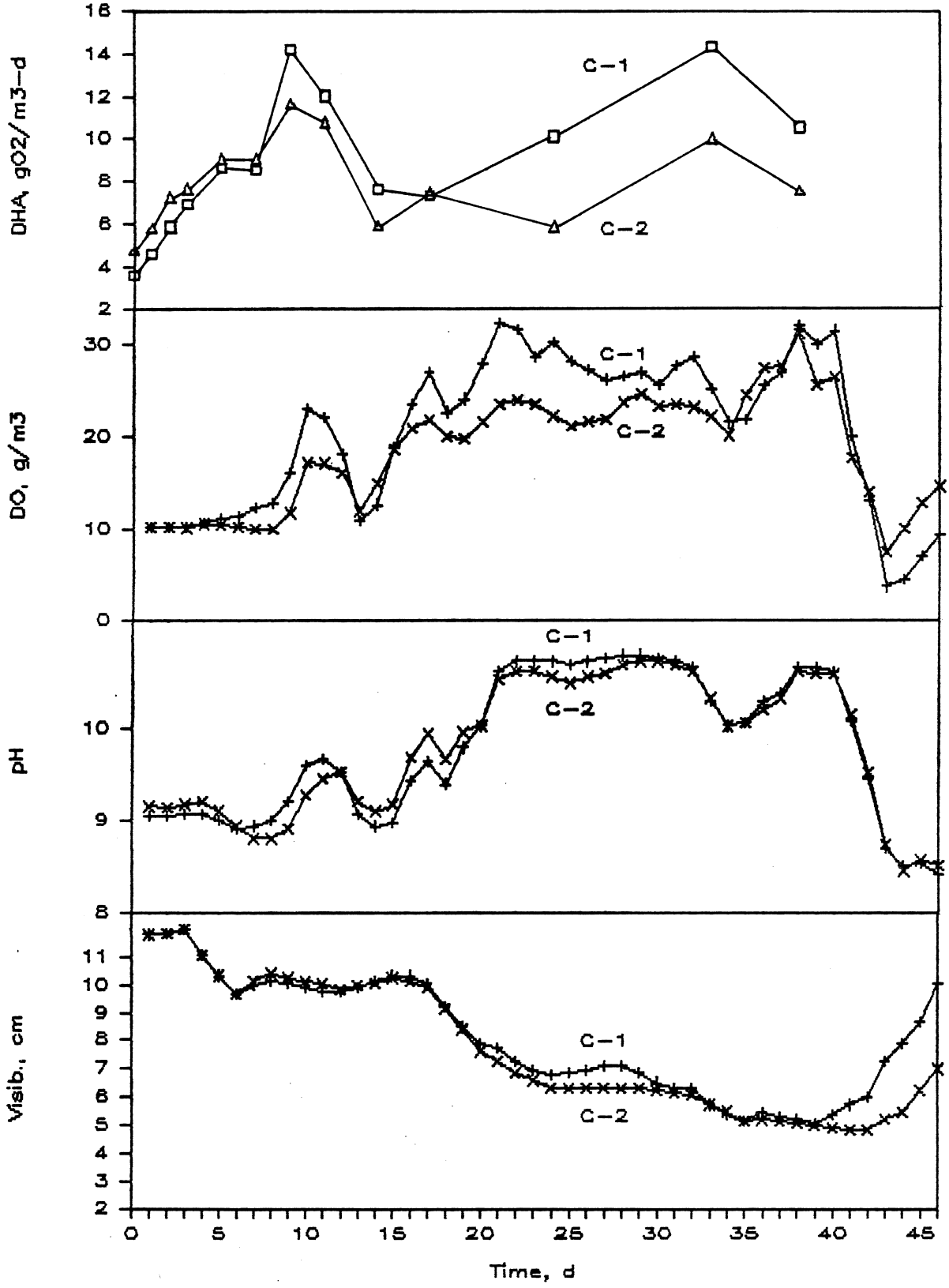

Figure 4-5. Temporal variation of DHA, DO, pH and visibility in mixed (C-2) and unmixed (C-1) cultures. Exp. 2.

Table 4-15. Effect of mixing on photosynthetic community composition in bioflocculation ponds, exps. 4, 12

Exp.	Day	Pond	Normalized cell count (#/grid)			
			Chlor.	Monod.	Synec.	Thiop.
4	4	C-1	30.2	6.2	38.8	4.8
		C-2	29.9	6.4	40.0	0.4
	8	C-1	33.5	5.7	32.4	0.4
		C-2	29.8	7.2	40.6	0.0
	13	C-1	40.4	29.8	23.4	0.0
		C-2	44.4	6.9	26.8	0.0
	18	C-1	35.7	7.7	3.4	0.4
		C-2	34.8	3.1	4.4	0.1
12	-1	C-1	92.2	63.4	0.3	0.3
		C-2	90.4	63.4	0.3	tr
	6	C-1	107.8	78.0	0.5	0.0
		C-2	107.9	75.7	5.4	0.0
	13	C-1	106.6	45.0	0.6	0.2
		C-2	118.2	39.8	0.1	0.0
	24	C-1	75.2	32.0	0.4	0.0
		C-2	49.6	16.6	0.0	0.0

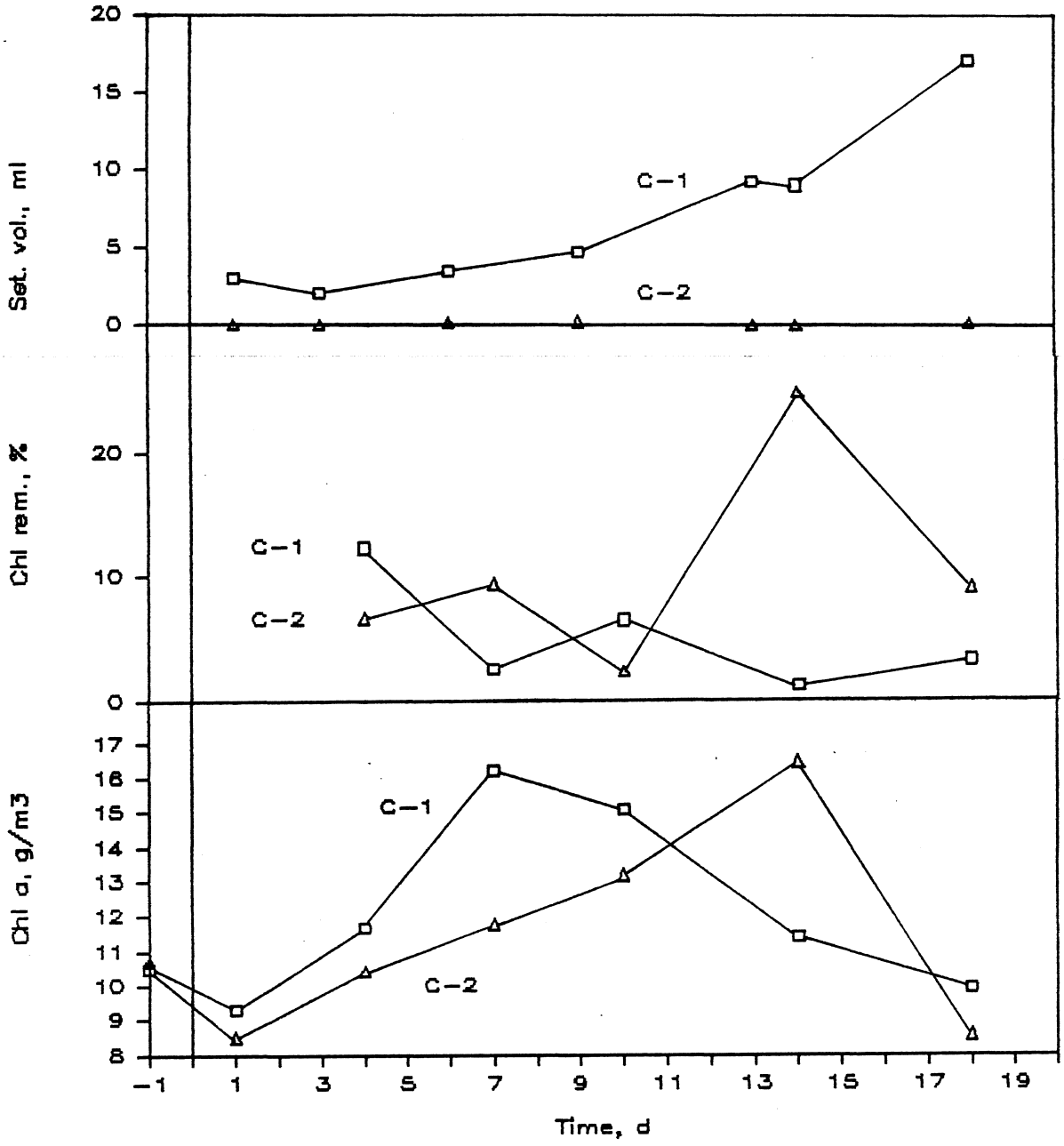

Figure 4-6. Temporal variation of settleability and chl. a in mixed (C-1) and unmixed (C-2) cultures. Exp. 4.

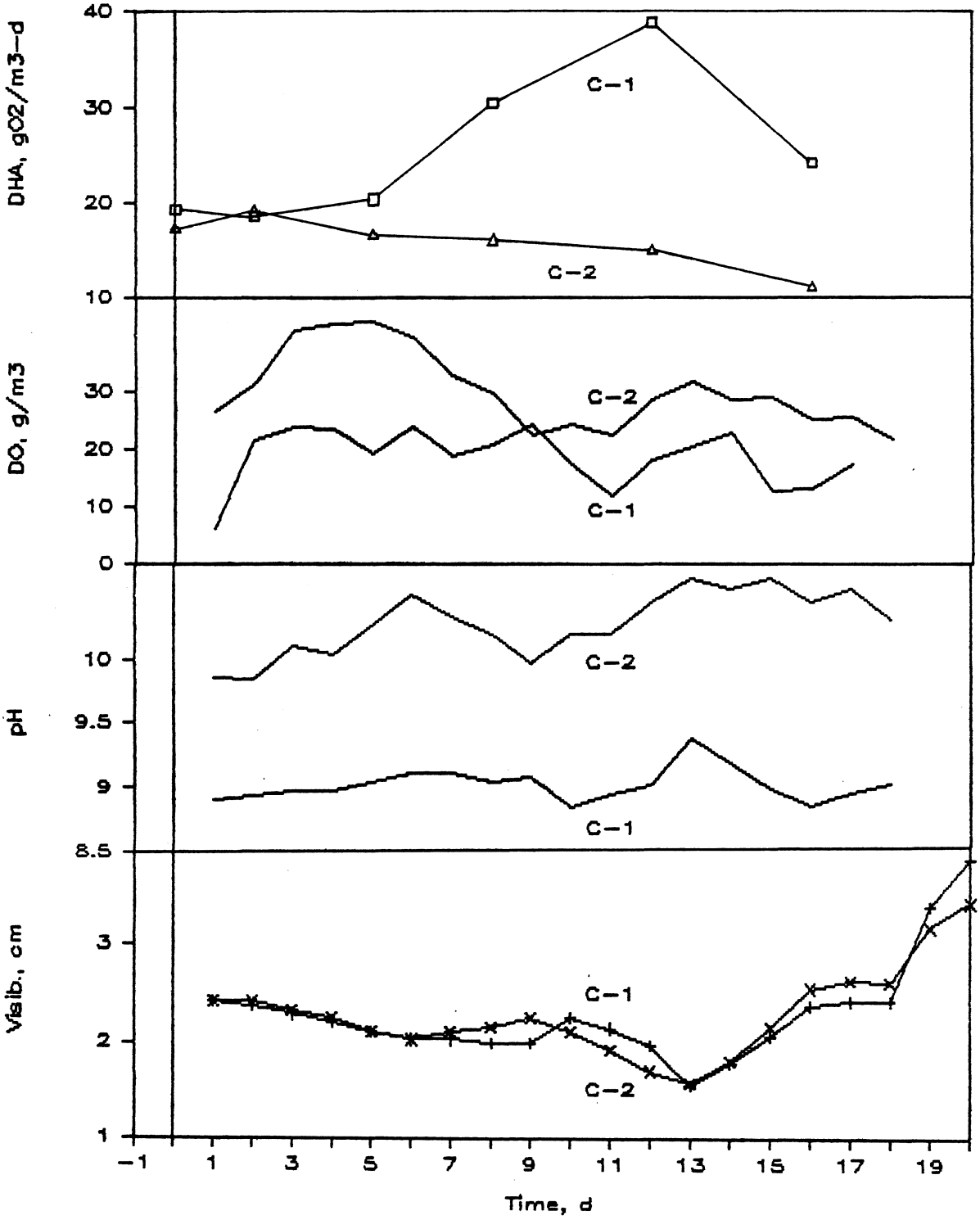

Figure 4-7. Temporal variation of DHA, DO, pH and visibility in mixed (C-1) and unmixed cultures (C-2). Exp. 4.

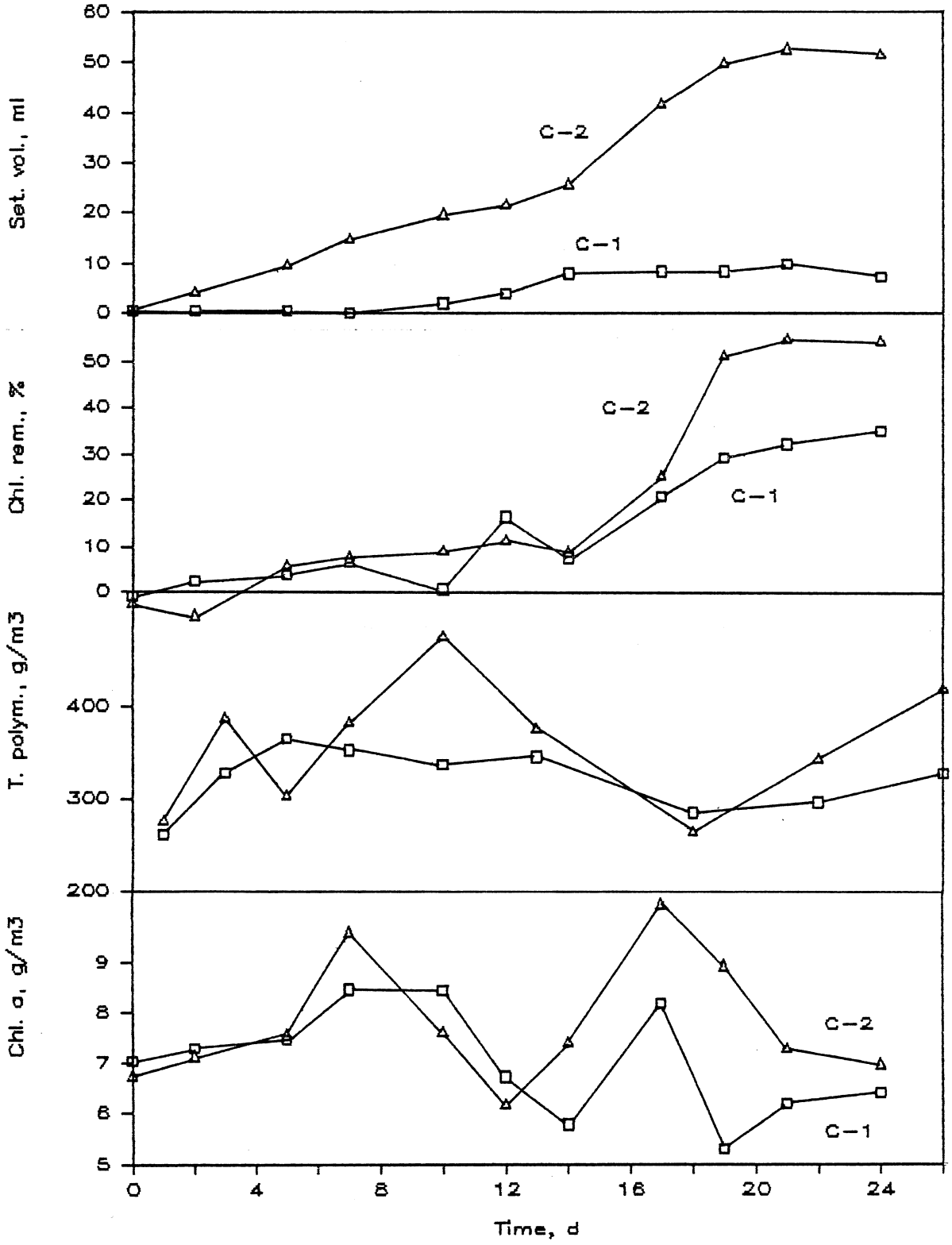

Figure 4-8. Temporal variation of settleability, biopolymer, and chl. a in slow mixed (14 cm/s, C-1) and fast mixed (30 cm/s, C-2) cultures. Exp. 12.

indicates, the production of settleable matter was greater in C-2. A maximum settleable solids volume of 53 ml/l was measured in C-2 medium, compared to 10 ml/l in C-1 medium. Algal removals by sedimentation were similar in both cultures through day 14, after which they became significantly greater in C-2 medium (max. 56% vs. 36%). Total biopolymer concentrations were similar. DO, pH, and visibility were generally greater in the slow mixed culture, whereas DHA was greater in the fast mixed culture (Fig. 4-9).

4.3 Effect of Waste Loading

The effect of waste loading was investigated in 4 experiments. A loaded culture was compared to a non-loaded culture in exp. 5 (with Synechocystis dominant initially) and exp. 11 (with Chlorella and Monodus co-dominant initially). Different waste loading rates were evaluated in exp. 6. The influence of waste type was studied in exp. 10.

4.3.1. Loading versus non-loading

The waste-loaded culture (C-2) in exp. 5 became visibly flocculent after 6 days, whereas flocs did not appear in the non-loaded culture (C-1). Microscopic examinations showed the flocs in C-2 medium to have diameters on the order of hundreds to thousands of microns, and to consist almost exclusively (approx. 95%) of Synechocystis cells. Smaller flocs ($\leq$ 100 μm in diameter) were observed microscopically in C-1 medium. Additional observations made using a steroscopic dissecting microscope showed the C-2 flocs to be irregularly or stellate shaped, with distinct lobes. C-1 flocs were roughly spherical in shape.

Synechocystis counts increased initially, then declined in both cultures (Table 4-16). The extent of decline was somewhat greater in C-2

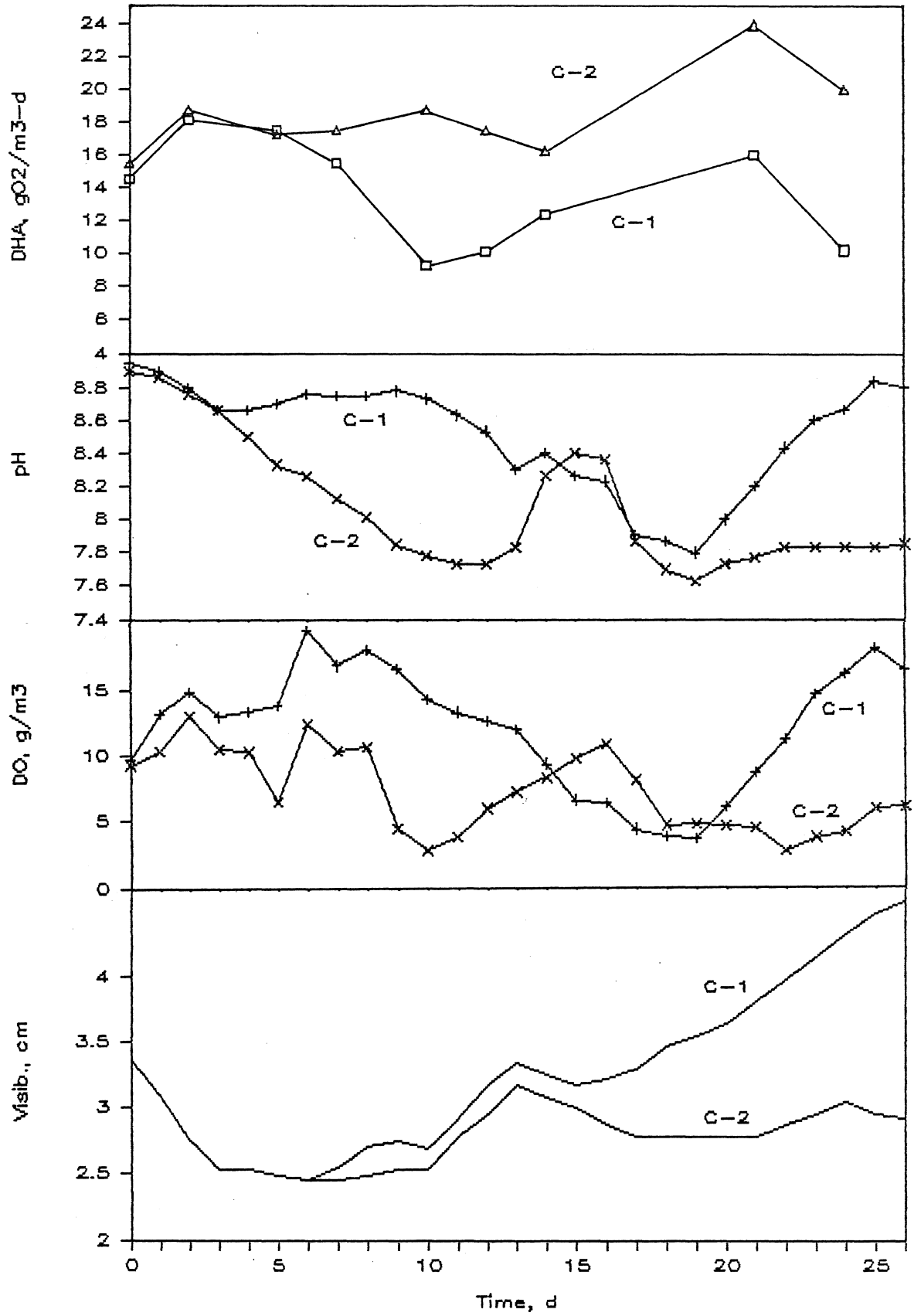

Figure 4-9. Temporal variation of DHA, pH, DO and visibility in slow mixed (C-1) and fast mixed (C-2) cultures. Exp. 12.

Table 4-16. Effect of waste loading on photosynthetic community composition in bioflocculation ponds, exps. 5, 11

Exp.	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monod.	Synec.	Thiop.	Micra.[a]
5	-1	C-1	11.3	1.8	69.8	1.9	0.0
		C-2	10.6	0.0	69.6	1.9	0.0
	3	C-1	9.8	3.8	109.4	0.0	0.0
		C-2	10.6	3.7	109.8	0.0	0.0
	10	C-1	8.5	3.2	118.2	0.0	0.0
		C-2	8.2	2.4	118.8	0.0	0.0
	18	C-1	7.6	4.1	50.6	0.0	0.0
		C-2	8.8	3.6	111.6	0.0	0.0
	25	C-1	3.8	1.3	61.2	0.0	0.0
		C-2	9.3	1.5	77.4	0.0	0.8
	30	C-1	1.6	0.7	73.0	0.0	0.0
		C-2	19.2	4.4	52.0	0.0	1.6
11	0	C-1	122.4	77.8	0.0	9.0	0.0
		C-2	129.0	75.8	0.0	6.2	0.0
	9	C-1	149.6	68.2	0.0	4.9	0.0
		C-2	148.8	71.4	0.0	4.7	0.0
	15	C-1	126.0	83.2	0.0	2.9	0.0
		C-2	111.8	85.6	0.0	1.5	0.0
	23	C-1	210.0	91.2	tr	2.8	0.0
		C-2	209.0	84.8	0.0	0.0	0.3
	30	C-1	172.8	70.0	0.0	0.0	0.0
		C-2	143.0	64.0	0.0	0.0	0.0

[a] Micractinium

medium than in C-1 medium (terminal counts of 52 vs. 73 cells/grid). Synechocystis was still the dominant genus in both cultures at the end of the experiment.

Production of settleable matter was greater in the waste-loaded culture, as shown in Figure 4-10. Maximum settleable volumes attained were 35 ml/l in C-2 and 4.5 ml/l in C-1. Algal removals were initially similar, but became significantly greater in the waste-loaded culture after 20 days. Maximum removals in C-2 and C-1 media were 43% and 20%, respectively. DHA, DO and visibility were generally greater in the loaded culture than in the control, whereas pH was less (Fig. 4-11).

In exp. 11, differences between the waste-loaded (C-1) and non-loaded (C-2) cultures were generally less pronounced. The algal communities in both ponds consisted almost exclusively of Chlorella and Monodus throughout the experiment (Table 4-16). Both cultures became visibly flocculent after approximately one week. Production of settleable matter was similar for the first three weeks, then became somewhat greater in C-2 than in C-1 (max. 54 vs. 40 ml/l) (Fig. 4-12). Algal removals in media from the two ponds were nearly equal throughout the experiment, whereas the biopolymer concentration in C-1 was somewhat greater than in C-2 (avg. 415 vs. 363 g/m^3). DO, pH and, eventually, DHA and visibility became lower in the waste-loaded culture (Fig. 4-13).

4.3.2. Waste-loading rate

Average waste-loading rates of 72 1/d and 217 1/d were applied to ponds C-1 and C-2, respectively, in exp. 6. Synechocystis was dominant initially (Table 4-17). Counts of this algal declined throughout the experiment, ending near zero. A limited regrowth of Chlorella and Monodus occurred.

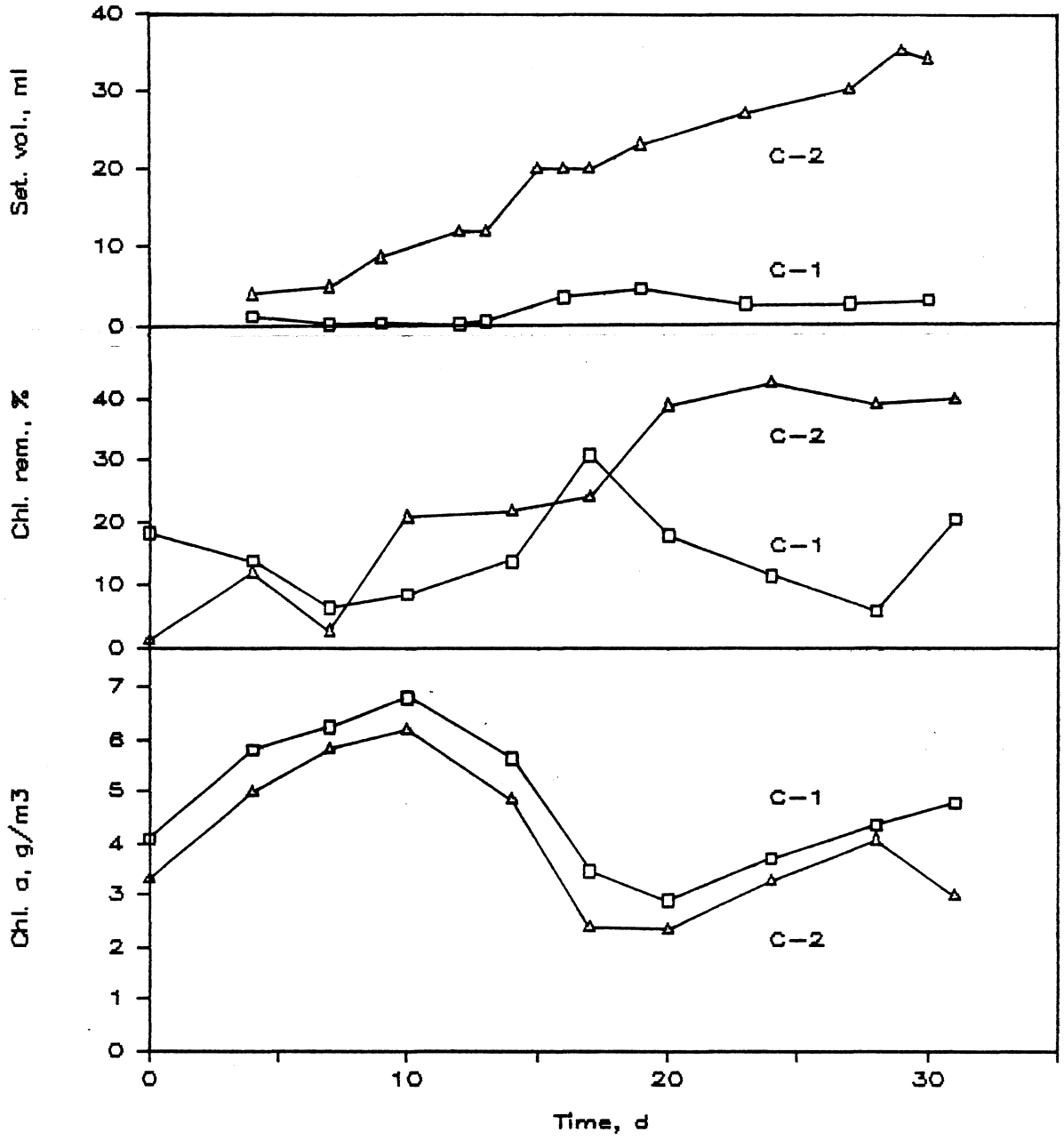

Figure 4-10. Temporal variation of settleability and chl. a in waste-loaded (C-2) and non-loaded (C-1) cultures initially dominated by Synechocystis. Exp. 5.

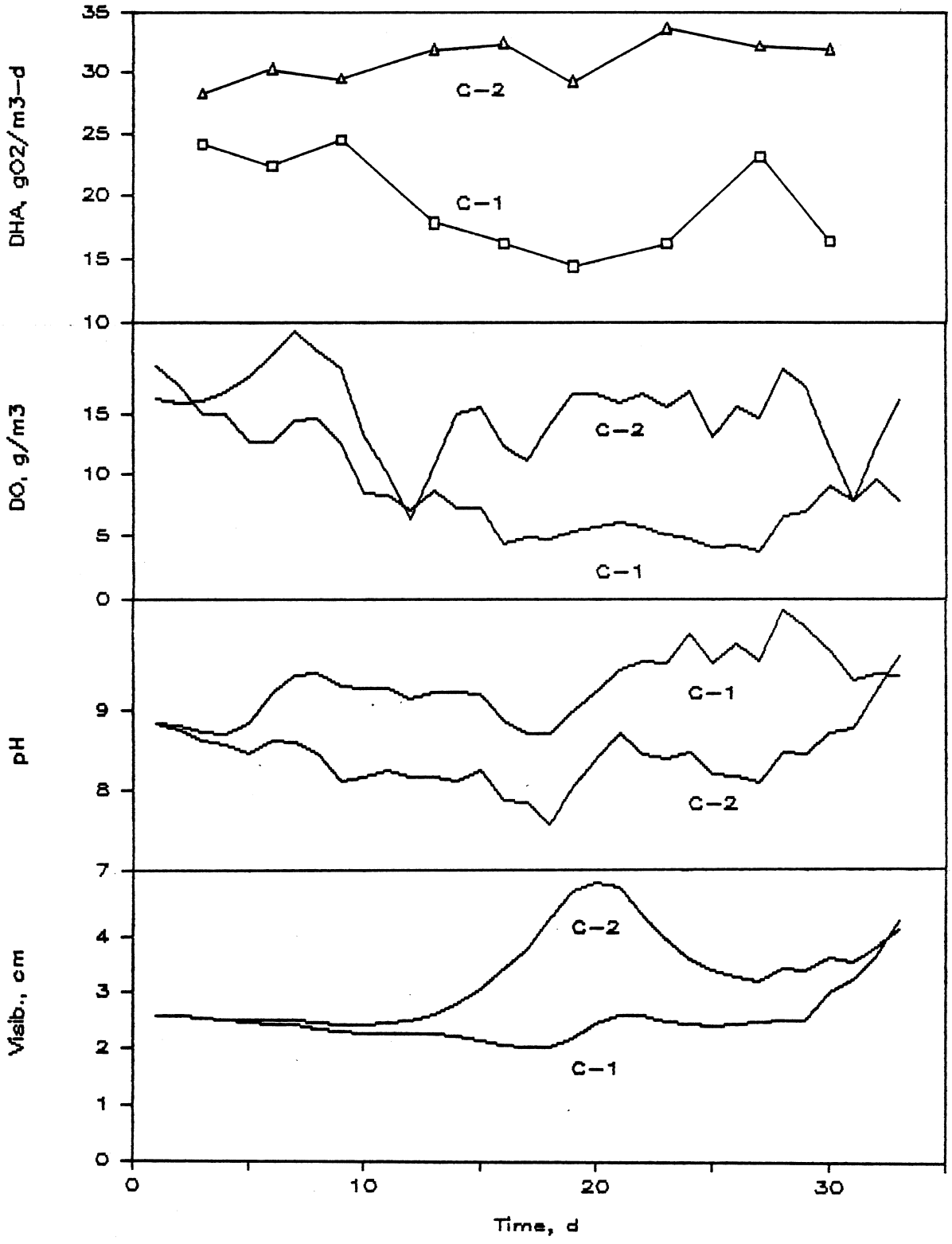

Figure 4-11. Temporal variation of DHA, DO, pH and visibility in waste-loaded (C-2) and non-loaded (C-1) cultures initially dominated by Synechocystis. Exp. 5.

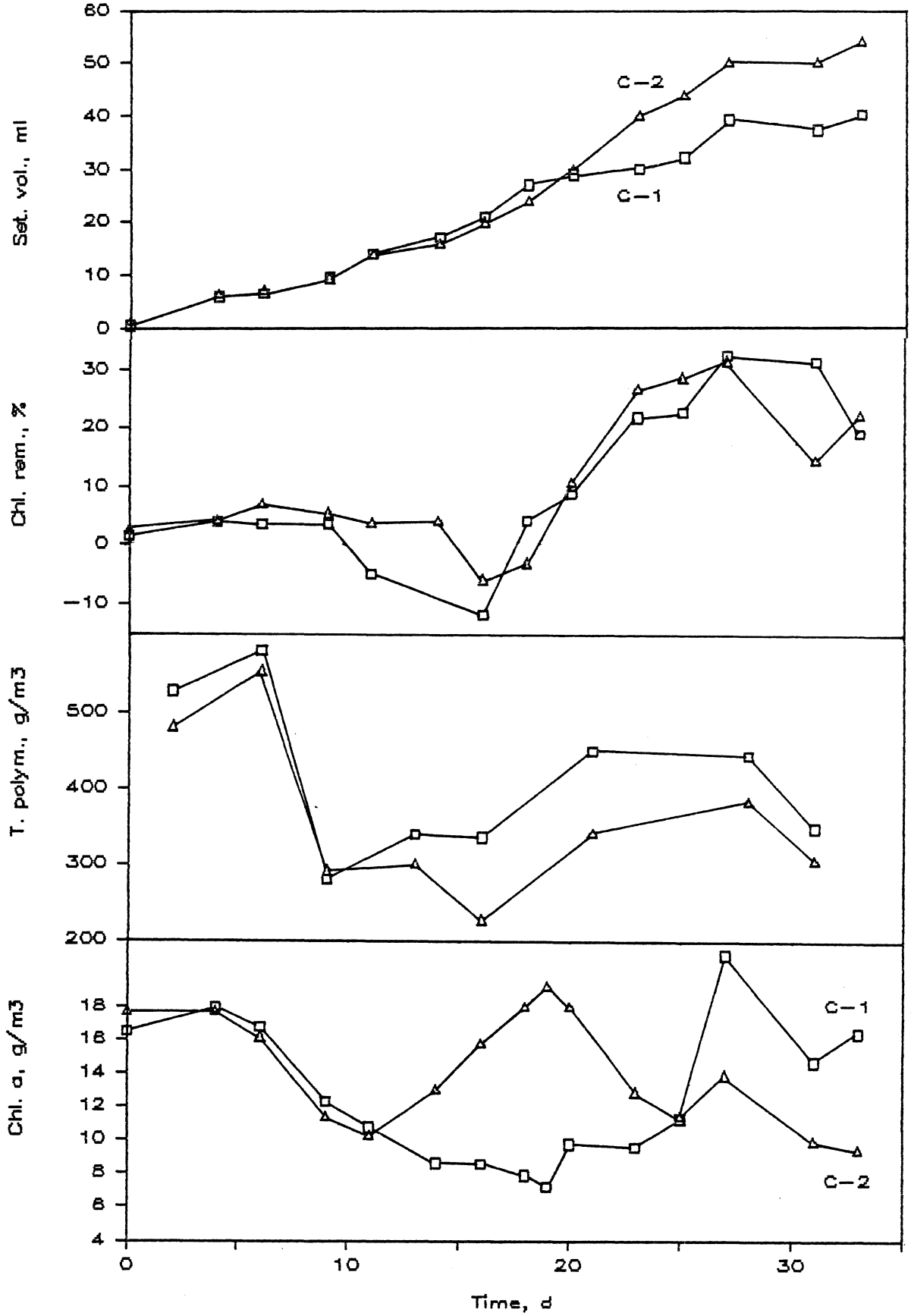

Figure 4-12. Temporal variation of settleability, biopolymer and chl. a in waste-loaded (C-1) and non-loaded (C-2) cultures initially dominated by Chlorella and Monodus. Exp. 11.

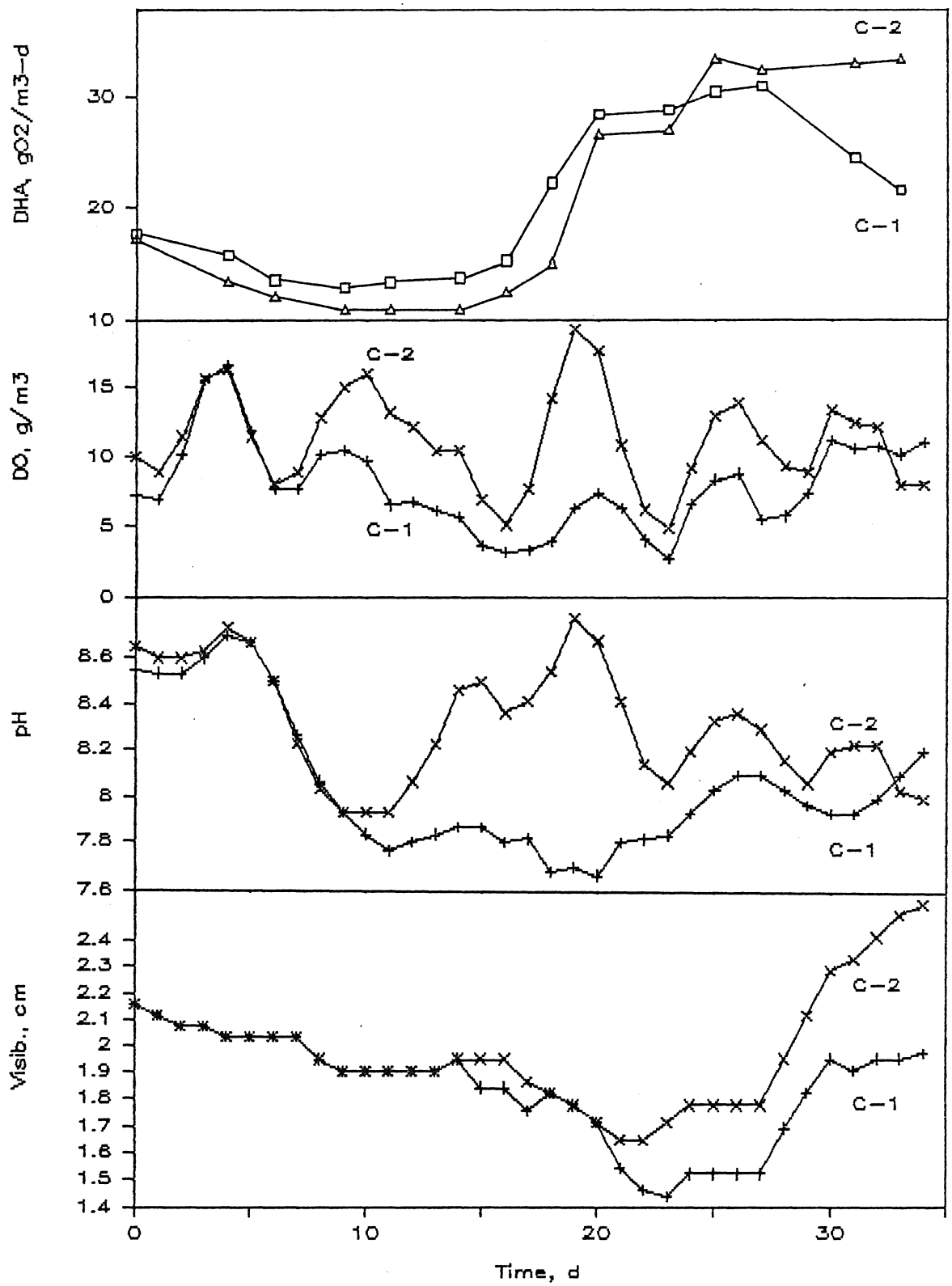

Figure 4-13. Temporal variation of DHA, DO, pH and visibility in waste-loaded (C-1) and non-loaded (C-2) cultures initially dominated by Chlorella and Monodus. Exp. 11.

Table 4-17. Effect of waste loading on photosynthetic community composition in bioflocculation ponds, exps. 6, 10

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

Extensive flocculation was observed in both ponds. Flocs appeared to be more numerous in C-1 than in C-2, however, production of settleable matter was almost identical in the two ponds (Fig. 4-14). Algal removals were also very similar, except near the end of the trial. A somewhat greater maximum removal was eventually attained in C-1 medium (90%) than in C-2 medium (74%). DHA was greater and visibility less in the more heavily loaded culture, whereas DO and pH were similar in both ponds (Fig. 4-15).

4.3.3. Waste type

Settled waste supernatant and fixed bed reactor effluent were added to ponds C-1 and C-2, respectively, in exp. 10. Chlorella and Monodus were co-dominant initially (Table 4-17). The settled waste supernatant had a stimulatory effect on Chlorella, as indicated by an increase in the relative proportion of this alga during the experiment. This waste also sustained a relatively high population of Thiopedia rosea in C-1. Relative proportions of Chlorella and T. rosea decreased in C-2 during the experiment.

Settleable matter production and algal removal were greater in the pond loaded with FBR effluent (Fig. 4-16). Maxima for these parameters in C-2 medium were 30 ml/l and 42%, respectively. Maximum settleable solids volume and algal removal in C-1 medium were 15 ml/l and 14%, respectively. Biopolymer concentrations in the two cultures were similar. DHA levels were consistently greater in C-1 than in C-2 (Fig. 4-17). DO, pH and visibility were similar in the two cultures for the first 20 days, then diverged. Terminal DO and pH were greater in C-1, whereas terminal visibility was greater in C-2.

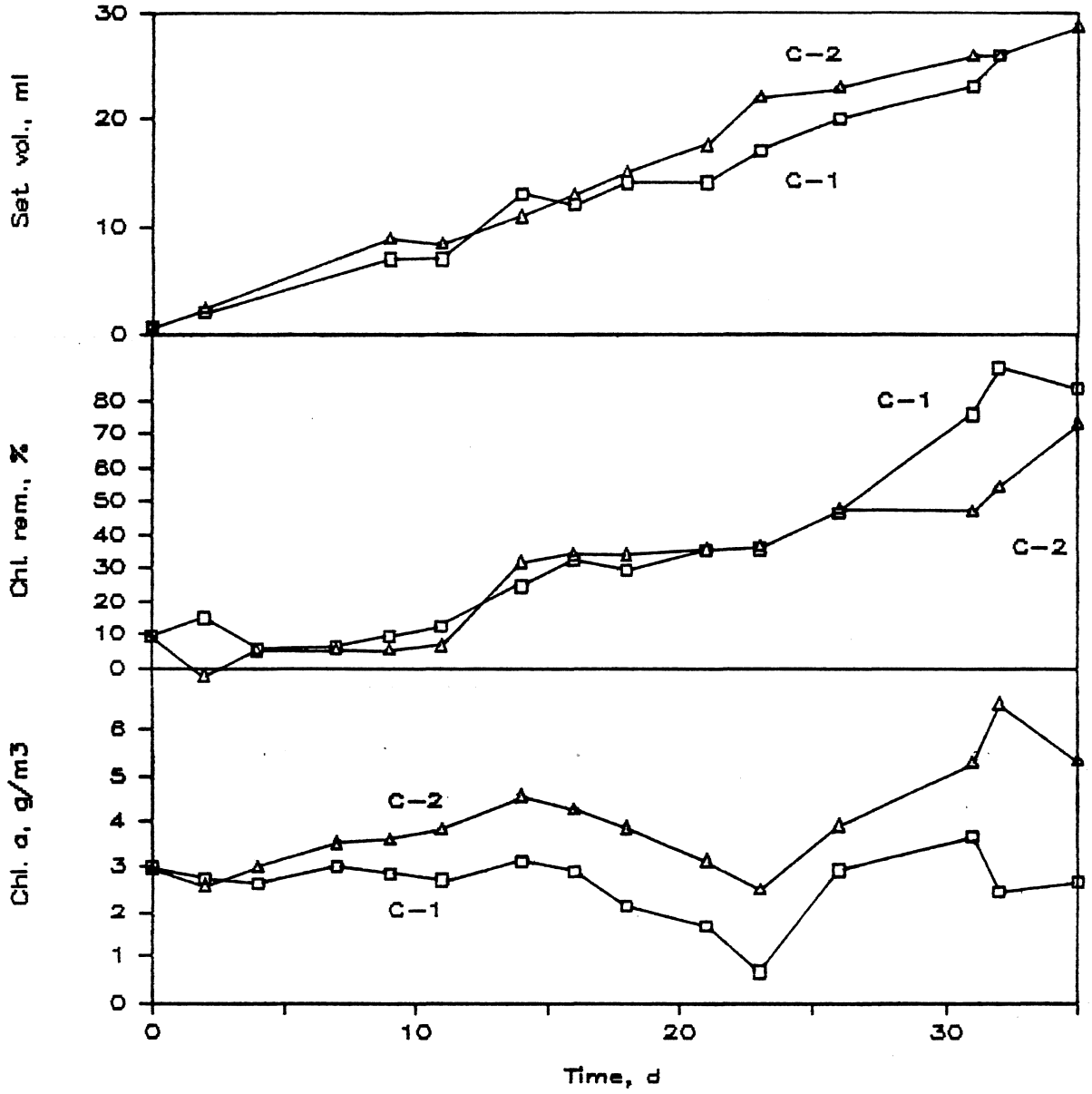

Figure 4-14. Temporal variation of settleability and chl. a in cultures loaded at low (72 l/d, C-1) and high (217 l/d, C-2) rates. Exp. 6.

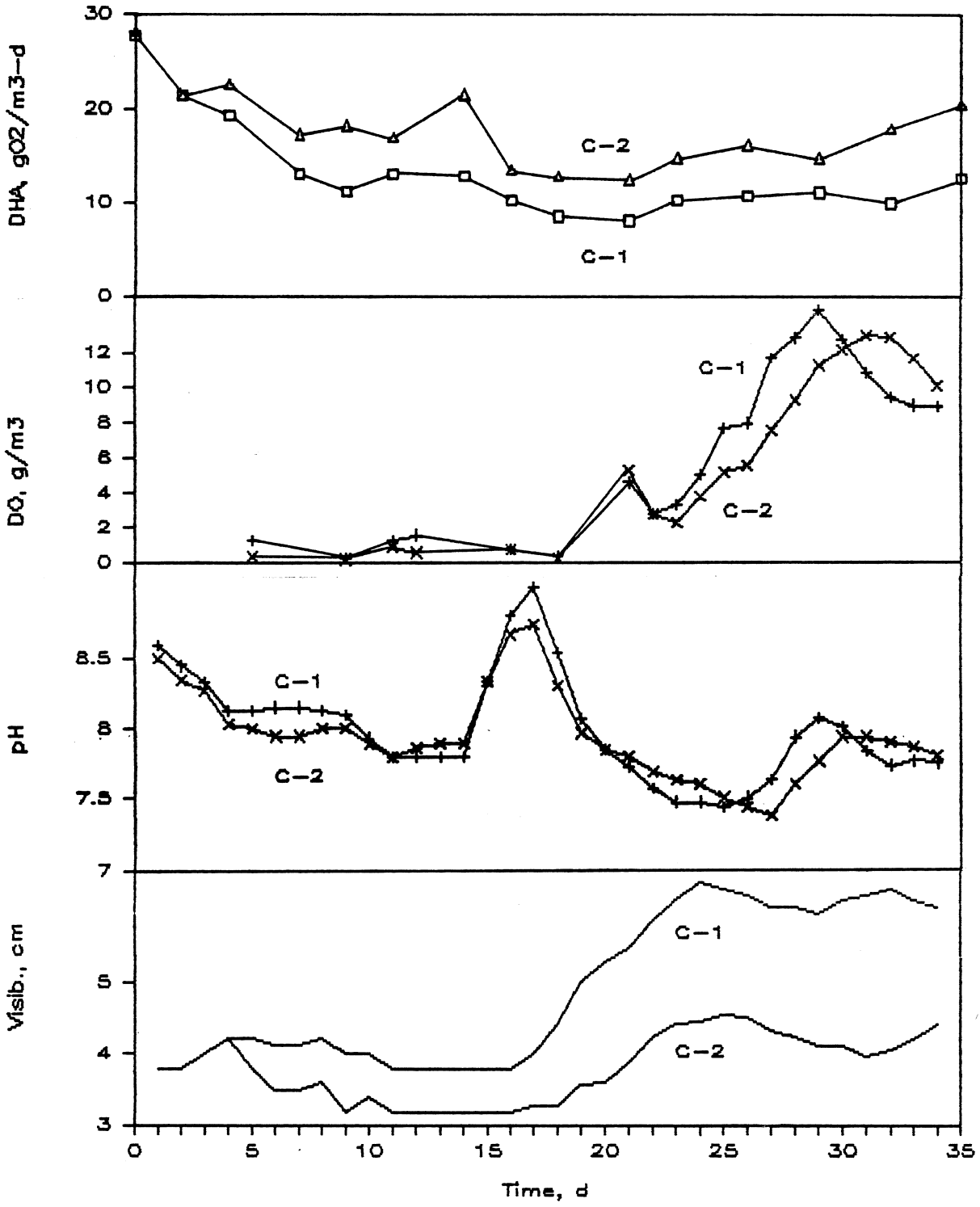

Figure 4-15. Temporal variation of DHA, DO, pH and visibility in cultures loaded at low (C-1) and high (C-2) rates. Exp. 6.

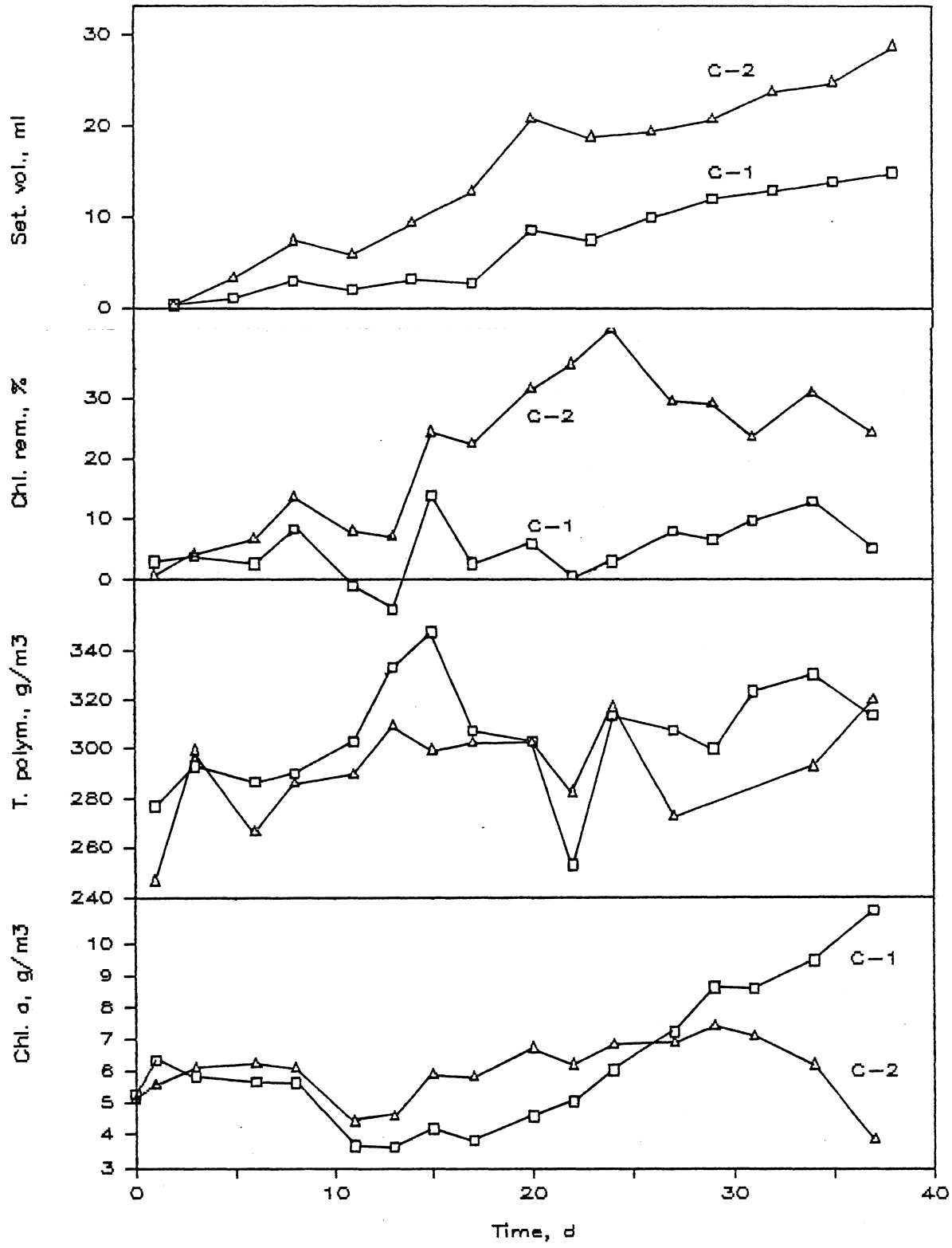

Figure 4-16. Temporal variation of settleability, biopolymer and chl. a in cultures loaded with settled waste supernatant (C-1) and FBR effluent (C-2). Exp. 10.

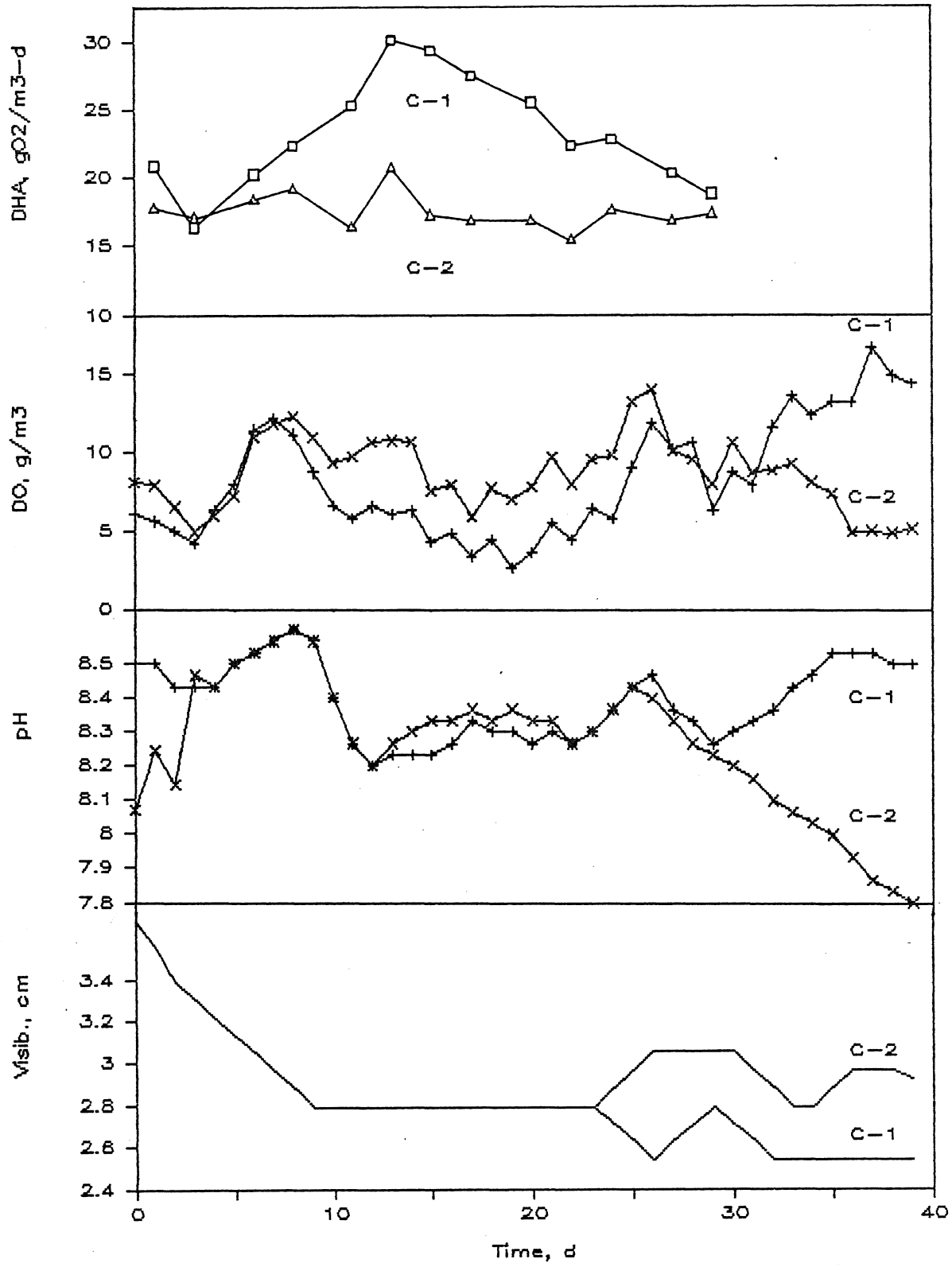

Figure 4-17. Temporal variation of DHA, DO, pH and visibility in cultures loaded with settled waste supernatant (C-1) and FBR effluent (C-2). Exp. 10.

4.4. Effect of Carbonate-supplementation and pH Reduction

4.4.1. Carbonate supplementation

Carbonate addition did not have a positive effect on the photosynthetic community in terms of succession or bioflocculation. The algae dominant initially were Chlorella and Monodus (Table 4-18). These genera remained co-dominant throughout the experiment, although their population density was eventually reduced. C-2 began browning after 12 days and both cultures were olive brown from day 14 on. Extensive flocculation was evident in C-1 by day 26, with a clear supernatant upon settling. Flocculation was also extensive in C-2 by that time, but settling did not leave a clear supernatant.

Settleable matter production was greater in the non-dosed pond (C-1) (Fig. 4-18). Carbonate addition did appear to stimulate DHA, but DO, pH and visibility remained virtually identical in the two cultures throughout most of the experiment (Fig. 4-19).

4.4.2. pH reduction

The effect of intermittently reducing pH in a culture dominated initially by Chlorella and Monodus was tested in exp. 8. The target pH was 6.0, but values significantly less than this (as low as 3.9) were reached after some of the acid additions. Despite experiencing extremes in pH, algal density in the experimental culture (C-2) exceeded than in the control (C-1) (Table 4-18). Settleable matter production and algal removal were similar in the two cultures until day 13, when C-1 was dosed heavily with ammonium hydroxide. Beyond this point, settleable solids and algal removal became greater in C-1 medium than in C-2 medium (Fig. 4-20). This trend was paralled by a progressive decline of chl. a in C-1. Maximum settleable solids volumes in C-1 and C-2 media were 19

Table 4-18. Effect of carbonate supplementation and pH reduction on photosynthetic community composition in bioflocculation ponds, exps. 3, 8

Exp.	Day	Pond	Normalized cell count (#/grid)				
			Chlor.	Monod.	Synec.	Thiop.	Micra.
3	0	C-1	26.9	19.6	0.0	15.5	0.0
		C-2	27.0	19.4	0.0	15.4	0.0
	2	C-1	25.1	17.8	0.5	13.7	0.0
		C-2	20.9	17.4	0.5	14.4	0.0
	3	C-1	29.9	17.6	0.0	11.8	0.0
		C-2	21.6	18.7	0.0	12.1	0.0
	9	C-1	29.6	29.4	0.0	8.7	0.0
		C-2	28.7	18.4	0.0	3.4	0.0
	14	C-1	25.2	39.9	0.0	2.6	0.0
		C-2	26.0	23.0	0.0	2.2	0.0
	20	C-1	11.5	57.3	0.0	1.8	0.0
		C-2	28.1	30.6	0.0	2.2	0.0
	24	C-1	5.8	76.5	0.0	0.0	0.0
		C-2	15.9	49.7	0.0	0.0	0.0
	30	C-1	0.4	71.4	tr	0.8	0.0
		C-2	21.0	43.9	tr	0.7	0.0
	36	C-1	0.2	0.4	tr	0.0	1.9
		C-2	2.5	7.4	0.0	0.0	0.0
	40	C-1	1.6	0.5	tr	0.0	0.8
		C-2	1.4	5.0	0.1	0.0	0.2
	45	C-1	7.8	6.8	0.0	0.0	0.2
		C-2	8.6	6.5	0.0	0.0	0.5
	49	C-1	5.5	21.4	0.0	0.0	1.1
		C-2	5.6	13.3	0.0	0.0	2.3
	56	C-1	1.7	19.9	0.0	0.0	1.3
		C-2	1.6	2.6	0.0	0.0	1.0
8	2	C-1	59.0	24.0	0.0	2.5	0.8
		C-2	62.0	22.0	0.0	2.2	1.1
	5	C-1	73.0	12.0	tr	2.1	0.9
		C-2	61.0	27.0	tr	1.8	2.1
	15	C-1	40.0	29.0	0.0	0.6	0.3
		C-2	58.0	44.0	tr	0.0	4.0
	23	C-1	32.0	25.0	0.0	0.0	0.0
		C-2	80.0	17.0	0.0	0.4	0.7
	28	C-2	83.0	19.0	0.0	0.7	0.0

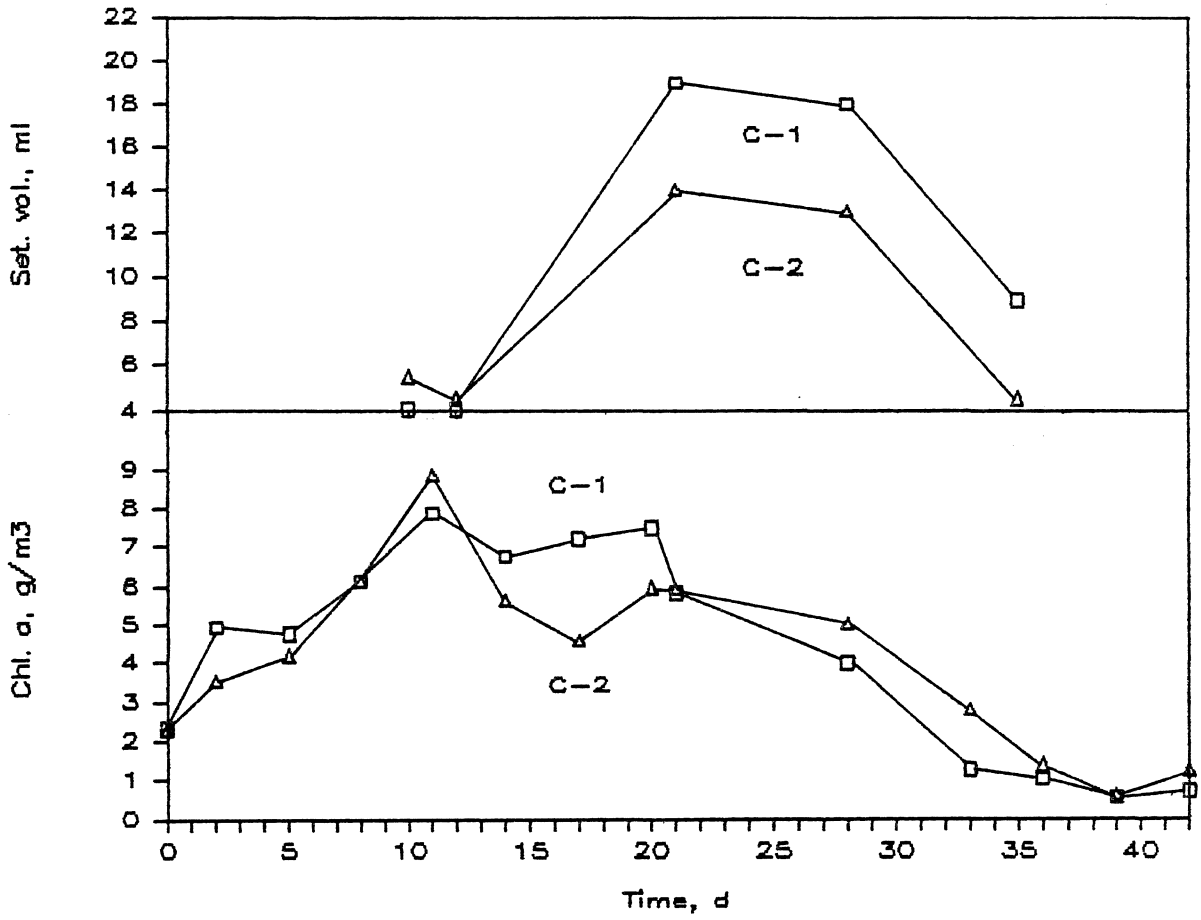

Figure 4-18. Temporal variation of settleability and chl. a in carbonate-supplemented (C-2) and non-supplemented (C-1) cultures. Exp. 3.

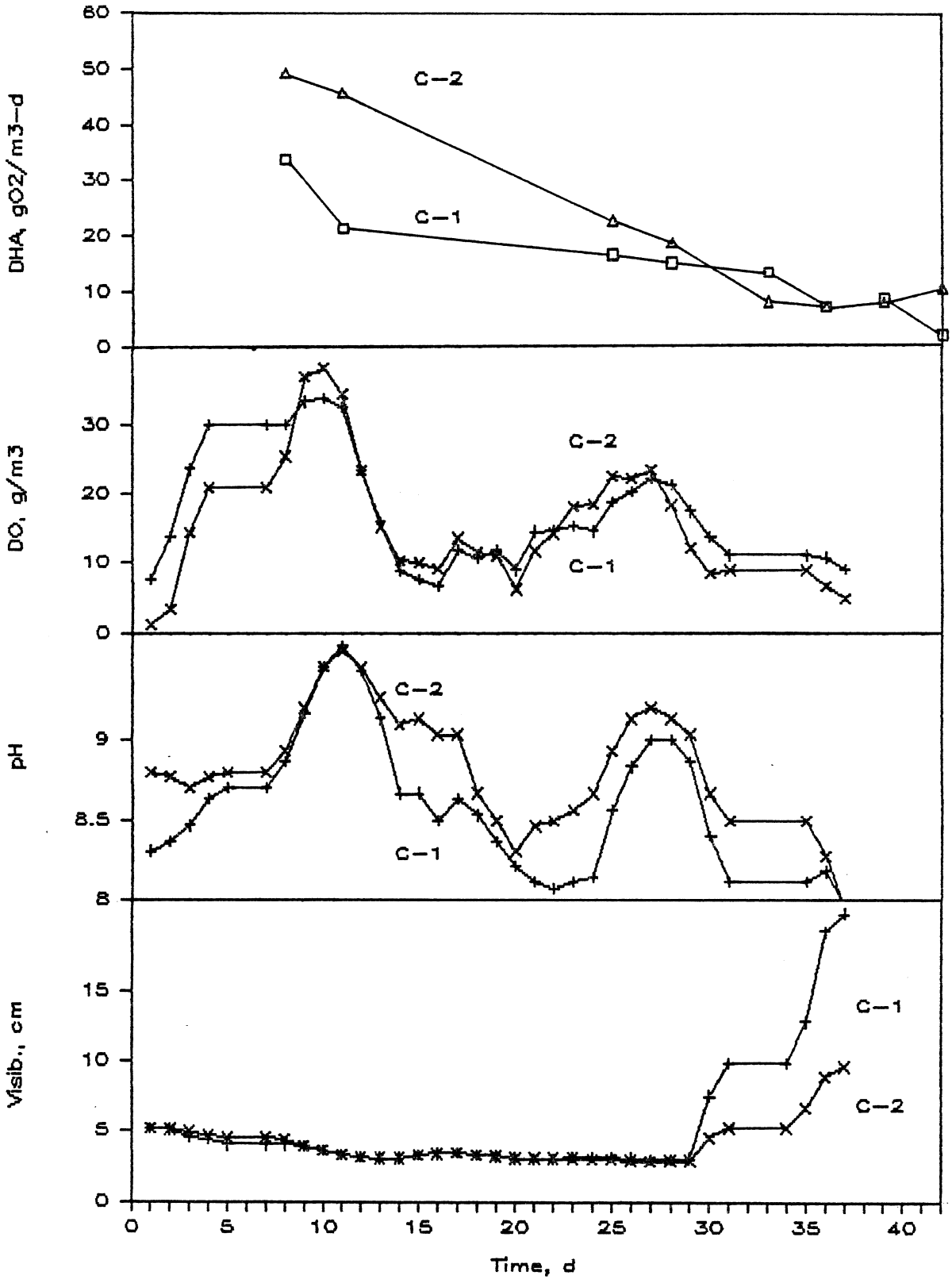

Figure 4-19. Temporal variation of DHA, DO, pH and visibility in carbonate-supplemented (C-2) and non-supplemented (C-1) cultures. Exp. 3.

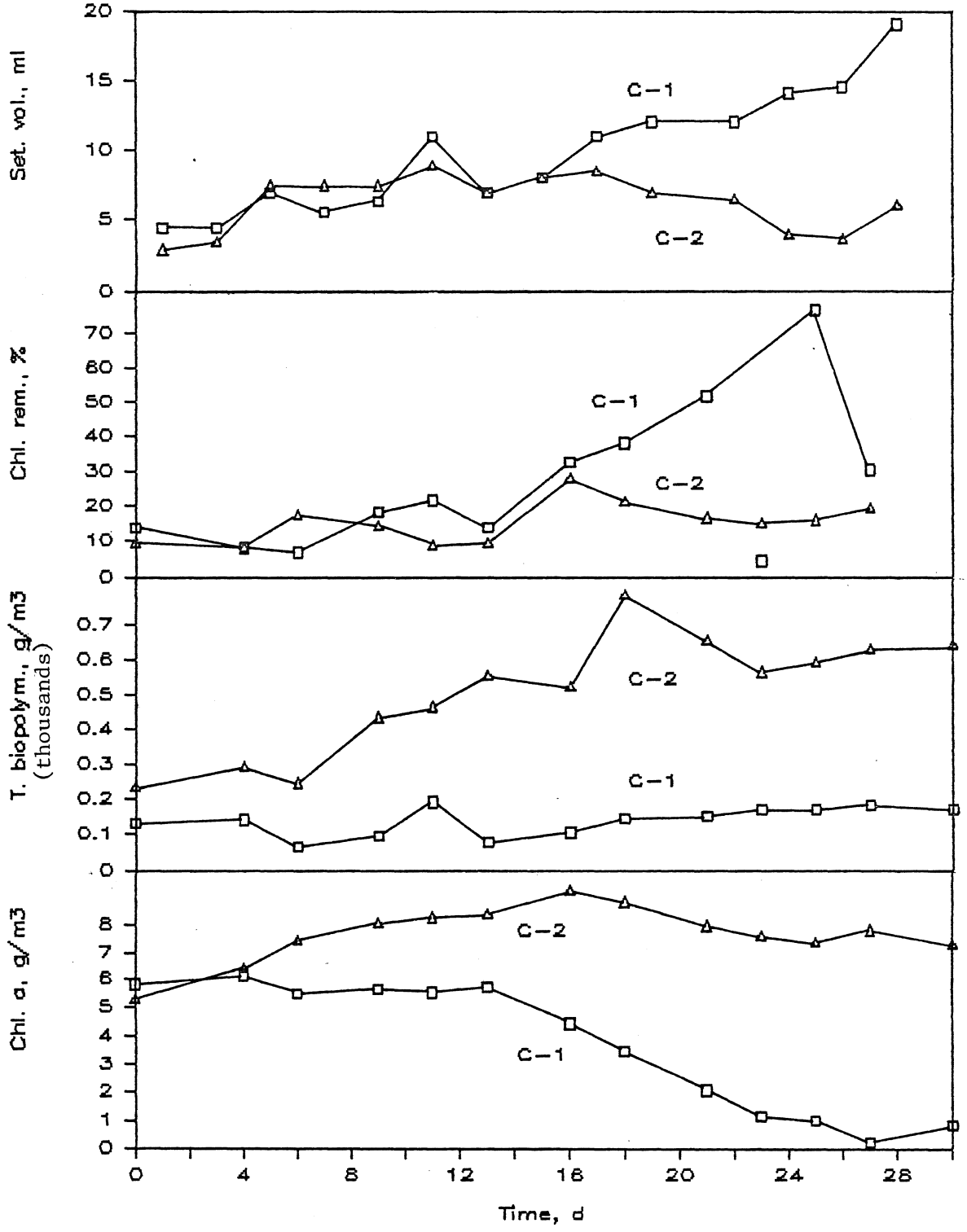

Figure 4-20. Temporal variation of settleability, biopolymer and chl. a in reduced-pH (C-2) and control (C-1) cultures. Exp. 8.

ml/l and 9 ml/l, respectively. Maximum algal removals in the two media were 76% and 28%, respectively. DO was greater in the pH adjusted pond whereas DHA, pH, and visibility were less, as compared to the control (Fig. 4-21). A rapid drop of DO in pond C-1 followed closely the heavy ammonium hydroxide dosage on day 12.

4.5. Biofloc and Bacterial Seeding

4.5.1. Biofloc seeding

Culture medium distributed between the two bioflocculation ponds at the start of exp. 7 was co-dominated by Synechocystis and Chlorella. The C-1 culture was then seeded with Synechocystis biofloc developed in the previous experiment. As a result, settleable matter was present in C-1 from the beginning of exp. 7. Limited flocculation of C-2 became apparent after 18 days. Cell density of Synechocystis and Chlorella declined to near-zero levels in both the experimental and control (C-2) cultures by the end of the experiment (Table 4-19).

The floc seed increased the rate of settleable matter production in C-1 initially relative to C-2 (Fig. 4-22). The difference in rates eventually diminished, but the quantity of settleable matter remained greater in C-1. Algal removals in C-1 medium were also greater than in C-2 medium initially. However, this situation was reversed after day 16. Total biopolymer and chl. a were similar in the two ponds. DHA was consistently greater in C-1, whereas DO, pH, and, eventually, visibility were greater in C-2 (Fig. 4-23).

4.5.2. Bacterial seeding

The culture seeded with photosynthetic bacteria exhibited a progressive increase in settleable matter but algal removals decreased

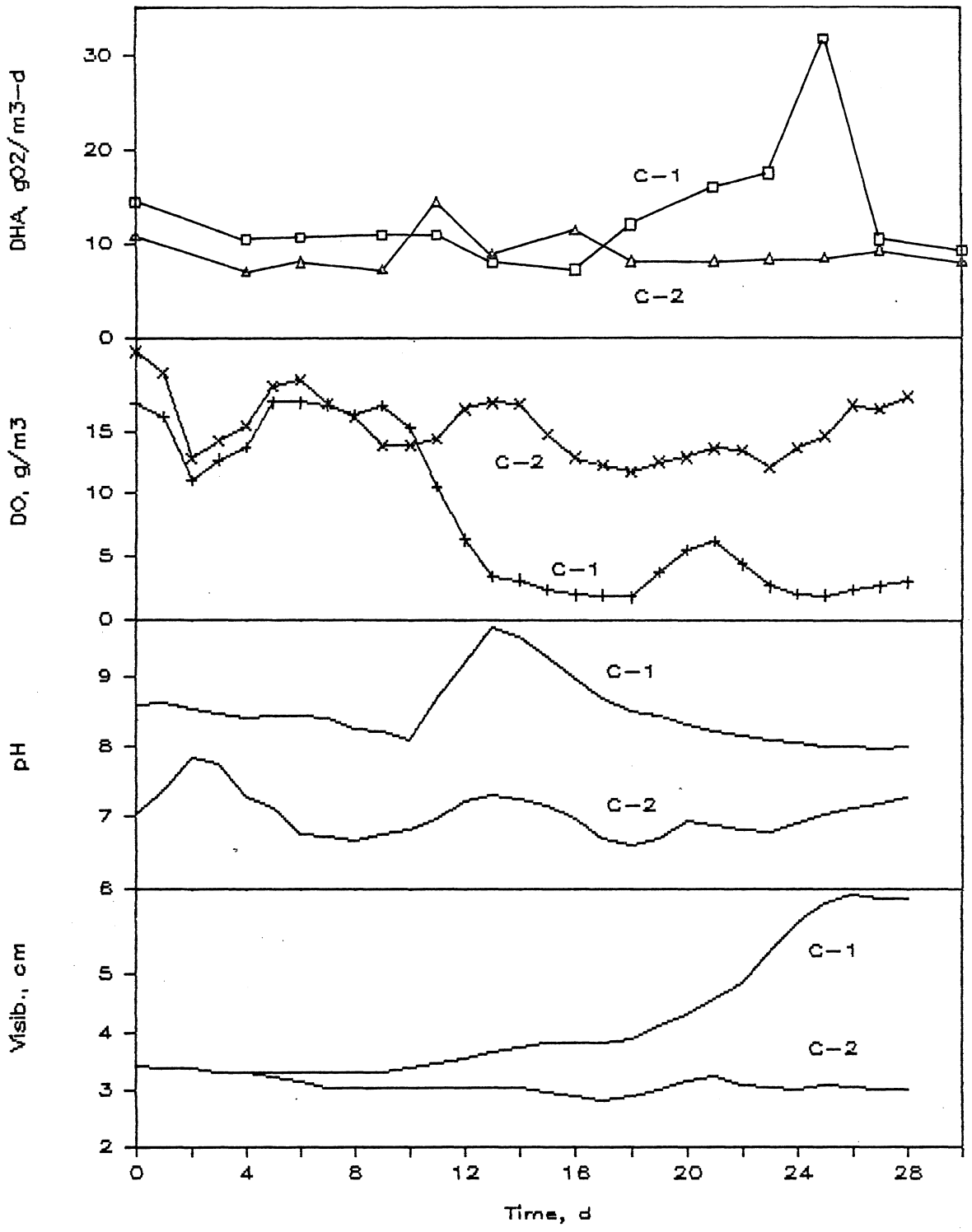

Figure 4-21. Temporal variation of DHA, DO, pH and visibility in reduced-pH (C-2) and control (C-1) cultures. Exp. 8.

Table 4-19. Effect of biofloc and bacterial seeding on photosynthetic community composition in bioflocculation ponds, exps. 7, 9

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

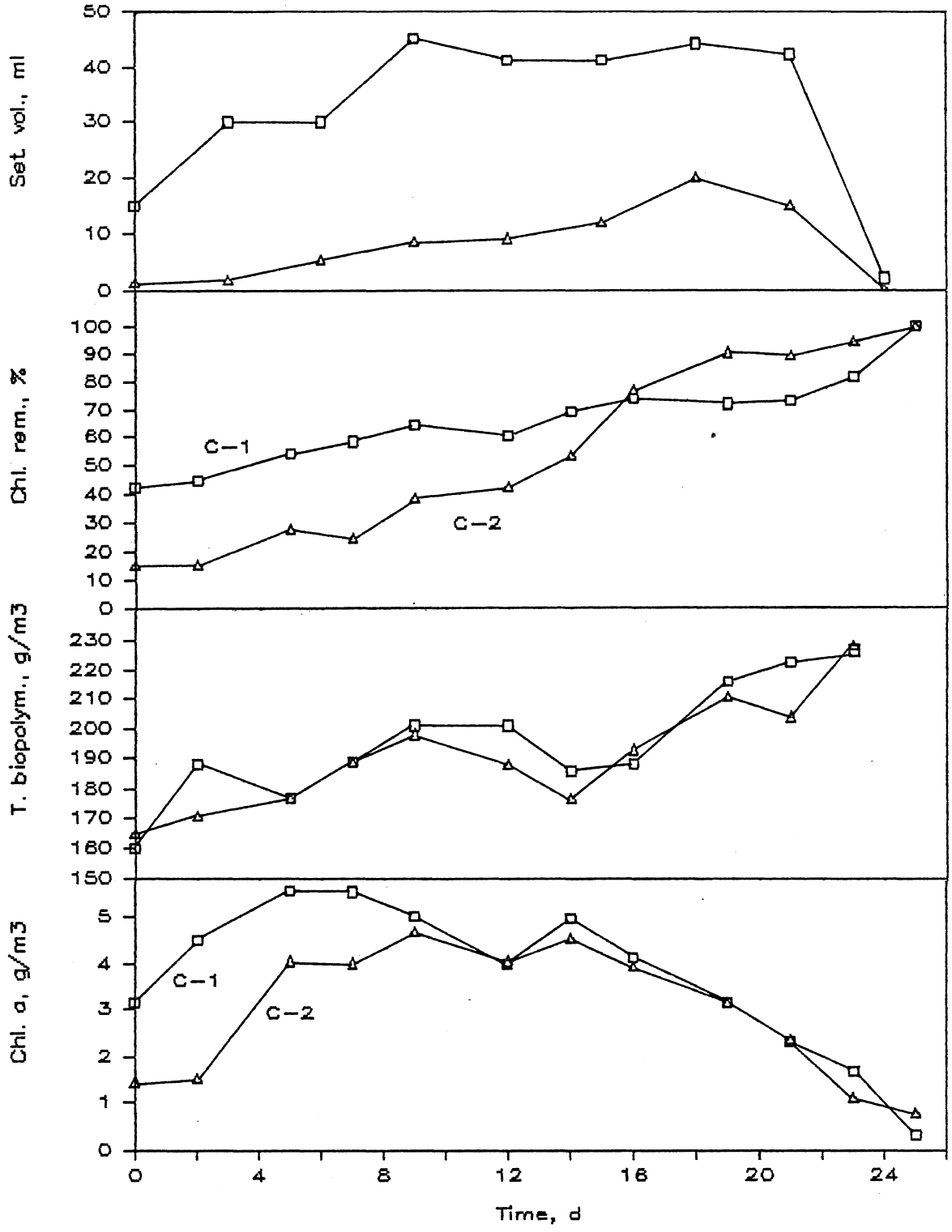

Figure 4-22. Temporal variation of settleability, biopolymer and chl. a in biofloc-seeded (C-1) and non-seeded (C-2) cultures. Exp. 7.

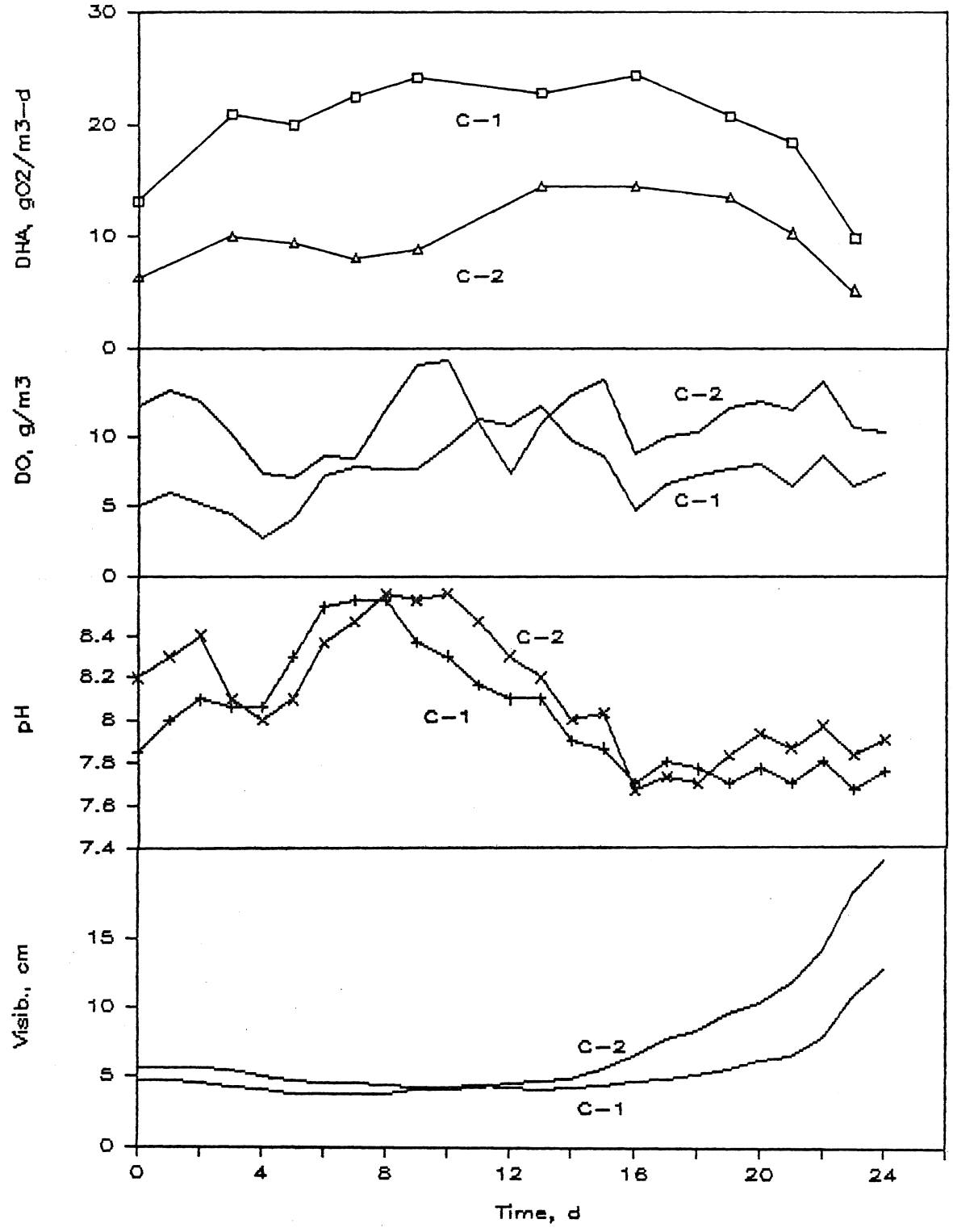

Figure 4-23. Temporal variation of DHA, DO, pH and visibility in biofloc-seeded (C-1) and non-seeded (C-2) cultures. Exp. 7.

(Fig. 4-24). Chlorella and Monodus, which were co-dominant initially, remained at significant levels throughout the experiment (Table 4-19). Microscopic examination of the C-2 medium revealed the presence of flocs consisting largely of T. rosea cells. Approximately 20% of the flocs consisted of Chlorella and Monodus cells. Biopolymer concentration increased in parallel with settleable matter. DHA, DO and pH dropped off towards the end of the experiment, while visibility remained approximately constant (Fig. 4-25).

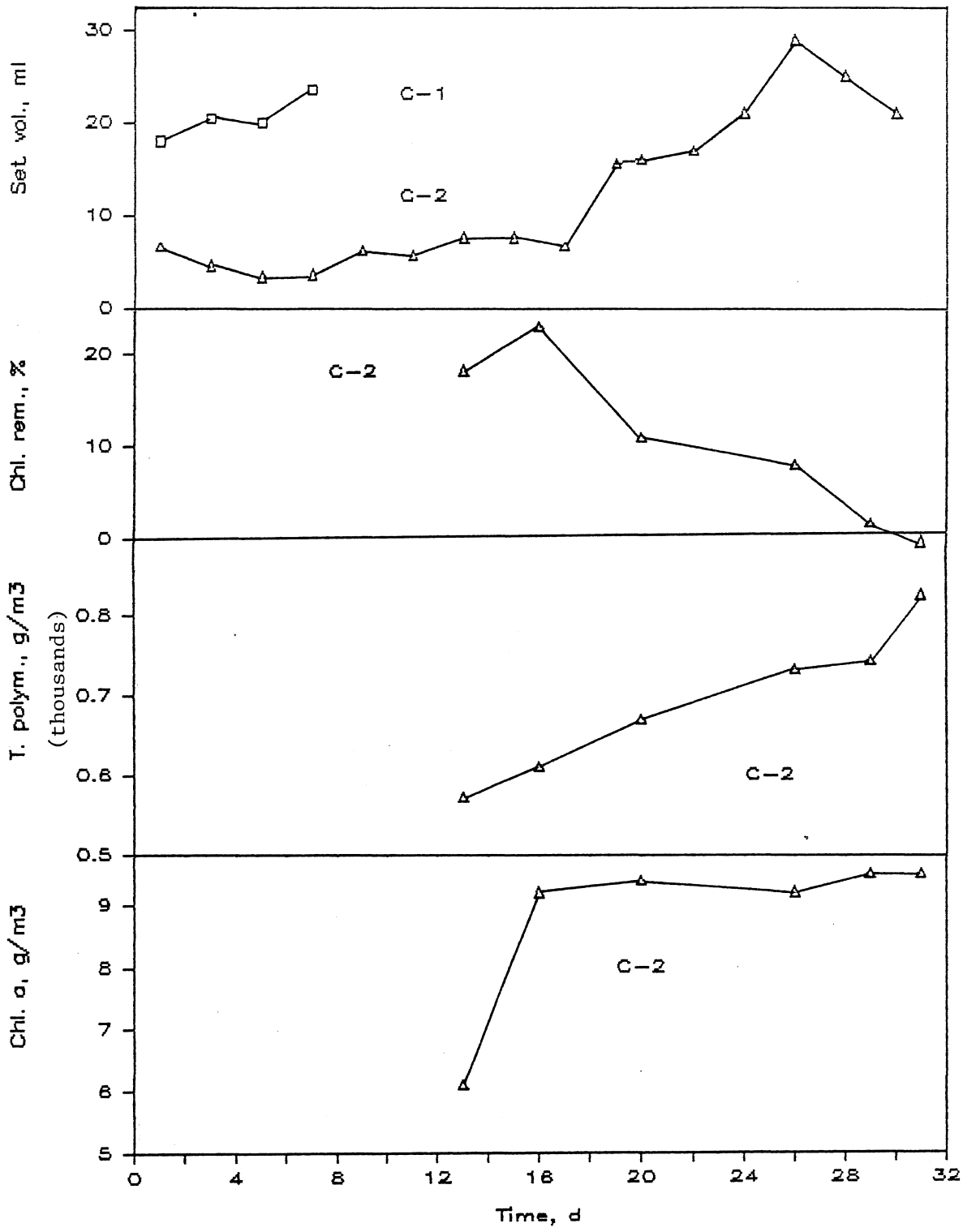

Figure 4-24. Temporal variation of settleability, biopolymer and chl. a in a culture (C-2) seeded with photosynthetic bacteria. Exp. 9.

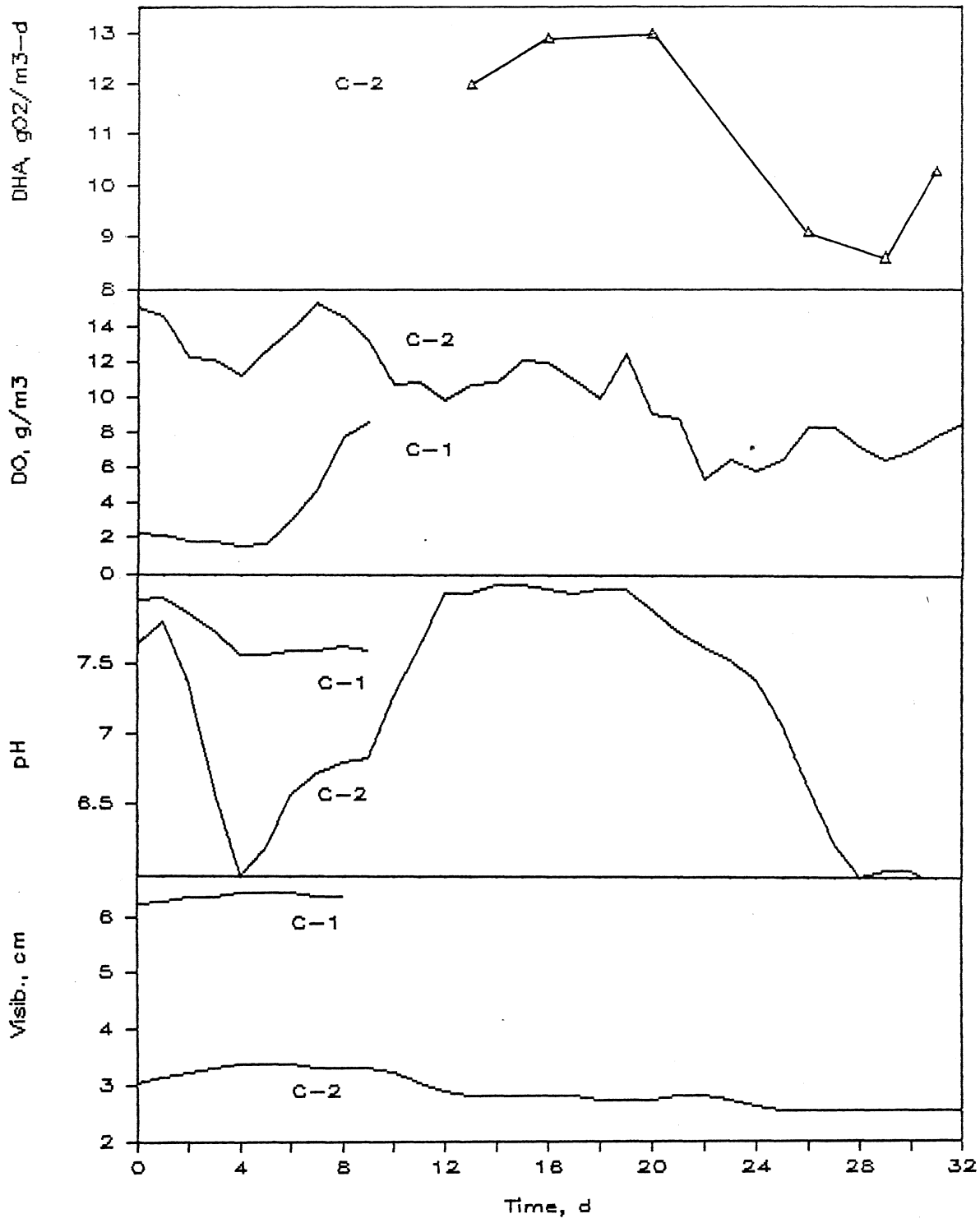

Figure 4-25. Temporal variation of DHA, DO, pH and visibility in a culture (C-2) seeded with photosynthetic bacteria. Exp. 9.

5. DISCUSSION

5.1 Photosynthesis-driven Chemical Flocculation (Autoflocculation) versus Bioflocculation

During warm, sunny weather it is possible for the pH in a shallow waste pond to rise as high as 11 if the removal of CO_2 by algal photosynthesis exceeds the rate of supply by atmospheric diffusion and bacterial respiration. The following reaction is favored at low CO_2 concentration:

$$HCO_3^- \longrightarrow CO_2 + OH^- \qquad (5\text{-}1)$$

The resulting accumulation of OH^- ions can lead to precipitation of $Mg(OH)_2$ and concomitant enmeshment and settling of algal cells. Golueke and Oswald (1965) observed that this phenomenon, which they termed "autoflocculation," sometimes occurred in high-rate ponds when the pH exceeded 10. Moelmer (n.d.) and Gordan and Chapman (1979) also observed autoflocculation of algal cells at elevated (10.3-11.0) pH.

Golueke and Oswald (1965) deduced that, at elevated pH, phosphate precipitation occurred at the same time as $Mg(OH)_2$ formation because greater removals of phosphate from solution were measured than could be accounted for by algal assimilation. Gordon and Chapman (1979) identified calcium, magnesium and phosphate as the major ions in phosphate precipitate based on the stoichiometry of the reaction. Sukenik (1985), using energy dispersive X-ray analysis, determined that calcium phosphate was the major agglomerating substance in algal suspensions flocculated at pH 8.5-10.0 in a brackish medium.

The latter finding indicates that phosphate precipitation was probably responsible for some observations of spontaneous algal flocculation at pH values less than 10, e.g., Hemens and Mason (1968) (pH

9-10), Sukenik and Shelef (1984) (pH 8.5-10.0), and Bogan et al. (1960) (pH 8.2). McGriff and McKinney (1972) operated an "activated algae" system in which the algal biomass was highly flocculated. They attributed flocculation of algal cells to enmeshment in a chemical matrix. Their results, however, indicated that the efficiency of algal settling was independent of pH at levels as low as 7.9, whereas a pH of 9.0 is the minimum at which extensive phosphate precipitation will occur. It is thus more probable that spontaneous flocculation in the activated algae system was exopolymer-mediated.

A number of factors make it unlikely that spontaneous flocculation achieved in the present research involved a chemical enmeshment mechanism. First, development of settleable algal suspensions was in many cases accompanied by declining pH trends (e.g., exps. 1, 5, 6, 11, 12). Second, cultures having approximately the same pH sometimes differed in settleability (e.g., exps. 2, 3, 7, 10). Third, in cases where pH differed, it was possible for greater flocculation to occur in the culture having the lower pH (e.g., exps. 1, 5, 12). Fourth, the extent of flocculation was generally influenced by algal species composition. Finally, microscopic observation of Synechocystis and Chlorella/Monodus flocs showed cells in tight adhesion with no evidence of a chemical matrix. These flocs were quite different from chemical flocs formed with aluminum sulfate in which algal cells made up less than half the floc volume.

5.2 Significance of Algal Species

Microalgal genera previously identified in bioflocculating systems include Ankistrodesmus (Koopman et al. 1978), Chlorella (McKinney et al. 1971), Micractinium (Eisenberg et al. 1981), Scendesmus (Oswald et

al. 1978, Koopman et al. 1978, McKinney 1985 personal communication), and Synechocystis (Lincoln et al. 1984). The principal algal genera encountered in the present investigation were Synechocystis, Chlorella, and Monodus. Of these three, the former genus had the greatest bioflocculating tendency. In experiment 1, where Synechocystis made up more than 95% of the biomass, agglomeration of approximately 90% of the cells was achieved and the resulting floc particles were made up almost entirely of this alga. Settling tests indicated close to 90% removal with settling velocities on the order of 5 m/h (Fig. 4-3). Maximum algal removals achieved in all experiments in which Synechocystis was dominant or co-dominant averaged 55%. The bioflocculating tendency of Chlorella and Monodus were co-dominant was less, but still led to significant algal removals. The best performance was in experiment 12, where a maximum settling efficiency of 56% was observed in the pond mixed at 30 cm/s, compared to an efficiency of 36% in the more slowly mixed (14 cm/s) pond. Maximum algal removals achieved in all experiments in which Chlorella and Monodus were co-dominant averaged 38%. This average does not include the performance of pond C-1 in exp. 8, where excessive ammonium hydroxide dosage killed the algal population, yielding an unusally high culture settleability.

5.3. Role of Mixing

5.3.1. Species control

One role of mixing in algal bioflocculation is species selection. Mixing is one of the most effective operational techniques for algal species control in mass cultures. Eisenberg et al. (1981) reported that continuous flow-mixing of domestic sewage-fed, 0.1-ha ponds at 15 cm/s promoted dominance of bioflocculating Micractinium spp. (M. pusillum and

M. quadrisetum). Azov et al. (1980) monitored algal genera in relation to environmental and operational parameters in a number of sewage-fed, small scale ponds. They found that mixing by pumps or jet aerators favored Chlorella, whereas mixng by cage aerators favored Scenedesmus and Micractinium as well as Chlorella. Complete absence of mixing encouraged Euglena.

Based on the above, Lincoln and Koopman (1984) introduced continuous flow mixing in the growth (high-rate) pond of the swine waste treatment system in an attempt to promote bioflocculating species. This action was initially successful: Micractinium spp. became dominant and settleable matter in the culture increased. The settleable matter consisted of phototrophic bacterial and Micractinium floc. There was little co-flocculation (i.e., mutual enmeshment in flocs) of these two types of microorganisms. Other algae present in the culture (notably Chlorella) did not flocculate. Eventually the Micractinium-dominated culture was lost.

The converse strategy of maintaining quiescent conditions in the growth pond would be advantageous if cultivation of Synechocystis were desired, as Lincoln et al. (1984) observed that continuous flow-mixing was inhibitory to Synechocystis. Flow-mixing to induce bioflocculation would then be applied after the culture was transferred to a secondary, mixed pond.

5.3.2. Particle interaction

The second role of mixing in algal bioflocculation is to facilitate bridging of polymers between cells. Algae-algae bridging is one possibility, but bridging may also occur between bacterial and algal cells. In fact, a crucial role of mixing might be to keep floc-forming bacteria in

suspension, available for interaction with algal cells. Turbulence is always present in water bodies, particularly larger ones where the fetch is significant, and may be sufficient to promote the aggregation of algae and bacteria without mechanical supplementation. Koopman et al. (1981) achieved bioflocculation of Ankistrodesmus, Scenedesmus and Micractinium in secondary ponds without mechanical mixing. From a practical standpoint, however, natural turbulence would generally be insufficient to keep flocs in suspension after they have formed. If the algae are to be harvested in a separate sedimentation basin, mechanical mixing must be provided in order keep floc in suspension during the transfer operation.

In the present research, mixing promoted the production of settleable matter in each of the three experiments (exps. 1, 2, 4) where mixed and unmixed ponds were operated in parallel. Mixing enhanced algal removal in two of these trials (exps. 1, 2). Settleable matter production and algal removal were found to be proportional to mixing velocity in exp. 12.

5.4. Role of Bacteria

If bacteria play a role in algal bioflocculation in wastewater cultures, then it should be beneficial to provide organic substrate to a culture undergoing this process. This hypothesis was tested in experiments 5, 6 and 11. Results were mixed. With Synechocystis initially dominant, application of FBR effluent had a pronounced, positive effect on both settleable matter production and algal removal (exp. 5). Bacterial activity, as reflected by dehydrogenase activity (DHA), was substantially greater in the waste-loaded pond. With Chlorella and Monodus co-dominant, application of FBR effluent had a slightly negative

effect on settleable matter production, while not affecting algal removal (exp. 11). Explanation for this result may lie in the fact that bacterial activity (DHA) was not stimulated by the waste addition. In experiment 6, with Synechocystis dominant initially, two different rates of loading with FBR effluent were tested. Again, there was little difference between the experimental (highly loaded) pond and the control in terms of settleable matter production or algal removal. The more heavily loaded culture exhibited only a small increase in dehydrogenase activity.

The mixed results with FBR effluent could perhaps have been due to the nature of the waste. Through the anaerobic digestion process, much of the biodegradable matter is removed, thus limiting the value of this waste as a substrate for floc forming bacteria. Undigested waste should contain a higher fraction of biodegradable matter, and promote greater prouction of floc forming bacteria. This hypothesis was tested in exp. 10, where the effect of FBR effluent was compared with that of settled waste supernatant. Contrary to the hypothesis, it was found that FBR effluent promoted greater settleable matter production and algal removal than the settled only waste. As expected, dehydrogenase activity was greater in the culture loaded with settled waste supernatant. However, most of this activity was apparently due to proliferation of phototrophic bacteria. These bacteria flocculated, but there was little tendency for algal cells to become enmeshed in their floc.

5.5. Algal Biofloc and Bacterial Seeding

Provision of seed material to promote floc development has been successful in chemical flocculation methods as well as the activated sludge and anaerobic contact processes. Experiment 7, conducted with

Synechocystis and Chlorella co-dominant initially, showed that settleable matter production and algal removal were improved at first by seeding with algal floc. Longer term results, however, were not as favorable.

It was noted that phototrophic bacteria (T. rosea) introduced into either the high-rate or bioflocculation ponds with anaerobic lagoon effluent or facultative pond effluent bioflocculated within relatively short periods of time. The question was asked whether these bacteria could interact with algal cells in a mixed pond environment, accelerating algal bioflocculation. Experiment 9, in which Chlorella and Monodus were initially dominant, was conducted to answer this question. Consistent with previous observations, the phototrophic bacteria bioflocculated, but there was only moderate interaction with algal cells. The phototrophic bacterial flocs contained an algal component of no more than 20% on a biovolume basis. This result indicates that bacterial exopolymers can be quite specific to bacteria, limiting the degree of algal-bacterial interaction in floc formation with these algal species.

5.6. Other Operational Variables

Two experiments were conducted to determine whether or not parameters important to autoflocculation had a signficant effect on flocculation observed in the field pond system. The first of these (exp. 3) involved addition of carbonate. Moellmer (n.d.) and subsequently Sukenik and Shelef (1984) and Sukenik et al. (1985) reported that the autoflocculation of algae was more pronounced in an alkaline medium. Results from exp. 3 showed a somewhat lesser settleable matter production in the carbonate supplemented pond, however. Interestingly, bacterial activity was stimulated by carbonate addition, even though culture pH was not

appreciably changed.

pH is a critical parameter in autoflocculation. In exp. 8, the pH of one of the cultures was lowered to determine whether or not this action would inhibit flocculation. In fact, lowering of pH did inhibit flocculation. It did not appear that low pH per se was not the most significant factor, however. Algal growth was stimulated at the lower pH, and it is known that more actively growing microbial cultures have less bioflocculating tendency than more slowly growing or static cultures (e.g., the control). Reduction of pH limited bacterial activity relative to the control culture.

5.7. Application of Bioflocculation

Application of bioflocculation as a sole means of algae harvesting is judged to be very promising in systems where Synechocystis is dominant throughout most of the year. This alga, because of its small size (1.5-2 μm diameter), forms very stable suspensions which require high flocculant doses for removal by chemical flocculation. In contrast, bioflocculation of Synechocystis via flow mixing with no chemical additions was highly effective. The significance of this result was particularly well demonstrated by the fact that 6.3 kg of cellular biomass of this species, free of chemical contaminants, was obtained at the end of experiment 1 simply by draining the experimental channel, allowing the settled biomass to air dry, and shoveling dried matter from the pond bottom. An estimated additional 14.7 kg of biomass was left in the pond and eventually discarded. The total crop was thus 21 kg, corresponding to a recoverable concentration of 0.23 kg/m^3. Because of the near neutral density of Synechocystis, previous attempts to obtain uncontaminated samples of this species by centrifugation resulted in dry

weight quantities of no more than a few grams, even with the expenditure of considerable effort.

Prospects for the harvest of Chlorella and Monodus via bioflocculation are less promising than those for Synechocystis when considered on a percent removal basis, but may be better in terms of biomass recovery. Harvests of these genera performed after experiments 10 and 11 yielded 35 kg and 46 kg, respectively, of air-dry algal solids corresponding to recoverable concentrations on the order of 0.5 kg/m^3.

It would be desirable to decrease the time required for bioflocculation to take place while also improving the separation efficency achieved, especially when Chlorella and Monodus are dominant. One means of accomplishing these objectives would be to increase mixing speed. Unfortunately, operation at greater mixing speeds imposes more severe energy and equipment demands. Initial dilution of the influent wastewater to the pond system might also improve flocculation. It has been observed in conventional wastewater treatment systems that concentrated wastes tend to inhibit bioflocculation. Further dilution of wastewater with potable water would not be economically feasible, but recycling of treated effluent may be practicable. Because nutrients are in excess in the system, this could be done without lowering final algal density, which should be kept high. A third possibility would be to combine bioflocculation and polyelectrolyte (natural or synthetic) addition. Initial aggregation of algae (to 50-100 μm diameter flocs) in the bioflocculation ponds occurred rapidly. At this point, flocculant could be introduced. Because flocculant requirements are a strong function of particle size, the initial aggregation achieved by mixing could possibly reduce the overall cost of harvesting.

More fundamental advances in bioflocculation are also possible. As indicated by this research, the interactive tendency of algal and bacterial exopolymers appears to be, at least in some cases, species specific. If a low cost chemical treatment could be made to lessen the specificity of these polymers, then the effectiveness of waste loading or bacterial seeding could be increased.

5.8 Preliminary Economic Evaluation

Based on the results of this study, the energy cost of the bioflocculation process can be compared to the cost of chemicals for conventional (alum) flocculation. Energy and chemical are the most significant inputs of the respective processes. It was found that the AC motor driving the paddlewheel of the high-rate pond (C-4) used 380 W at a flow mixing velocity of 20 cm/s. With continuous mixing, energy consumption was 9.12 kWh/d. At \$0.06/kWh, the energy cost would be \$0.55/d. Although the combined volume of the paired bioflocculation channels (C-1 and C-2) is less than the volume of C-4, this same energy cost is assumed for bioflocculation. At an average flocculation time of 21 days, the cost of mixing would thus be \$11.55 for 100 m^3 of culture volume. Experimental harvests of the bioflocculating cultures yielded approximately 500 g/m^3 (50 kg from 100 m^3 of culture volume) air-dry solids on three occasions. The corresponding mixing energy cost amounts to \$0.23 per kg of algal solids. Using alum priced at \$200/tonne, and a typical alum/TSS ratio of 1.2, the chemical cost would be \$0.24 per kg of algal solids.

Thus, it can be seen that chemical flocculation and the biological flocculation actually obtained under field conditions are comparable in cost. It is also evident that if the bioflocculation process could be

shortened (e.g., to 5 days) its cost would be one-fourth that of alum flocculation, and no doubt be economical. Also, the biomass recovered would be free of the substantial ash content imparted by alum precipitation. The use of flocculation aids, such as organic polmers, could perhaps reduce bioflocculation time to two days or less. If mixing were to reduce the required polymer dosage, a minimum cost might be realized by the combined effect of short mixing time and polymer addition.

6. CONCLUSIONS

The following conclusions were reached based on the results of the present investigation and their relationship to previous studies of microbial flocculation.

1. Self-aggregation of microalgae observed in the present research was due to exopolymer-mediated flocculation, not enmeshment in chemical precipitate (autoflocculation).

2. Algal species composition is the strongest variable in bioflocculation of microalgae grown in concentrated wastewater from a swine rearing unit.

3. Floc-forming bacteria and their exopolymers can enhance algal bioflocculation, but are not primarily responsible for causing this phenomenon.

4. Exopolymers of algae and bacteria can be species specific, i.e., bioflocculation of specific microbial types in mixed cultures is possible.

5. Wastewater composition is of fundamental importance to the bioflocculation process: concentrated wastewaters tend to inhibit bioflocculation, and wastewater composition influences the types of microalgae and bacteria which grow in the culture medium.

6. A better understanding of the physical chemistry of exopolymers, particularly in regard to their tendency for species-specific interactions, would facilitate advancement of bioflocculation technology. A goal of this research should be to develop methods of reducing the specificivity of exopolymers.

7. The greatest immediate potential for utilization of the bioflocculation phenonmenon in algae harvesting lies in combining this process with natural or synthetic polymer addition as a means of reducing the dosage of chemical flocculant which is required to effect separation.

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