HARVESTING SOME AGRICULTURALLY PROMISING ALGAE

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ABSTRACT

HARVESTING SOME AGRICULTURALLY PROMISING ALGAE

By

John Benham Gerrish

Several microscopic algae were harvested by each of several methods. Combinations of species and method were sought which might make algal culture an attractive alternative for a limited-resource agricultural enterprise. Genera represented are Chlorella, Oocystis, Scenedesmus, Coelastrum, Chlamydomonas, Anabaena and Spirulina. The algae were produced in light-limited 10-liter continuous culture. Harvesting methods investigated include sedimentation, centrifugation, electroflocculation, filtration, electrodecantation and predation. Quantitative measure of harvesting success is attempted and a new parameter based on entropy is presented. A stochastic model for sedimentation provides an incomplete description of the process. There are significant differences in harvesting behavior between cells grown at different growth rates in the light-limited cultures; old cells harvest generally more easily than young cells. Energy costs are estimated for accelerting the harvest operation.

Approved: W. G. Bickert
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HARVESTING SOME AGRICULTURALLY PROMISING ALGAE

By

John Benham Gerrish

A THESIS

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To Sandra,

and our four children:

Deborah
Philip
Elizabeth
Matthew

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Glossary of Symbols

A	area
a	a variable number of standard deviations
b	a slope of a filtration graph, t/V versus V
C_F	concentration of algae in liquid before harvest (g dry wt./liter)
C_L	concentration of algae in the lean fraction after separation has begun (g/l)
C_R	concentration of algae in the rich fraction after separation has begun (g/l)
C_{max}	the maximum concentration of algae achievable in a given process
D	dilution rate (liters/liter/hour)
D_H	hydraulic diameter (defined in Table 14)
E	enrichment C_R/C_F
F	flow rate through a continuous culture (liter/hour)
F	(as subscript) mixed state or inlet condition
G	a one dimensional parameter expressing harvesting success
g	acceleration due to gravity (9.81 m/sec^2)
H_a	a normalized parameter related to E, $H = \frac{C_F}{C_R}\frac{(C_{max} - C_R)}{(C_{max} - C_F)}$
h	height of interface above bottom of settling vessel; expressed as a queue length
I	purity index
K	constant growth in light-limited culture (liter gram/hour)

k_B	Boltzmann's constant, 1.38×10^{-23} joules/°K
k	growth constant in $hours^{-1}$
L	a constant proportional to illumination level (liter gram/hr. m^2)
L	(as subscript) lean fraction
ln	Naperian logarithmic operator
M	electrophoretic mobility (μ/sec/volt/cm)
N	a population, see subscripts F,L,R,Q; also a number of observations
N_o	a number of sites available for occupancy
$\bar{N}_{RQ}$	the number of objects in the average queue in the fraction R
n	a number of moles, type and location indicated by subscripts
P	pressure ($newtons/m^2$)
$P_r\{\}$	a probability {event in brackets}
Q	(as subscript) indicates participation in a queue
R	recovery; the fraction of algae initially present which is recovered in a harvest
R	(as subscript) rich fraction
r	recycled fraction of harvested material, also a radius
RI	a recovery index, two dimensional
S	entropy (joules/°K)
T	time, a fixed value
t	time, a variable
t_r	retention time

Glossary of Symbols - Continued

V	volume (m^3 or liters)
v	volume (usually liters) see R,L,F as subscripts
W	work or energy (joules)
X	culture density in grams dry weight per liter (g/l)
X_f	a fixed concentration
X_i	concentration of a culture just after inoculation (g/l)
X_s	the lowest concentration at which light-limited growth takes place (g/l)
x	a fraction of occupied sites
Y	a mole fraction
y	the vertical dimension in a settling vessel (m)
α	a constant expressing the effect of input energy on (liters/joule)
Δ	a small increment
κ	instantaneous growth constant in $hours^{-1}$, may be a function of time
Λ	cell age (units of time, usually hours)
$\bar{\Lambda}$	mean cell age
Λ_d	cell age at division
λ	death rate, $1/\lambda$ is the mean time between cells leaving a population
μ	10^{-6}m. usually μm
μm	10^{-6}m
μ^3	$10^{-18}m^3$
ν	a mole fraction, one dimension of RI
ε	Rony's index, extent of separation

Glossary of Symbols - Continued

π	osmotic pressure expressed in milliOsmols/kg.
ρ	bulk density
σ	standard deviation
ϕ	a mole fraction, one dimension of RI
ω	angular velocity (radians per second)

CHAPTER I

INTRODUCTION

The overcrowded planet foreseen by demographers is a distinct possibility in the not-too-distant future. Competition for land area between habitation and food production will force a trend toward unconventional agriculture: exploitation of insects, aquatic animals, bacteria, non-vascular plants, and the like.

The cultivation of microscopic algae is considered unconventional agriculture. The algae were extensively studied in connection with the space program of the 1960's when it was thought that algal culture might provide the multiple needs of oxygen regeneration, food supply and waste treatment in a man-alga partially-closed symbiosis. Interest seems to have waned with the news that only for missions of greater than 400 man-days would an algal culture in space have any cost advantage over the brown-bag lunch (Lachance, 1968).

The two-species culture is thought to be inherently less stable than a culture of many interdependent species. This has not deterred modern agriculture from its trend toward local cultivation of single species. The

fail-safe control necessary for prolonged operation of the less stable few-species cultures poses an urgent technical challenge.

Exploitation of the algae for food or feed has attracted some research effort. As a protein source, the algae can be managed to yield an impressive quantity of protein per hectare, albeit basically a plant protein with the known deficiencies in amino acids essential to the human diet.

Since unwanted algae are a symptom of water pollution, it seems logical that the cultivation of algae in wastewater might be a useful way to remove the plant nutrients before they find their way into an unfertilized lake or stream. Moreover, it is tempting to anticipate a windfall in food or feed production at the same time. More will be said of this.

In spite of the promising agricultural prospects, the microscopic algae have not been accepted as a crop on a commercial scale in more than a few isolated cases that smack of profligate research endeavors. The reasons for non-acceptance of algal cultivation are nutritional, economic and historic. While the algae promise a high protein yield of a fair quality, the digestibility and availability of the protein is less than desired. There appear to be limits to what the human system can accept;

taste and texture problems remain to be solved. For algae as animal feed, these same problems exist.

In 1970, a ton of algae cost more to produce than the ton of soybeans it might replace in an animal diet. In several cost projections, the harvesting and drying of the algal product amounts to about seventy-five percent of the total cost of production. Half of this 75% cost is assigned to harvesting, i.e., getting the algae enriched from the typical 1 gram dry matter per liter of culture to the forty grams per liter suitable for a drum dryer. Costs have often been discovered too late. For example, a closed culture is relatively contaminant-proof, but it lacks the evaporative cooling which keeps open culture at a reasonable temperature for good growth. So a cooling cost was found to be a large percentage of production cost in closed culture. The need for agitation, large illuminated-surface-to-volume ratio, and efficient carbon dioxide transfer seems to have been overlooked by optimistic cost forecasters. So the meal costs more than the menu indicated.

In the space program, culture volume was to be minimized. Consequently, the species possessing the maximum of growth rates was sought. For this reason, species of the genus Chlorella seem to have attracted the most attention - in spite of the attendant nutritional and technical problems. There is perhaps a second reason for

the Chlorella connotation in the word "algae." It is a laboratory weed which can only be avoided by careful management of the culture in which it is not wanted. I suggest that Chlorella is analogous to crabgrass in many respects. I cannot imagine a sensible farmer cultivating crabgrass, however.

The algae, then, have received a bad press coverage not only for the hopes which the Chlorella episode shattered but also for the appearance of algae as a symptom of water pollution.

There are about 370 genera and 10,000 species among the green algae alone. If technical and nutritional problems disqualify Chlorella from further consideration as a potential agricultural crop, there is certainly a good chance that a more promising species can be found. The sacrifice involved in accepting a lower growth rate means only that a larger cultivation area must be planned. It is difficult to take full advantage of a very high growth rate anyway; efficient introduction of light to the culture becomes a serious problem or else the surface-area-to-volume ratio gets out of hand.

In this thesis, I will concentrate my efforts on the technical problems of harvesting the algae. I will touch on the associated problems of cultivation and washing the harvested product. Except for an occasional remark, I will avoid the nutritional aspects of algae as food or

feed. Hopefully, the wolf will not come to the door, but if he should, perhaps this work will help those who must keep him at bay.

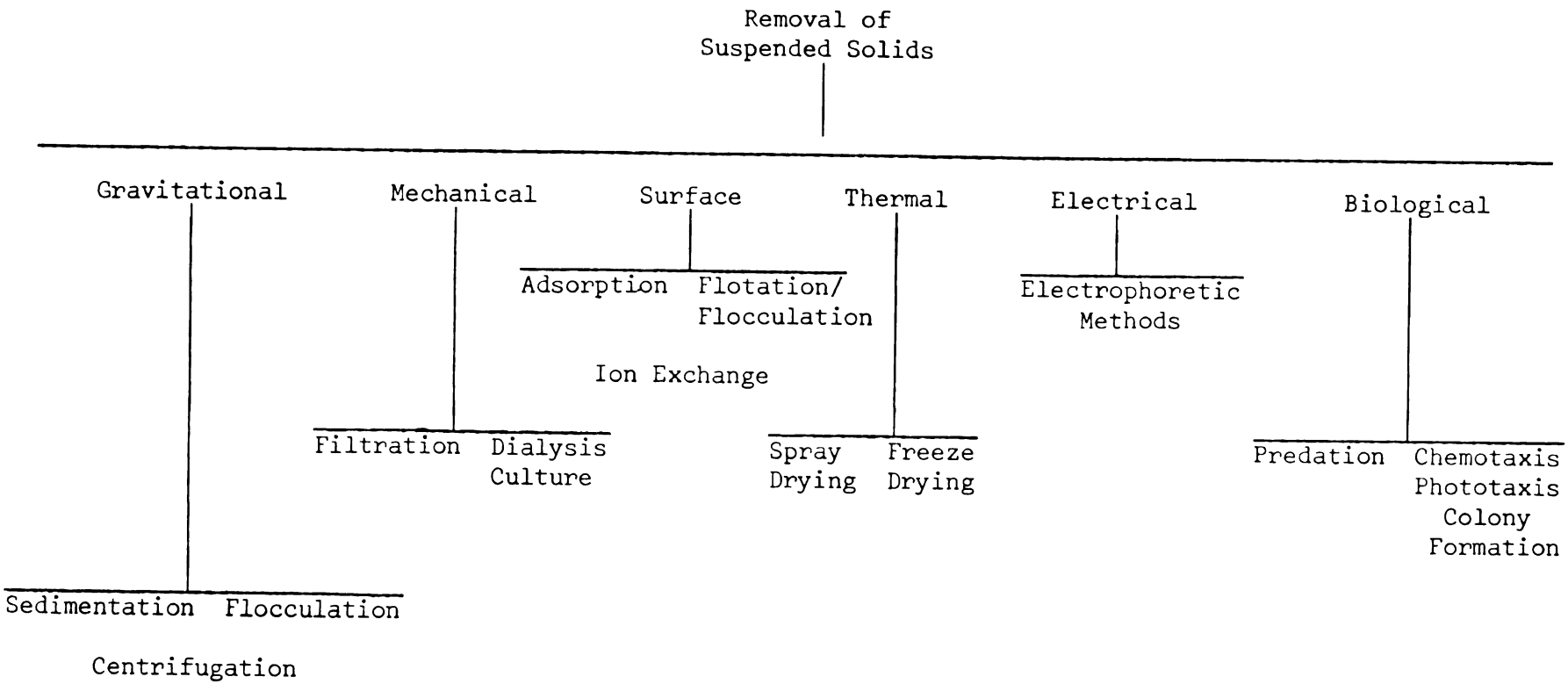
CHAPTER II

OBJECTIVES

Harvesting the algae deserves investigation since by the present harvesting methods, the cost of harvesting is a large percentage of the total production cost. There may be a thermodynamic limit which prevents improvements in harvesting efficiency.

I propose to study the possibilities for increased harvesting efficiency by exploring methods for separating the algae from their culture media and enriching the algal suspension to the point where it can be considered "harvested" - or practically so. Classical liquid solid separation methods will be examined in order to quantify the alleged "difficulty" associated with harvesting. Novel methods will be explored for their potential promise (Figure 1). Using several algal species will provide an interesting variable which seldom appears in published quantitative studies. Ways in which a particular algal species can cooperate or hinder harvest will be emphasized. The species I have selected represent a diversity in characteristics; most have been tried in large scale culture. Indeed a method may evolve for more accurate

FIGURE 1.--A Scheme Classifying Separation Methods



selection of promising species based on harvestability. Cheap and easy harvest is a necessary criterion for acceptance of algal cultivation into the agricultural setting.

CHAPTER III

LITERATURE REVIEW

The problem of algal harvest is casually classed into the broader engineering category of liquid-solid separation. The conventional techniques which apply for solutions containing low concentrations of suspended solids are the ones one might expect to be successful: centrifugation, flotation, filtration, chemical flocculation and sedimentation.

There is a voluminous literature devoted to the theoretical and practical aspects of these separation methods. Poole and Doyle (1968) edited a 1.6 Kg. tome entitled Solid-Liquid Separation. It includes 5,181 references in abstract form. Mathematical models abound for the separation of small discrete particles from liquids by settling and filtration. Chemical flocculation and flotation are less well understood.

Examples of biological solids in Poole and Doyle are brewers yeast and activated sludge; algae are barely mentioned. Aiba et al. (1965) devote a chapter to the separation of microorganisms from culture media. They have selected several models which seem to have worked well in what they term "biochemical engineering". Aiba et al.

have also considered energy expenditure in the cultivation operation, i.e. mixing and aeration. Little or no reference to the algae appears in their book, however. It seems that engineers have assumed that the technical problems of harvesting the algae are not unique. Such an assumption may be correct; it may be only that the cost of recovery is too great using expensive techniques in which case less expensive techniques should be sought.

Freeman (1964) treats the recovery of cells from fluids in much the same way as do Aiba et al. Freeman mentions in addition a few unconventional methods of harvesting - or avoiding harvesting - which caught my interest, viz. dialysis culture, surface culture, biphasic growth and electrokinetic methods. Electrokinetic separation takes advantage of the fact that most growing cells including some algae (Ives, 1959) possess a negative charge. The conventional separation methods depend on a difference in specific gravity between cells and medium for their success or in the case of filtration, on cell size and rigidity.

Bier's adaptations (Cooper et al., 1965) of Pauli's electrodecantation idea (Pauli, 1924) seem to be the most promising of the electrokinetic techniques. The suitable configuration for liquid solid separation involves use of the electric field to prevent the accumulation of charged solids on a filter. Filtration runs (i.e. times between

back flushings) are much longer than for filtration without the electric field. The conductivity of the liquid phase economically limits the applicability of the method. Cooper et al. filtered a clay suspension and an algae-laden lagoon effluent. They estimated for a water of 1000 μmho/cm a cost of 35 Kw hr/1000 gal. for a filter handling 1 gal/hr sq. ft. Latex has been electrokinetically concentrated on an industrial scale for years (Murphy, 1942). Some electrokinetic pumping of fluid through the filter takes place at the same time as electrodecantation. It is a very uneconomic substitute for the same amount of hydraulic power (Osterle and Farn, 1967).

The interesting contributions of Kolin (1953, 1955) appear to me to be inapplicable for separation of cells from their growth media. They involve elegant techniques - electrophoretic isoelectric focussing, electromagnetophoretic migration - but are more on the order of laboratory curiosities. Electrophoretic focussing seems unpromising since it takes place in a combined pH and density gradient (Kolin, 1955). It moreover requires an isoelectric point which Ives (1959) failed to find for Chlorella. Electromagnetokinetic phenomena work even on uncharged particles and bubbles (conductivity of the particles must be unequal to the conductivity of the medium) but much energy is required (Kolin, 1953). The method is suitable for measuring

conductivity of cells but the possibility of economic separation of cells from fluid is remote.

Eckenfelder and O'Connor (1961) consider the separation of liquid and activated sludge, a mixed culture of floc-forming bacteria. The treatment is crude but quantitative enough to facilitate engineering design as was intended. Emphasis is on gravity settling. Eckenfelder and O'Connor point out that settling of discrete particles in a tank is quite different from the settling of floc where time is required for floc formation.

Most work on sedimentation harks back to the classic contribution of Hazen (1904). Hazen showed that in continuous sedimentation the settling tank depth had no effect on the degree of separation achieved; only overflow was significant. Overflow is the bulk flow into a tank divided by the horizontal cross-sectional area of the tank. Fitch (1957) contradicted Hazen and pointed out that for suspensions in which flocculation occurs during settling, the detention period can be of greater significance than the overflow rate. The standard method (_____, 1965) for determining settleability of activated sludge involves measuring the downward velocity of the sludge-clear water interface in a one-liter graduated cylinder. The interface is usually well-defined and in 30 minutes has typically subsided to about the 250 ml. level. The supernatant is swept virtually clear of particulate matter.

Wang (1968) presents classical models for centrifugation and filtration in cell recovery operations. He writes primarily about yeasts and bacteria and recognizes species differences. He points out the difficulties encountered in filtration of unicellular organisms directly from their fermentation broths. He also brings up the need for washing of the cells if they are to be food or feed; at each washing operation, another harvesting operation is required. For reasons unknown (or untold), cells that have been washed can be successfully filtered. Wang makes cost estimates for cell recovery. A 5.5 μm yeast can be recovered for 1.5¢ per Kg when the inlet concentration to the recovery operations is ten grams dry weight per liter. Cells are washed once. The investment costs are two thirds of that figure, and are included in it. The cost is inversely proportional to inlet concentration; doubling the washing water volume increases the price by half. Wang suggested that future work be directed towards growth of giant cells and use of polyelectrolytes as flocculating agents.

Where polyelectrolytes have been studied (e.g. Bargman et al., 1958, Kuźkin and Nebera, 1960), the problem has usually been waste water purification or ore recovery, so the edibility of the polyelectrolyte (or other flocculant) is not a problem. Alum and ferric chloride are important inorganic coagulants, now long in use. The

chemistry of Fe (III) and Al (III) is considered by Stumm and Morgan (1962). In the pH range between 4 and 7, the aluminum ion forms a tri- or tetra-positive polynuclear species. Reasonably successful stochastic models have been worked out by Vold (1959, 1960, 1963), Sutherland (1967) and Harris et al. (1966) for the elusive concepts involved in flocculation. The basis for the models is that as particles settle at different rates in a fluid, faster particles overtake slower ones and stick together. The models generally fail to predict sediment volume. Anderson (1956) used this same working hypothesis as did Camp and Stein (1943). Camp and Stein proposed initiation of velocity gradients by stirring rather than relying on the velocity differential between particles settling at different speeds. I did not pursue the history any further, but the Camp and Stein theory was apparently found in need of only slight modification in a thesis by Ritchie (1955, cited by Poole and Doyle, 1968). Anderson (1957B) showed that settling, flocculating cellulose fibers touched only at their concave surfaces. In a startling short paper, Calleja (1970) gave evidence suggesting that in the yeast Saccharomyces pombe defloccu-lation by heat (~60°C) was reversible. Calcium ions were apparently uninvolved.

As an adjunct to focculation, flotation has been used for many materials. Dissolved air coming out of

solution forms miniscule bubbles which are trapped in the floc particles. In the 50 - or-so flotation abstracts in Poole and Doyle (1968), flocculation is always mentioned as part of the separation process. The larger the floc particle (with a trapped bubble) the faster it rises (Katz and Wullschleger, 1957).

According to Lewis, (1922) filtration theory was found to be in disagreement with industrial experience. The method of Ruth (1933) for constant pressure filtration is used to estimate filterability of various slurries even though industrial filtrations are seldom carried out at constant pressure. A recent opinion (Tiller, 1968) is that each material to be filtered needs to be tested; the best theories still fail to span the range of filterable materials. The subject of filter aids and conditioners is related to the phenomenon of flocculation. The character of the filter cake can be altered to advantage by the addition of polymers which bridge the solid particles.

Boucher (1946/1947) suggested a filterability index which was to be measured at constant flow instead of constant pressure. It is more difficult to obtain this index with on-the-shelf laboratory gear than it is to do the more usual constant pressure test. Ives (1960) has composed a moving-front type of computer simulation for making sand filtration predictions from laboratory data.

Predation as a means of separating solids from liquid has been considered by Rashevsky (1959) who determined that there was a minimum size particle which a large organism - say, a fish - could afford to go out of its way to eat. Rotifers are said (Schulze, 1966) to play an important role in the removal of turbidity from wastewater. Most of the eligible predators produce by-products other than their own growth. These may constitute contamination. Predator growth is a one step food chain and is 90% inefficient in terms of protein conservation. If predators are used merely as traps - that is, they are starved until being fed the solid-to-be-recovered and harvested immediately afterwards - one must then consider the economy of keeping the necessary quantity of starved predators on hand (Schulze's turtles, Schulze, 1966). Fecal pellets may be easier to harvest than the disperse uningested solids.

A magnetic method of recovering phagocytic predators (such as leucocytes) was proposed by Cutts (1970). Particulate iron or α-ferric oxide was fed to the cells. After the iron was ingested, the cells were retrieved as they passed near a magnet.

Another interesting scheme was proposed by Fulwyler (1965). The slurry passed through an acoustic droplet generator which was tuned to make electrically charged droplets that could hold at most a single cell.

Most droplets would be empty in a dilute suspension. Droplets containing cells were detected and electrostatically deflected as they fell. The anticipated maximum rate was 1000 droplets per second.

Up to this point, I have limited attention to a miniscule sampling of references in the general area of liquid-solid separation. The general literature seldom recognizes the algae as posing any unique technical separation problems. In Poole and Doyle (1968), for example, only twelve of the 5000 abstracts mention algae; another three or four refer to plankton. On the other hand, proponents and opponents of algal cultivation do recognize the difficulty:

> Geoghegan (1951, Chlorella vulgaris as food), "Harvesting by ordinary filtration proved difficult as the cells bed down tightly; but centrifuging is quite satisfactory."
>
> Groggins (1953, Chlorella as protein source), "Harvesting of large scale culture units containing 1% of algae, dry weight, is a costly procedure, particularly if a super centrifuge is used."
>
> Mackenthun & Ingram (1967) studied algal removal from lake water for drinking water supply. "Five algal genera consistently passed through the microstraining fabric. These were the blue-greens Anabaena and Aphanizomenon, the diatoms Cyclotella and Navicula and the green flagellate Phacotus."
>
> Borchardt and O'Melia (1961) studied "algae" for waste-water nutrient stripping. They used sand filters 24 inches deep. "When an algal suspension was filtered, a low percentage of the algae was removed by each increment of the filter bed depth and some of the initial material left the bed continuously, this amount increasing with time."

Borchardt (1958) (algal removal of pollutants) claimed that Chlorella, Scenedesmus and Ankistrodesmus were too small for filtering. "Several organisms seem to respond well when treated as though they were activated sludge." He presented a 5-minute sequence of photographs to show settling speed of a filamentous alga. But he failed to mention the name of the promising species.

Bogan (1961) reported that Stigleoclonium (sic) stagnatile, a branching filamentous green alga, flocculated well and settled well. Settling was actually improved by recycling the settled floc as if it were activated sludge. At temperatures above 20°C, however, Chlorella and Scenedesmus tended to predominate the culture. Settling characteristics remained good, however, due to the coagulation effect of the insoluble phosphate salts produced at high pH levels. Golueke and Oswald (1965) report the same phenomenon and call it auto-flocculation since the high pH level is achieved at high photosynthetic rates in high light intensities (CO_2 starvation). Bogan calculates that lime can be applied for one-tenth the cost of using artificial illumination to achieve the required pH of 9.5.

Wachs et al. (1968, Chlorella and Euglena, deep waste treatment lagoons) recorded an effluent biochemical oxygen demand which was higher than that of the untreated waste water influent unless the algae were removed from the effluent stream.

Golueke and Oswald (1965, Chlorella, Scenedesmus, wastewater treatment, by-product used as animal feed)

have thoroughly researched the possibilities for harvesting and processing sewage-grown planktonic algae. They adequately distinguish between the purposes of wastewater renovation and feed or food production. Different levels of separation treatment are compared which would suit the quality required of the algal by-product. They call the harvest process in which I am interested "initial concentration" and work with an enrichment of from 200 mg. dry algae per liter of culture to 20 grams per liter. They studied the following methods: Centrifugation, pH flocculation, ion-exchange, chemical flocculation, flotation, micro-straining, passage through an electric field, sonication and filtration. Most economically promising of these were centrifugation and flocculation. I will review their work in some detail since my own work is modeled somewhat after theirs.

Centrifugation. A continuous centrifuge was used in the field scale experiments. It was a disc type centrifuge with solids discharge nozzles. The disc angle was a sensitive parameter in the figure of merit - energy consumption per ton of algae. Extent of algal removal was determined optically and by decrease in suspended solids. With increasing length of separation run, they noticed an increasing concentration of algae in their enriched harvest stream (as high as 36.5 g/l after a four hours' run - something short of steady state). Their best figure was

3200 Kw. hr. per ton of algae, 45° disc angle, 3000 rpm, 280 gallons per minute throughput, 200 mg. dry algae per liter of feed stream. This corresponds to 2300 joules per liter.

pH Flocculation. Both Chlorella and Scenedesmus seemed to flocculate and settle best at a pH of around 3. Ives (1959) found, however, that the minimum charge density for Chlorella occurred at pH 7 in deionized water.

Chemical Flocculation. The cationic flocculants Purifloc 601 and Purifloc 602 were 95% effective at 3 mg/l. A crude experiment showed no immediate deleterious effects on rats fed massive doses of the Puriflocs. Inorganic flocculants included $Ca(OH)_2$ at 120 mg/l and $FeSo_4$ at 40 mg/l in combination enough to raise the pH to 10.6. Filter alum (80% $Al_2(SO_4)_3 \cdot 18\ H_2O$) was also used. 70 mg/l was best in terms of mg. algae per mg. alum used (about 12 mg/mg). Three minutes mixing at a blade tip velocity of 12 inches per second helped flocculation; settling took 15 minutes. At a conference in 1961, Oswald apparently presented a slide which showed a very puckered horse's mouth just after administration of some alum-flocculated algal feed. Autoflocculation was suggested to be a very promising technique. The natural rise in pH which was noticed to occur on sunny California afternoons, is thought to be responsible. A settling pond of 12.7 cm. depth was suggested.

Flotation was found unsuccessful; 18 flotation agents were tried. The work of Levin et al. (1962) which was referenced described a method of harvesting algae (Chlamydomonas spp. and Chlorella sp.) by reducing the culture pH to 4, then bubbling air through a 5μ sparger. Essentially complete recoveries were achieved in 20 minutes with concentration factors ranging from 50 to 200. The richest harvest was 59 g/l - dense enough for direct application to a drum dryer. The cost was too great primarily due to the cost of pH adjustment. At pH greater than 4, the foam is not stable.

Microstraining was generally considered a failure in pilot plant scale trials. Either the cells passed through the screen (aperture not given) or it was predicted they would clog a finer mesh screen. As mentioned before Golueke and Oswald had relative success with filamentous algae but Berry (1961) reported "effective" algal removal for various genera - including Anabaena, a filamentous blue-green alga. Berry used a stainless steel mesh with 23 to 65μ aperture. Mackenthun and Ingram (1967) reported that during an Anabaena bloom in a Wisconsin lake, a 35μmicro-strainer retained only 16 percent of the algae.

Golueke and Oswald's treatment of electrokinetic phenomena is very cursory. The electric field accomplished little or no separation. They did notice, however, that

use of aluminum and cooper electrodes resulted in excellent floc formation.

Sonic vibration had a dispersing effect on the cells. Filtration was disappointing: usually the algae passed through the filter medium (cloth, paper, teflon cloth 14-20μ). Too much filter aid was required to be economic and still effective. Even cornstarch was rejected as a filter aid since the algal enriched cornstarch is not that much better a feed so that it justifies the process costs.

Most of this good engineering work by Golueke and Oswald was done with cultures of sewage-fertilized Scenedesmus and Chlorella. In Golueke's efforts with other species (Golueke, 1961), he seems to have avoided filamentous blue green-algae because of the toxins they reportedly produce. Porphyridinum cruentum and Synechocystis sp. were among the other species tried; both are unicellular, the former a red alga, the latter a blue-green alga Porphyridium was harvested by addition of an equal volume of 80%-90% ethanol and stirring until the stringy coagulum could be withdrawn. The high cost of alcohol recovery constitutes the major disadvantage to this method.

Mass cultivation of several species has been tried. Success was reported for Spirulina maxima, Chlamydomonas reinhardtii, Scenedesmus obliquus, Chlorella sorokiniana, Scenedesmus acutus, and Scenedesmus quadricauda, among others. Table 1 gives particulars with references.

TABLE 1.--Some Examples of Microscopic Algae Which Have Been Cultivated in Cultures Larger than 100 Liters.

Species		Yield (Kg/day)	Scale	Open or Closed Culture	Batch or Continuous	Purpose	Harvesting Method	Source
Chlorella sp.	U.S.	0.62	5000 l 56 m^2	closed	continuous	food	centrifuge	Milner (1953)
Scenedesmus acutus 276-3a	Germany	5.6	30000 l 200 m^2	open	continuous	food	-	Stengel (1970)
Scenedesmus qaudricauda & S. obliquus	Czech.	31.5	36000 l 900 m^2	open	continuous	-	-	Stengel (1970)
Chlorella ellipsoidea	Japan	-	50000 l dark	closed	batch	food industry	centrifuge	Stengel (1970) heterotrophic 2-3% Glucose grows to 50 g/l
Chlamydomonas reinhardtii	Romania	0.24	28.8 m^2	closed	continuous, occasional harvest	not stated	doctor blade	Salageanu (1970)
Spirulina sp.	Mexico	-	120 m^2	open	continuous	food	-	Clément and van Landeghem (1970)
Spirulina sp.	Mexico	-	700 m^2	covered	continuous	food	-	
Spirulina maxima	France	1.5	6300 l 90 m^2	open	continuous	food	filtration	Clément et al. (1968)

TABLE 1.--continued

Species		Yield (Kg/day)	Scale	Open or Closed Culture	Batch or Continuous	Purpose	Harvesting Method	Source
Spongeococcum eccentricum	U.S.	-	dark heterotrophic	closed	-	feed, chickens	-	Oswald and Golueke (1968)
Phaeodactylum	England	-	1000 l 2.5 m^2	open covered	batch	feed	centrifuge	Ansell et al. (1963) marine
Chlorella vulgaris pyrenoidosa ellipsoidea chlamydossionas	Israel	0.06	2000 l 4 m^2	open	continuous	not stated	centrifuge	Mayer et al. (1964)
Scenedesmus obliquus (Turp) Kruger	Germany	-	1000 l	open	batch	vitamins B_1 & B_2	not reported	Kraut & Rolle (1968) aging cells
Euglena sp.	U.S.	not harvested	$2x10^8$ l 4300 m^2	open	continuous	wastewater treatment	not harvested	Caldwell (1946)
10 Scenedesmus/ 1 Chlorella (wild)	U.S.	40	10^6 l 4000 m^2	open	continuous	wastewater	centrifuge (other methods tried)	Oswald & Golueke (1968)
Scenedesmus sp. (wild occasionally) Chlorella or Oocystis	U.S.	20	$4x10^5$ l 660 m^2	open	continuous	wastewater treatment	coagulation	Mattoni et al. (1965)

The subject of continuous cultivation must be considered. Physiological differences exist between young and old cells, i.e. between cells which are actively dividing and those which are starved for a nutrient (Fogg, 1959, Kok, 1952). In a continuous steady state culture, nutrient addition constantly replenishes the supply as fast as cell growth assimilates the nutrients. One can appreciate the potential differences in cells which would be produced in batch growth or continuous growth. The mathematics of continuous cultivation has been treated extensively in the literature. Aiba et al. (1965) give representative coverage; the nutrient which limits growth or fermentation is always assumed to be one of the nutrients supplied in the replacement medium. As is more often the case with algal cultures, growth is limited by light or carbon dioxide - both of which enter the culture by another route, generally at a constant rate - flat out. Pipes (1962; Pipes et al., 1962) treats both the light-limited and CO_2- limited growth kinetics. His simple approach will be revisited in a later section, but his basic conclusions are appealing: (1) productivity in light limited cultures is proportional to the ratio of illuminated area to culture volume in a light limited culture and (2) in a CO_2 limited culture, the rate of CO_2 transfer controls the productivity, of course.

The continuous cultivation of microorganisms is usually considered a single-species proposition, that is, there are no predators or competing species. Oswald and Golueke (1968) conclude that closed mass cultivation of algae is too expensive because of the refrigeration equipment which must replace evaporative cooling. They also allude to the difficulties in controlling the predominance of the desired species in an open culture. There is the additional threat of contamination by predators (Tamiya, 1956), fungi (Masters, 1971), pathogenic bacteria and viruses (Safferman and Morris, 1963). Oswald and Golueke propose careful manipulation of the niche for the desired species and a massive continuous inoculum provided by recycling some of the harvested material. Mayer et al. (1964) contend that despite massive inoculation, any local species particularly favored by the conditions for growth will become established. Minkevich et al. (1969) consider the mathematics of a complete-mixing cultivator with biomass recycling.

Complications arise when a second organism enters the scene - either as harvester or as an uninvited guest. Symbiotic bacteria are known to inhabit open cultures of Chlorella (Fitchfield et al., 1969). The bacteria were thought to be thriving on extracellular amino acids produced by the algae. Lewin (1956) found that Chlamydomonas mexicana exuded 25 percent of its total organic

carbohydrate production. This would be a factor in addition to the O_2-CO_2 exchange usually cited as the reason for the bacterial-algal symbiosis. Humenik and Hanna (1970) created an intentional symbiosis between Chlorella and activated sludge for light-driven wastewater nutrient removal. The Chlorella cells were trapped in the sludge floc so that harvesting was no problem. Although algal oxygenation via artificial light is economically out of the question, solar energy might be cheap enough. The surprising result of their research was the establishment of a steady-state population with both algae and bacteria well represented.

For the case in which the second organism is a predator, a pertinent mathematical model was composed by Canale (1970). The predator must naturally take to an algal diet if it is to serve as a harvester. Compatible combinations which I have come across in the literature are recorded in Table 2. The size of the algal cell seems to be the predominant factor in determining whether a small predator can ingest an alga. Digestibility is generally good except in a few cases; senescent Chlorella cells were found to be growth depressing - if not toxic - to Daphnia magna. Dense suspensions of algae tend to foul the gills of larger fish that might possibly serve as the intermediary in the food chain.

TABLE 2.--Some Grazers and the Algae They Eat.

Predator	Alga	Source
Daphnia pulex copepod	Chlamydomonas reinhardtii	Richmond (1958)
Daphnia magna copepod	Chlorella vulgaris- (young cells only)	Ryther (1954)
	Chlamydomonas snowiae Scenedesmus spinosus - (but S. quadricauda was only fair food)	Edmondson (1957)
Keratella cochlearis rotifer	Stichococcus minutissimus	Edmondson (1965)
Polyarthra vulgaris rotifer	Cryptomonas oreta,* Chlorella sp.	Edmondson (1965)
Brachionus rotifer	12 algae < 18μm	Pourriot (1957)
Chilodonella cucullulus protozoan, wild	Chlorella sp.	Tamiya (1956)
Diurella tigris rotifer, wild	Chlorella sp.	Tamiya (1956)
Calanus finmarchicus copepod, marine	Diatoms & dinoflagellates	Cushing (1968)
Artemia marine	Dunaliella tertiolecta Chlorella stigmatophora Phaeodactylum tricornatum	Reeve (1963)

*a large brown colored unicell which can grow to 16 μm x 48 μm.

Used as a trap, starved marine copepods become sated in 4 to 12 hours (Mullin, 1963). At their optimum ingestion rate the starved copepods might have harvested 2×10^5 cells apiece ($2.3 \times 10^3 \mu^3$ per cell). Gibor (1957) calculated a 53 percent conversion efficiency of wet algal biomass to wet zooplankton (Artemia) biomass; at high cell counts per animal, most of the ingested cells ended up in the green fecal pellets. Chlorella and Chlamydomonas have been shown inadequate as the sole diet for Daphnia pulex (Taub and Dollar, 1968).

This, of course, brings up the question of nutritional quality of algae for humans and economic livestock. Briefly, the nutritional promise of species in the genera Chlorella, Scenedesmus, Oocystis and Spirulina has been reported. Table 3 should underline the similarity in quality which leads me to conclude that the search for algae that are nutritionally more desirable is perhaps less fruitful a venture than the search for improved harvestability. In general, algal protein is at best a dietary supplement. The protein is usually deficient in lysine and the sulfur amino acids - as are most plant proteins. Poor digestibility of the algae is attributed to the cell wall (Lachance, 1968). Digestibility seems to improve when the algae are used only as a feed supplement (Hintz, 1966). Hintz and Heitman (1967) successfully fed mixed Chlorella and Scenedesmus spp. (waste-grown) at the

TABLE 3.--Gross Nutritional Characteristics of Some Algae.

	Crude Protein (N x 6.25) % of dry wt.	Carbo-hydrate % d.w.	Lysine % d.w.	Methio-nine % d.w.	Apparent Protein Digestibility	Comments, Sources
Chlorella sorokiniana (lyophilized)	55-56.5	18-26	3.50	0.81	86% freeze-dried, fed to rats	Dam et al. (1968) unpleasant sharp taste Mateles & Tannenbaum (1968) Lubitz (1961)
Scenedesmus obliquus (lyophilized)	50-56	16-18	2.92	0.77	68% humans	Dam et al. (1965) bitter taste, bloated feeling, upset stomach, halitosis Soeder & Pabst (1971)
Mixed Chlorella spp. & Scenedesmus spp.	51	--			73% ruminants 54% monogastric	grown on municipal sewage. Hintz (1966) and Hintz & Heitman (1967) additional B_{12} needed in feed.
Oocystis Polymorpha	65	13.5	3.3	2.2		Richardson & Orcutt (1968) Knops NO_3 medium
Spirulina maxima (raw)	56-62	17	2.85	0.85	76	Clement et al. (1967) food for humans. Lake Chad. taste inoffensive Mateles & Tannenbaum (1968)

10 percent level with steamed barley. There is immediate market potential for algal feed if the price is right.

In the pilot plant trials of the 1950's, the price for a pound of dried Chlorella was estimated at 25.8 cents (Tamiya, 1956, algae produced in Japan, 82 acres, open culture) and 24.8 cents (Fisher, 1956, algae produced in U.S., 100 acres, closed culture, uncooled). In the former case about 3 cents is the harvest cost; in the latter case harvest costs about 4 cents or 25% of the production cost (16.6¢). Pre-harvesting entails about 50% of the total harvest running cost and investment. 25% of the total capital investment in the U.S. system was to be tied up in harvesting and pre-harvesting equipment. Although there are limited possibilities for a cheaper harvest to influence the total product cost, I feel this is a good place to start. Not only is a reduced running cost desirable; the capital investment of $450,000 (Fisher, 1956) for harvest equipment is sufficient to prevent entry by those already engaged in agriculture who might consider algal cultivation as a profitable use of the land they control.

Algal genera other than Chlorella may well hold the answer to a cheaper harvest. Myers suggested this back in 1956 - and he was probably not the first. Hindák and Príbil (1968) examined filamentous green algae (Hormidium sp., Ulothrix sp., Uronema gigas, Uronema sp., and

Stigeoclonium sp.) which Tamiya (1957) had suggested might be easily harvested. Hemerick (1967) selected some blue-green algae which grew attached to the bottom of polyethylene lined containers. He was able to scrape off kilogram quantities of Anabaena cylindrica, Calothrix membranocea, Fremyella diplosiphon, Lyngbya sp. Hydrocoleum sp. and Tolypothrix tenuis. Gaur et al. (1960) tried to grow Oscillatoria in organic wastes in order to end up with an easily harvestable product.

In Rumania, Chlamydomonas reinhardtii was grown on a continuous polyethylene band (Sălăgeanu, 1970) thus avoiding the harvest from dilute suspension. Spirulina maxima is considered very promising not only because of its acceptance in human diets, but also because it is easily harvested. (Clément et. al., 1968). Coelastrum proboscideum is presently under study since it filters remarkably well (von Witsch and Heussler, 1970).

CHAPTER IV

EXPERIMENTATION

Examination of the effectiveness of several different harvesting methods on several different algal types should serve to confirm or negate the literature's evaluation of the difficulty involved. Such study may reveal a novel combination of species and method which could lead to a more economical harvest. I have chosen seven algal species, somewhat representative of the diversity available. They were chosen for their agricultural promise as potential food, feed, or nutrient traps, keeping in mind the relative ease of harvest and a history of successful outdoor mass cultivation.

Algal Species

Chlorella pyrenoidosa. Chick (division Chlorophyta; order Chlorococcales). This illustrious species thrives at 40°C, a temperature too high for most other algae (Sorokin and Myers, 1953). It is a classic genus and served here as a basis for comparison of my work with work reported in the large literature. The cells are relatively small (2μm to 12μm depending on age), non-motile, ovoid-to-spherical, and can divide under favorable conditions about

10 times per day (Sorokin, 1965). Golueke & Oswald's (1965) Chlorella (species uncertain) was found to foul glass surfaces and to harvest with great difficulty by means other than centrifugation and flocculation. Its high temperature optimum ensures a niche that is relatively free of competition by other algae even in open culture. It also makes closed culture less expensive by obviating refrigeration equipment and energy.

Scenedesmus obliquus (Turp.) Kruger. (division Chlorophyta; order Chlorococcales) is also somewhat of a classic. Features for which the genus is well known are as follows: Scenedesmus (quadricauda) can apparently utilize bicarbonate for its carbon source; Chlorella cannot (Osterlind, 1950). It shows up in wastewater treatment perhaps for this reason (Oswald, 1968). It has been successfully mass-cultivated and fed to humans (Soeder and Pabst, 1970). Culture conditions can determine whether it grows as 4-celled colonies or unicells (Trainor and Rowland, 1968). Tufted spines (S. quadricauda, one strain) contribute to its floation; a motile stage may explain its world-wide distribution (Trainor and Burg, 1965).

Oocystis polymorpha (division Chlorophyta, order Chlorococcales) was reported by Richardson and Orcutt (1968) as having a maximum biomass growth rate comparable with that of Chlorella pyrenoidosa Chick. It does not

have the annoying habit of fouling glass surfaces. It grows well at 39°C, forms large cells and was considered a suitable choice for mass culture. Its nutritive characteristics have been discussed. This species was found as a contaminant in a photosynthetic gas exchanger. It was identified as a new species by Groover and Bold (1968) who declared it different from Scotiella oocystiformis (Lund, 1957) based on the absence of longitudinal ribs on the cell walls. A distinguishing characteristic is the frequent occurrence of mother cell-wall fragments still attached to a daughter cell. The scanning-electron-photomicrograph of Oocystis (Figure 9) may contradict Groover and Bold's placement of this alga since delicate ribs are clearly visible. This example is a typical cell from a slowly-growing (3000 lux) batch shake-culture (100 oscillations per minute) using a medium by Schultz (1963). Cells taken from the 10-liter continuous culture (growth rate 0.015 per hour, i.e. rather starved for light) in double-strength Schultz medium had smooth walls. It appears we are dealing with nutritional types rather than two different genera. I did not, however, examine a type culture of Scotiella.

Coelastrum proboscideum Bohlin (division Chlorophyta, order Chlorococcales), called to my attention by K. L. Schulze (Michigan State University), was singled out for its filtration and settling characteristics by von Witsch and

Heussler (1970). It is a colonial alga with colony diameters of as much as 50μm. Its biomass doubles about twice a day under favorable conditions. It is not a high-temperature alga. Work on defining its growth medium is in the early stages. Nothing has been said of its nutritional potential. Although H. von Witsch kindly sent me some fresh material from Germany, I apparently need not have been so particular. Fenwick (1962) found that a Coelastrum microporum Naeg. from Lake Huron gave rise to two daughter colonies which would pass as C. proboscideum Bohlin and C. cubicum Naeg. Trainor et al. (1971) review polymorphism in Coelastrum sp. as well as Chlamydomonas, Scenedesmus and others.

Chlamydomonas reinhardtii (division Chlorophyta, order Volvocales) is a biflagellate alga that has appeared occasionally as a nuisance scum on wastewater treatment ponds (Eppley and Macias R, 1963; Wiedeman and Bold, 1965). The species also grows in acid mine wastes (Round, 1965, p. 233). It is positively phototactic (swims towards a source of light) but an applied electric field will reversibly interfere (Marbach and Mayer, 1971). The cell wall is very fragile: one minute of sonication ruptured 80% of the cells (Miller et al., 1972). Borrowing from Round (1965, p. 37), "A fibrillar layered cellulose wall has been deduced for C. reinhardtii but details are lacking (Sager, 1957). In this species there is an outer

capsule containing polysaccharides (0.02μm to 0.06μm thick) appearing as a fibrillar felt with a fringed outer surface. The capsular material is continuously diffusing into the medium." Chlamydomonas has been observed in a quasi-colonial state known as the Palmella stage. Many cells are imbedded in a common mucilagenous mass.

Spirulina platensis (division Cyanophyta, order Nostocales) is a large, spiral-shaped multicellular filament. It is known as Arthrospira platensis in some circles. Filaments are typically 6-9μm in diameter, coils are typically 20-30μm in pitch and diameter. The filaments can attain a length of 350μm (Leonard and Compère, 1967). The alga thrives in media high in bicarbonate (pH 10) which pretty well describes the niche. Spirulina appears in the salt ponds of a table-salt company where the salinity ranged from 4.2 to 7.5 times that of sea water (Davis, 1968). It is found in almost unialgal culture in the dying lakes of Africa where it has been a part of the local human diet for untold ages (Leonard, 1967). Material collected from the same Lake Chad area was identified as S. maxima by Clement et al. (1968), who studied the nutritional aspects and mass-cultivated the alga. Farrar (1966) relates a fascinating history surrounding what was tentatively Spirulina. Tecuitlatl was the Aztec name for the edible green scum which grew in the now-extinct lake near Mexico City. The 1541 account is by Toribio, a

Franciscan missionary to the Aztecs. The algal mat was harvested with fine mesh nets and then sun dried on sand or ash beds. Interesting is the liklihood that the city's sewage was channeled directly to the lake. S. platensis fabricates small simple-membrane-bound organelles called gas vacuoles. The gas vacuoles impart buoyancy to the filaments (Walsby, 1968).

A short pressure pulse of a few atmospheres will irreversibly collapse the gas vacuoles (Klebahn, 1895) as will a few seconds' sonication (Lehmann and Jost, 1971). S. platensis exhibits gliding motility, an ability to move (without use of a visible organ) along a solid substrate or through a mat of other algal filaments. It advances like a cork-screw; in motion, the filaments do not twist about their filamental axis (Allen and Drum, 1968) as do other gliding blue-green algae (Halfen and Castenholz, 1971). It is not a swimming type.

Anabaena cylindrica Lemm. (division Cyanophyta, order Nostocales) is a filamentous blue-green alga, quite a bit smaller than Spirulina platensis. Filament diameter is 2.5 to 3μ; lengths are highly variable. The alga's claim on a niche derives from its capability for fixing atmospheric nitrogen. In aerobic conditions a number of filamentous blue-green algae possess such capability usually related to the occurrence of a differentiated cell called a heterocyst (Fay et al., 1968).

If a filament breaks, the break is usually at the heterocyst-vegetative cell junction (Borzi, 1878, cited by Wolk, 1966). The maximum doubling rate of the N_2-fixing alga is about once per day (Wolk and Wojciuch, 1971). It can grow faster in the presence of combined nitrogen. A few heterocysts are produced when NO_3 is the nitrogen source; no heterocysts with NH_3 and many heterocysts when N_2 is the nitrogen source.

A. cylindrica exhibits gliding motility; it has been clocked at 1.6μm/sec. by Walsby (1968). Moreover, in dense suspensions, Walsby reported a clumping phenomenon in which the algal mass withdrew from the sides of an 18 cm. diameter beaker at a rate of 2 mm/min. or greater. The clump usually settled unless exposed to high light intensity in which case photosynthetically-produced oxygen bubbles trapped in the clump caused it to float. Walsby related the clumping to gliding motility: a mucilage "ring" common to two filaments was pushed along the surface of each of the filaments. The filaments move in opposite directions relative to the ring. He could account for a clumping rate of 0.4 mm/min. by this model. The detergent Teepol prevented the process.

Gas vacuoles in this Anabaena have not been reported to the best of my knowledge. Prodigious quantities of mucilage are secreted into the medium that is very "rich". The nutritional aspects of Anabaena cylindrica are being

studied (Gordon, 1966). Hemerick (1967) grew A. cylindrica in 280-liter batch cultures and successfully fed the harvested algae to Japanese quail from hatching to maturity (Hemerick, 1968). Anabaena is a troublesome filter-clogger: it both clogs and gets through sand filters (Borchardt and O'Melia, 1961).

So much for an introduction to the species. Scanning electron photomicrographs of each alga follow (Figures 2-11). The generic name will identify each alga hereafter, the proper species names being used only as necessary. I will also dispense with the underlining of the seven genera except where the formality seems appropriate.

Harvest Methods

I consider the harvest to be the enrichment of the algal product from its concentration during growth to a concentration suitable for economical drying. Golueke and Oswald (1965) have called this a combination of pre-harvesting and de-watering. Clement et al. (1968) found that the initially harvested Spirulina could be dried directly without the intervening dewatering operation. Assigning numerical values, an algal culture having one gram of dry algae per liter will be enriched to a concentration of 20 grams dry weight per liter. A proper analysis of a complete cultivate-harvest-dry-package-

FIGURE 2.--Chlorella pyrenoidosa. Scanning Electron Microscope 10000X, photograph 2X. Lower cell is in late division. Some cell shrinkage was probably caused by the critical-point drying procedure. ⊢——⊣ = 1μm.

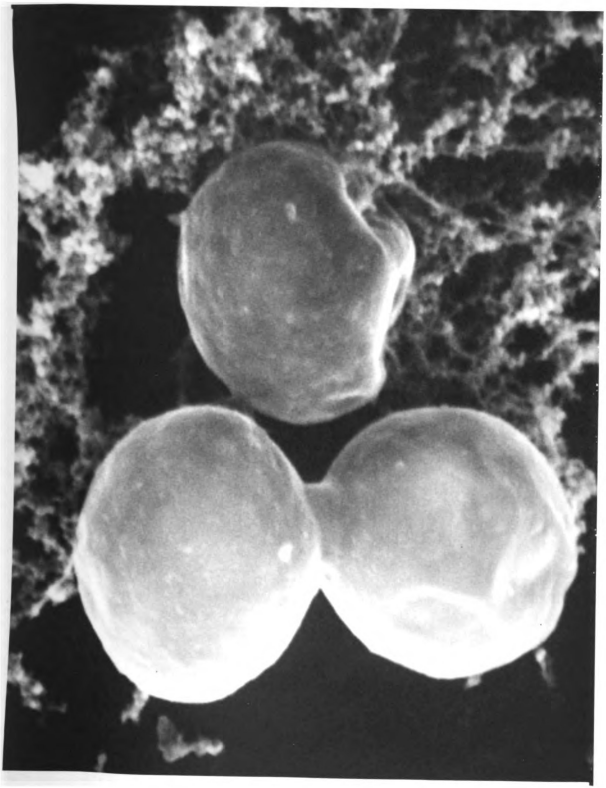


FIGURE 3.--Oocystis polymorpha. SEM 7500x, photo 2x. The cell wall remnant from the old mother cell is a distinguishing characteristic. Some vestige of surface ornamentation can be seen. This cell is typical of those grown in the fermentor with retention time 22 hours. ⟷ 1μm.

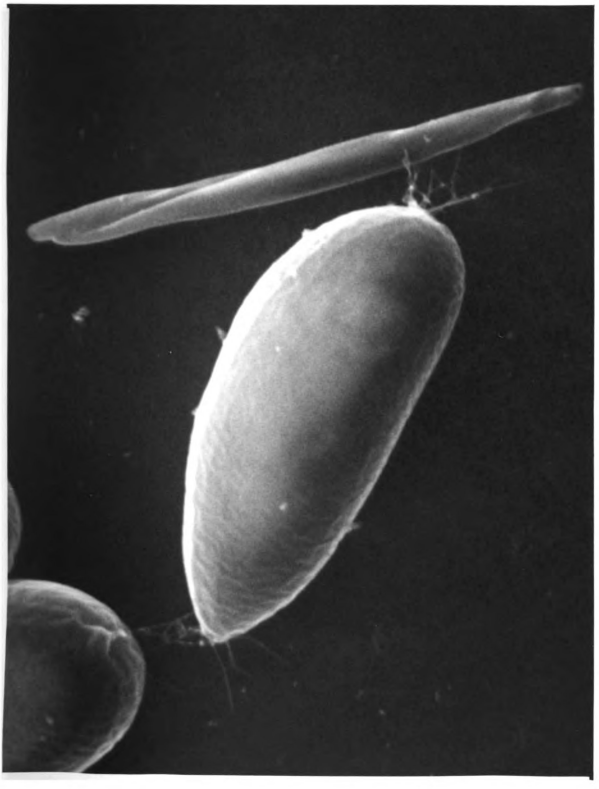


FIGURE 4.--Scenedesmus obliquus. SEM 7500x, photo 1.8x. The fast-growing cultures were predominantly unicellular. The surface is rather uninteresting. The surface ridges probably are the contact site when the organism is multicellular (Figure 9). Most cells lay on the slide so that the ridge is horizontal. ⟵⟶ 1µm.

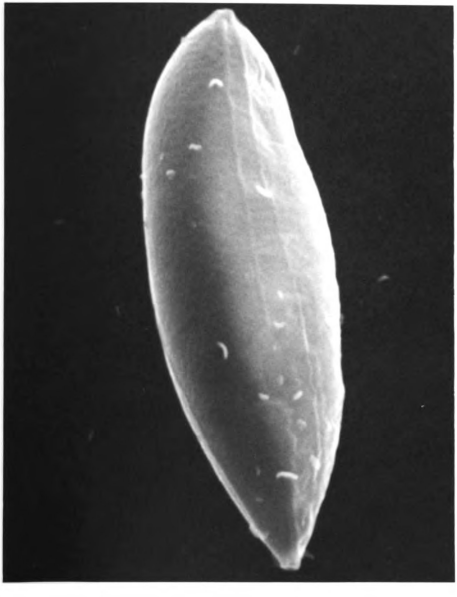


FIGURE 5.--Coelastrum proboscideum. SEM 3000x, photo 2.1x. Colonies are typically much larger than this one. The threads (probably dried mucilage) seem to be absent from the interior of the colony. ⟝⟞ 1µm.

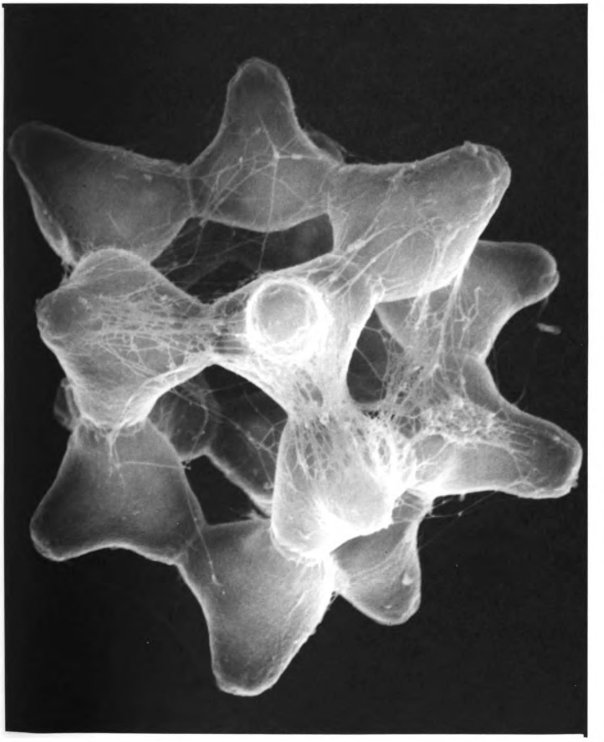


FIGURE 6.--Chlamydomonas reinhardtii. SEM 5000x, photo 1.5 x. Shrinkage is again evident in these delicate cells. Unless specimens were pre-fixed in 2% glutaraldehyde prior to dewatering in ethanol, the flagella broke off. Slight vibration in the SEM is responsible for the vertical streaks noticeable on the flagella. ⟼ 1μm.

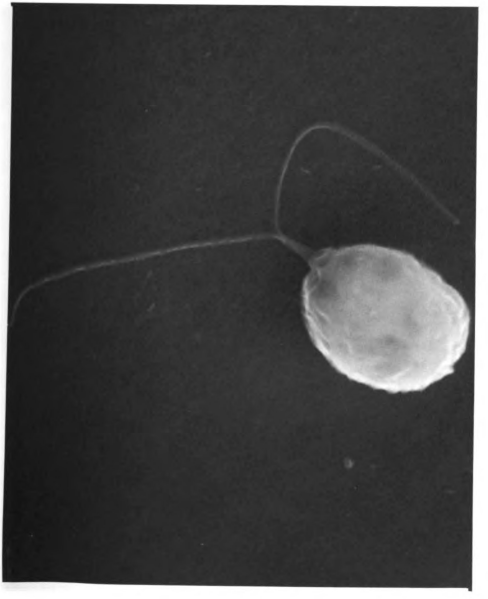


FIGURE 7.--Anabaena cylindrica. SEM 1000x, photo 2x. The gap and the pattern in the dessicated mucilage give an idea of the axial shrinkage which took place in preparation. The number of little coccoid bacteria on the slide suggests the extent of bacterial contamination.
⊢——⊣ 10μm.

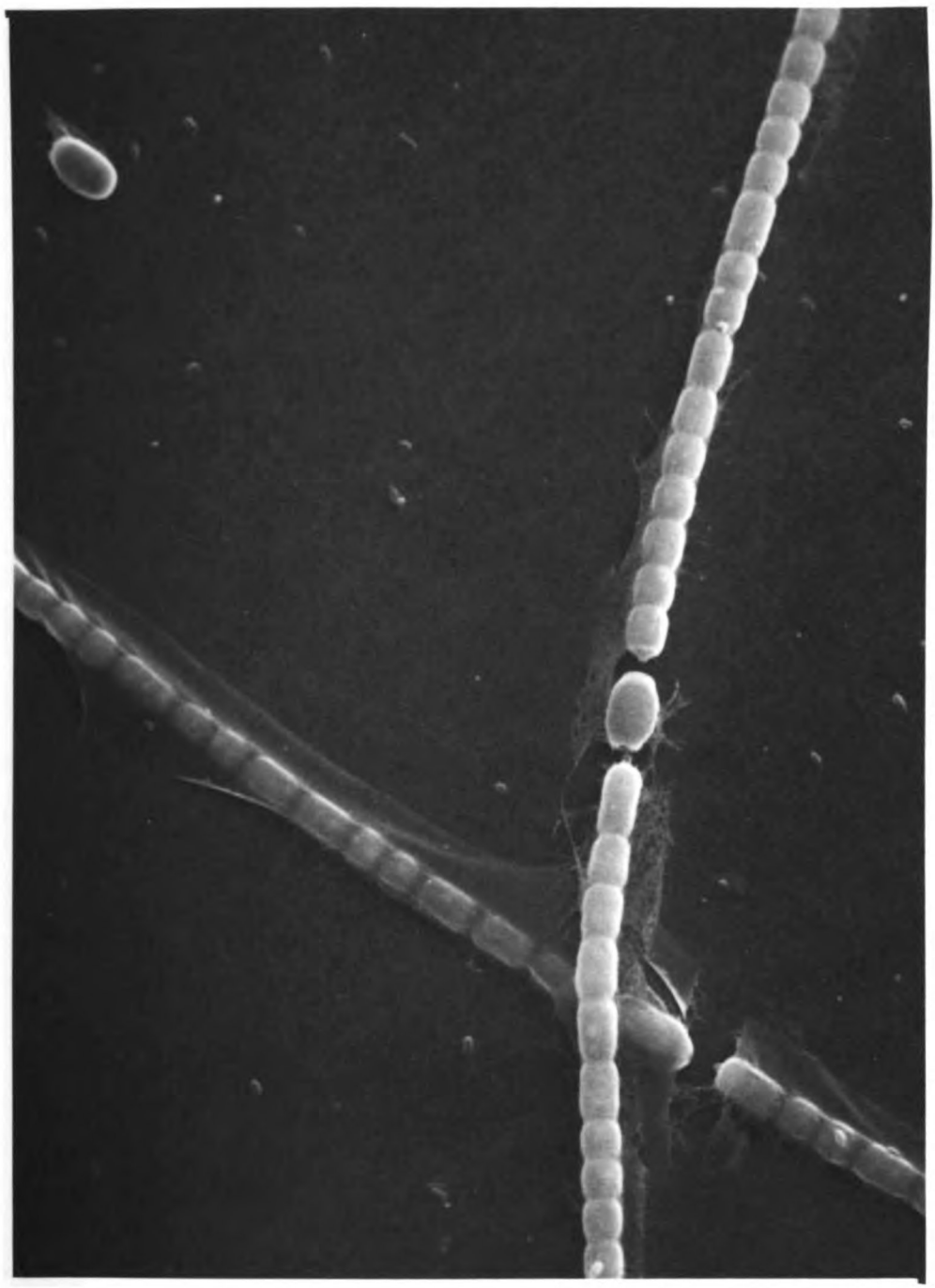
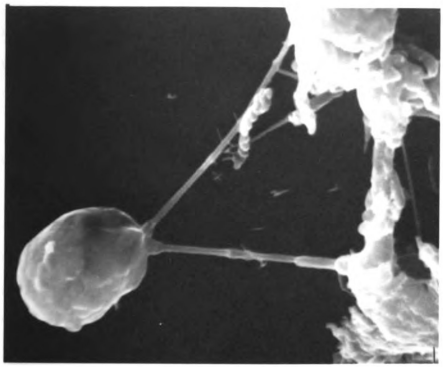


FIGURE 8.--Spirulina platensis. SEM 600x, photo 2x. tilt 45, magnification corrected. The surface pattern seems to be not structural, but rather the remains of mucilage. Septae between individual cells along the filament are barely discernable at very high power. ⊢——⊣ 10μm.

FIGURE 9.--Oocystis polymorpha. SEM 7500x, photo 1.6x. This cell was taken from a slow-growing shake culture, CO_2 at ambient concentration only. ⟝⟞ 1µm.

FIGURE 10.--Scenedesmus obliquus. (SEM 5000x, photo 1.4x) in classical multicellular form. Bristles and other ornaments are lacking (Trainor, 1966). ⟷ 1μm.

FIGURE 11.--Chlamydomonas reinhardtii (SEM 7500x, photo 1.1x) with detritus and unindentified bacteria. The thickened base of each flagellum may be an artifact caused by cell shrinkage. This specimen was not pre-fixed with glutaraldehyde. By chance, this cell was the only one to escape de-flagellation. ⟷ 1μm.



distribute system might result in different recommended concentrations so I will attempt to consider a range of values - say 0.5 to 2 g/l for the culture and 10 g/l to 30 g/l for the harvested material.

The harvesting methods I will try include sedimentation, semi-continuous centrifugation, filtration using a membrane filter, and variations on these. Each species will be subjected to each method. In addition I will investigate the possibilities for taking advantage of physiological quirks peculiar to certain species such as swimming, gliding motility, attachment to surfaces, autoflocculation, and as prey for a suitable predator. Addition of chemical flocculants will be avoided since I do not intend to perform the necessary nutritional evaluations needed to determine if the harvested algae could still be used for food or feed. Table 4 displays the harvest method-species intersections to be studied. In some cases, adequate and convincing research reports can be found in the literature; these will be acknowledged. Experimental investigation will proceed to the point where an intersection's promise can be estimated. In a few cases such as sedimentation and filtration, some quantitative results will be obtained since there is little by way of numerical comparison in the literature. Some of the intersections are absurd: vegetative Chlorella, for example, is non-motile. Nevertheless, Table 4 presents the gross experimentation plan.

TABLE II.—Experimentation Plan

TABLE 4.--Experimentation Plan

Species	Harvest method	Sedimentation	Centrifugation	Filtration	Phototaxis	Electrical Flocculation/ Flotation	Electro decantation	Adhesion	Predation
Chlorella pyrenoidosa					not possible				
Scenedesmus obliquus					not possible during most of life cycle				
Oocystis polymorpha					not possible				
Coelastrum proboscideum					not possible during most of life cycle				
Chlamydomonas reinhardtii									
Spirulina platensis									
Anabaena cylindrica									

Materials

Species

The algal material came from several sources: *Chlorella pyrenoidosa* Chick., *Scenedesmus obliquus* (Turp.) Krüger and *Oocystis polymorpha* came from the Culture Collection of Algae, Indiana University (Culture numbers 1230, 72 and 1645 respectively). H. von Witsch (Wiehenstephan) kindly sent me a sample of fresh *Coelastrum proboscideum* Bohlin. *Chlamydomonas reinhardtii* is from the laboratory of D.T.A. Lamport (MSU) and *Anabaena cylindrica* Lemm. was given to me by C.P. Wolk (MSU). *Spirulina platensis* is culture number 1475-4 of the Cambridge Culture Collection of Algae and Protozoa.

Media

The first five algae (all Chlorophyta) were maintained as slow-growing seed cultures in a medium designed by Schultz for long-term continuous culture of Scenedesmus obliquus (Schultz, 1963). The salt concentrations of the major nutrients are equal to their content in the ash residue of the algae at about 0.5 to 1.0 grams dry algae per liter. Measurement of the algal harvest should give an indication of make-up nutrient required. Spent medium can be re-used and no major salt should accumulate as a result. The medium has a further advantage: two of the

liquid stock solutions probably need no sterilization since they have extreme pH values.

Heat, stirring and time are required to get the salts dissolved into the iron solution. Molybdenum is absent since nitrogen is supplied as ammonia. Vanadium may be essential; Arnon et al. (1955) used it. I have added cobalt for good luck. Schultz apparently used boron for good luck (Dear and Aronoff, 1968) but it may be salutory for Chlorella.

The Schultz medium appears to be satisfactory for the growth of seed cultures of all five green algae. At the low growth rates maintained in the seed cultures (light-limited most of the time), Scenedesmus grew in both colonial and unicellular form, Oocystis behaved as expected and Coelastrum grew well in spite of Round's (1965, p. 154) observation that Coelastrum disappears from lakes near the end of summer when copper reaches 0.03 mg/l.

Anabaena was kept in the medium of Allen and Arnon (1955) (Table 6).

For Spirulina, a medium by Zarrouk (1966) was used (Table 7). I modified Zarrouk's recipe by adding vitamin B_{12}, and omitting W, Ti, Cr and Ni. Both blue-green algae were grown contaminated with bacteria; Spirulina has apparently not been grown free of its bacteria.

Others have had trouble growing Spirulina on defined media. In the culture collection at Cambridge it

TABLE 5.--Schultz Medium (for seed cultures)

Stock Solution:	1 liter distilled H_2O 89g KH_2PO_4 57g $MgSO_4$ 7g MgO 51.4 ml of 86% H_3PO_4	1 ml.
Ammonia Solution:	800 ml. distilled H_2O 200 ml. of 58% NH_4OH	1 ml.
Iron Solution: (store cold, dark and anaerobic)	1 liter dist. H_2O 10.6g Fe^{+++}-citrate 10.6g citric acid	5 ml.
Micronutrients: (A_5-Mo+Co+V)	100 ml dist. H_2O 286 mg H_3BO_3 121 mg $MnCl_2.4H_2O$ 10.5 mg $ZnCl_2$ 5.4 mg $CuCl_2.2H_2O$ 2.3 mg NH_4VO_3 *0.4 mg $CoCl_2.6H_2O$	1 ml.
Calcium:	36.7 mg $CaCl_2.2H_2O$	36.7 mg. 1 liter dist. H_2O
		1 liter Schultz medium

* 1/10 of what is usual

Medium was sterilized by filtration through a 0.22 μmembrane filter (Millipore Corp.)

Concentrations in the final medium are: N 42 mg/l, P 46.8mg/l, K 25.5mg/l, S 15.2mg/l, Mg 15.7mg/l, Ca 10mg/l, Fe 10mg/l, B 0.5mg/l, Mn 0.5mg/l, Zn 0.05mg/l, Cu 0.05mg/l, V 0.01mg/l, and Co 0.001mg/l.

The medium was used by Kraut and Rolle in 1000 liter batch experiments, non-sterile, open culture. They were studying the effects of cell again on vitamin B_1- and B_2-content (Scenedesmus obliquus (Turp.) Krüger).

TABLE 6.--Modified Allen and Arnon Medium (for seed culture). The Iron Source chelator is Citric Acid rather than EDTA.

1 liter dist. H_2O

0.120g $MgSO_4$

0.0735g $CaCl_2.2H_2O$

0.232g NaCl

0.348g K_2HPO_4

4mg Fe as Fe III-citrate-citric acid

1 ml micronutrient solution

Fe solution: 1 liter distilled H_2O

10.6g Ferric-citrate

10.6g citric acid

(1.7 ml = 4 mg Fe)

Micronutrient solution (A_5 + Co + V):

100 ml distilled H_2O

286 mg H_3BO_3

181 mg $MnCl_2.4H_2O$

10.5 mg $ZnCl_2$

5.4 mg $CuCl_2.2H_2O$

2.1 mg $(NH_4)_6Mo_7O_{24}.4H_2O$

2.3 mg NH_4VO_3

4.0 mg $CoCl_2.6H_2O$

TABLE 7.--Zarrouk Medium (for seed culture), slightly modified: B_{12} added; Ti, W, Cr and Ni omitted.

1 liter distilled H_2O
16.8g $NaHCO_3$
2.5g $NaNO_3$
0.6g $K_2HPO_3.3H_2O$
1.0g K_2SO_4
1.0g NaCl
0.10g $MgSO_4$
0.80g EDTA (Ethylene dinitrilotetraacetic acid)
0.01g $FeSO_4.7H_2O$
0.5µg vitamin B_{12}
1 ml micronutrient solution
0.53g $CaCl_2.2H_2O$ added last
Micronutrient solution (A_5 + Co + V)
100 ml distilled H_2O
286 mg H_3BO_3
181 mg $MnCl_2.4H_2O$
10.5 mg $ZnCl_2$
5.4 mg $CuCl_2.2H_2O$
2.1 mg $(NH_4)_6Mo_7O_{24}.4H_2O$
2.3 mg NH_4VO_3
4 mg $CoCl_2.6H_2O$

The medium is sterilized by filtration through a 0.22µ membrane filter.

is grown in Erdschreiber or a soil-sea water medium. Castenholz (1969) could not get a thermophilic species to grow. M. Homès (Jardin Botanique, Brussels: personal communication, 1970) encountered trouble in the mass-cultivation trials in Belgium - a progressive decrease in growth rate. I obtained poor growth with Spirulina until I included vitamin B_{12} in the medium. (I am indebted to Dr. R. D. Simon for suggesting this to me.) Its essentiality is far from proven. Notice that the cobalt concentration I used (early in the experiments) is one-tenth the amount used by Allen and Arnon (1955). Except for Spirulina and Anabaena, I had no trouble over this low cobalt concentration; the green algae can apparently tolerate it. The Spirulina problem, then, was solved by addition of vitamin B_{12} and a similar problem with Anabaena may have solved itself when I simply increased the cobalt concentration to what it ought to have been.

The recipes were altered somewhat for the ten-liter continuous cultures. In order to simulate the more gross conditions which will apply to large scale agricultural enterprises, tap water was used instead of distilled water. For the Spirulina culture, the calcium concentration in the Zarrouk medium was reduced to the highest concentration which would not cause a precipitate to form. In all other (subsequent) experiments, I found that

softened tap water could be used without any modifications in the calcium concentrations. It means that all the softened water media had somewhat more sodium than planned. The tap water was allowed to run for a while to clear the lines of water which may have had a high copper or zinc concentration. Table 8 lists the medium adjustments.

Anabaena grew quite yellow and finally died with Fe-citrate as the iron source. A change to $FeSO_4.7H_2O$ and EDTA as in the Zarrouk medium improved the growth of Anabaena. Perhaps the bacteria are thriving on the citric acid or perhaps citrate is inhibiting some process in Anabaena. I did not pursue the matter.

The algae were grown at 30°C except for Chlorella which was grown at 40°C. Light at the culture surface was 1800lux. 4 l/min air was bubbled through the cultures. It was enriched to 1.5 percent CO_2 for all algae but Spirulina and N_2-grown Anabaena.

Equipment

Algae were maintained as seed cultures - 125 ml in 250 ml Erlenmeyer flasks - which were constantly illuminated (3000 lux, cool white fluorescent) and shaken (rotary pattern, 100 rpm) in a Gyrotary Water Bath Shaker (New Brunswick Scientific Co., Model G76). The controlled temperature option was not exercised; room temperature varied from 20°C to 30°C. Seed cultures were treated as

TABLE 8.--Ten-liter Continuous Culture Media

Chlorella Scenedesmus Oocystis Coelastrum Chlamydomonas	Double strength Schultz, tap water (softened), not sterilized.
Anabaena	Allen and Arnon, tap water (softened), not sterilized. Iron source changed from Fe-citrate to 0.010g $FeSO_4.7H_2O$ and 0.1g EDTA per liter.
Spirulina	Zarrouk, tap water (hard) Ca reduced to about 0.35 g/l not sterilized.

if unialgal and bacteria-free. The two blue-greens and Chlamydomonas were in fact not growing free of bacteria. All were unialgal inasmuch as I could determine. Periodic checks for health and contamination were made with an Olympus model EH binocular microscope. The continuous cultures were maintained with a 14-liter Microferm bench top fermentor (New Brunswick Scientific Co., Model MF100). The fermentor provided for constant temperature, constant speed agitation, constant gas supply introduced below the impellers, illumination (18000 lux at the vessel surface, 24 fluorescent lamps, durolite, Optima 15 watts each), electronic level control. There were two Zero-max variable-speed peristaltic pumps for harvest stream and make-up medium.

For the continuous culture mode, the make-up medium pump was run at a pre-set speed. Whenever the level in the fermentor exceeded a set level for an adjustable (1-30 sec) time interval, the harvest pump would reduce the level to the electrode set point. Thus it was a fill-and-draw type of operation. The detention time in the vessel (volume/input flow rate) was only as accurate as the medium pump. So low was the range of detention times useful for the slow algal growth attainable with the available illumination that I modified the feed pump to operate on a percentage timer--one or two minutes every hour. Slight evaporative loss did occur in spite of the condenser in the gas output line. Cultures were started in a sterile batch culture fashion. When the desired operating density was reached, semi-continuous medium addition was begun. Data were not collected until at least one and preferably two retention times had elapsed.

Sedimentation tests were carried out using pyrex 1-liter and 500-ml conical lab separatory funnels (pyrex, cone angle 31-38°).

Centrifugation was done with a Sorvall SS-4 (SS-34 head fixed angle 34°, 4800g, 17000rpm) manual centrifuge fitted with a Szent-Gyorgyi and Blum continuous flow apparatus. In semi-continuous operation, the input flow to the centrifugal field was introduced at a radius of about 7.0 cm. The operation is termed semi-continuous

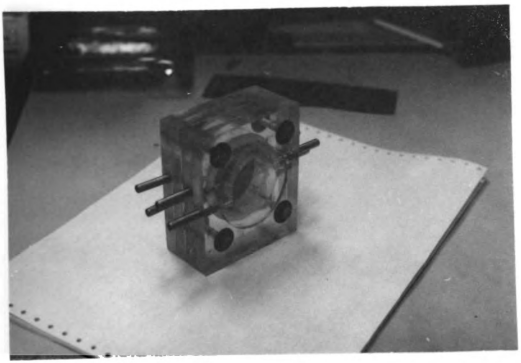
since although fluid flow through the machine was continuous, the accumulation of solids inside the centrifuge required periodic shut-down for unloading. Centrifuge power consumption was measured with a Weston wattmeter made more sensitive by using a current transformer (30:3). The wattmeter consumed 5 watts. The separating efficiency of the centrifuge was drastically affected by the flow rate through the machine. The pressure head of the algal-feed reservoir was maintained at a constant level throughout each test.

Filtrations were performed using a stainless membrane filter holder (Millipore Corp., No. XX2004720). All filtrations were vacuum filtrations. A variety of filter papers was used (0.22μ, 0.45μ, 3.0μ (MF series 18¢ ea.), and Microfiber Glass prefilters 0.011 inches thick (3¢ ea.) all from Millipore Corp. Filter area for the holder is 9.35 cm^2. Filterability (to be defined later) was measured using a stopwatch and a graduated cylinder inside the vacuum flask to catch the filtrate.

Filtration in an electric field was managed on a home-made apparatus shown in Figures 12, 13 and 14. Construction is of Lucite, inlet and outlet tubes are stainless steel of uncertain grade. Viton o-rings seal the filters or cellulose on one side only. Electrodes are platinum wires cemented in place with epoxy glue.

FIGURE 12.--Electrodecantation cell, all tubing disconnected.

FIGURE 13.--Close-up side view of the electrodecantation cell in operation. Filtrate flows toward the left, polarity, right +, left -.



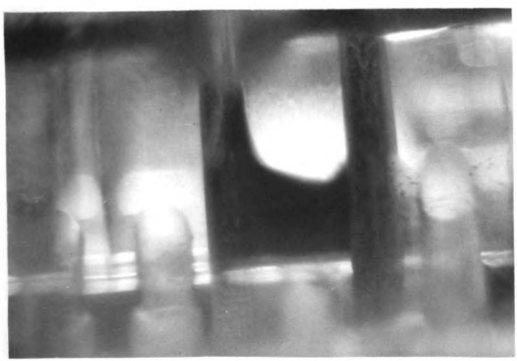
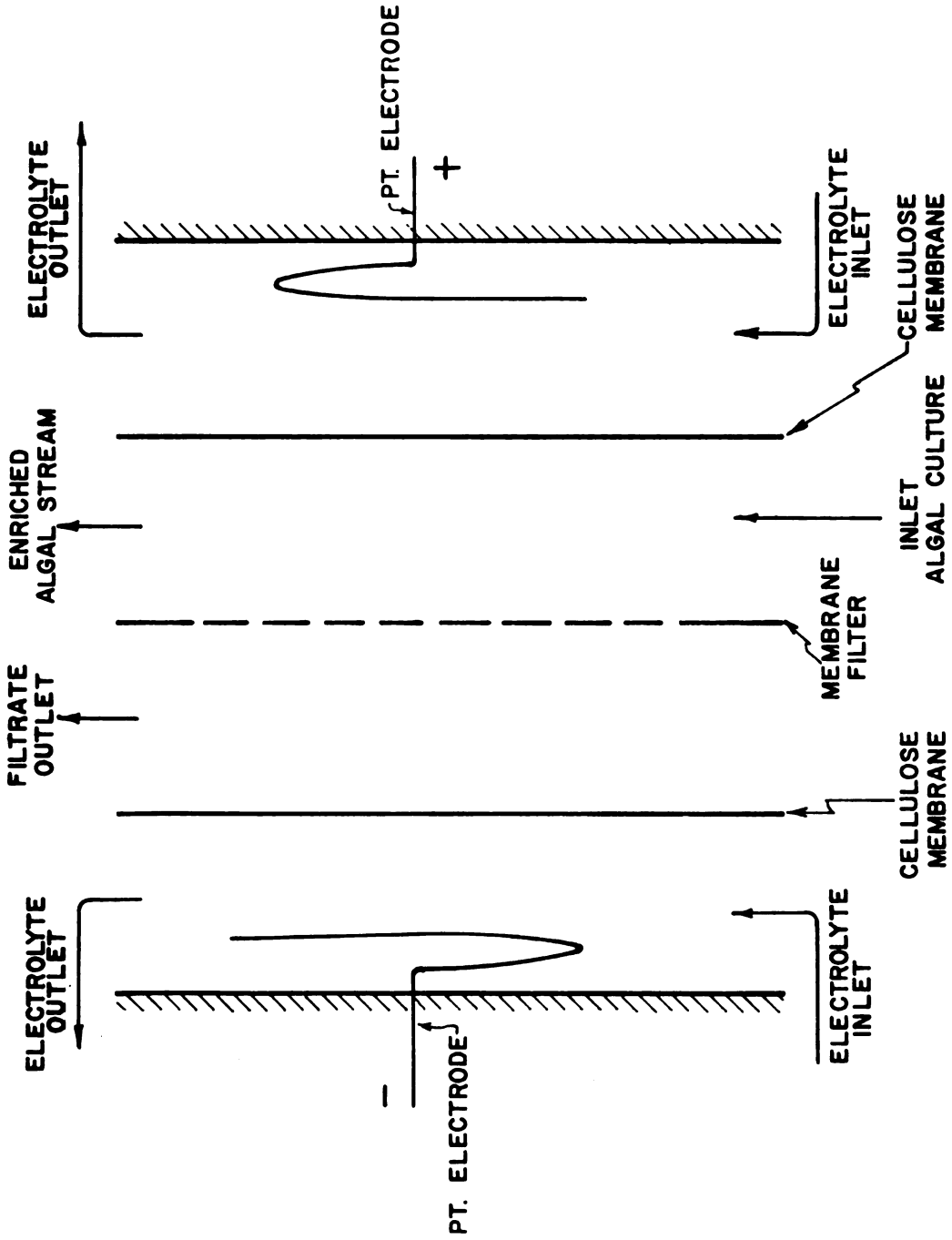


FIGURE 14.--Schematic view of the electrodecantation cell. Electric power, supplied with the polarity indicated, prevents filter cake buildup and simultaneously pumps filtrate.


ELECTROLYTE OUTLET
PT. ELECTRODE
+
ELECTROLYTE INLET
CELLULOSE MEMBRANE
ENRICHED ALGAL STREAM
INLET ALGAL CULTURE
MEMBRANE FILTER
FILTRATE OUTLET
CELLULOSE MEMBRANE
ELECTROLYTE OUTLET
ELECTROLYTE INLET
−
PT. ELECTRODE

The electrode chambers are isolated from the filtration chambers by DEAE cellulose membranes (pieces cut from dialysis bag stock). The filter membrane was usually a 3μ Millipore filter membrane. Filter area in this device is 7.5 cm^2. Peristaltic pumps circulated the electrode chamber electrolyte (0.1 M Na_2SO_4). The pressure head across the membrane was kept constant at 40 cm H_2O (positive pressure, no vacuum). In connection with the electrokinetic separation work, electrophoretic mobilities were measured using a microscope stage electrophoresis cell. The cell dimensions are 32.5mm x 13mm x 0.8mm thick. This was permanently mounted on a Bausch and Lomb microscope with a 21 x objective and 10x eyepiece. The method of Black and Smith (1962) was followed. The stationary level was ~160μm above the bottom surface of the cell. The electrolyte used was 0.1M $NaNO_3$. Fresh human red blood cells reluctantly donated by the author were used for calibration of the electrophoresis apparatus. The mobility of the algal cells was always measured in culture medium--"as-harvested" to estimate the potential for further exploitation of this property. The electric field was supplied by a 500 v d.c. power supply. Currents were not large enough to cause convective trouble in the cell except in the case of Spirulina when the medium conductivity was 21000 μmho/cm. In other cases (σ= 1040 μmho/cm) the cell could be operated continuously. The time required for a

298μm round trip (149μm each way) was measured with a stop watch. The field was abruptly reversed after the cell had traversed 149 μm from a starting point (eyepiece reticle). From the time and electric field were calculated the electrophoretic mobility.

The electrophoretic mobility of my red blood cells is 37% above the so-called reference value stated by Black and Smith. In spite of this, my value for Chlorella electrophoretic mobility (2.41 μm/sec/v/cm) is in fair agreement with that reported by Tilton, et al. (1972) (2.8 μm/sec/v/cm, std. deviation 0.5).

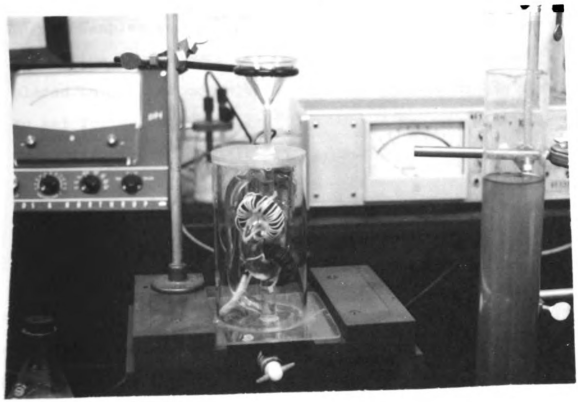
Electrical conductivity of the various media and solutions was measured by an electrodeless conductivity cell pictured in Figures 15 and 16 (Gerrish and Bickert, 1971). The conductivity cell is linear over a very wide range. A conventional phase-sensitive amplifier used for linear voltage displacement transducers was used. The meter was calibrated with a standard salt solution.

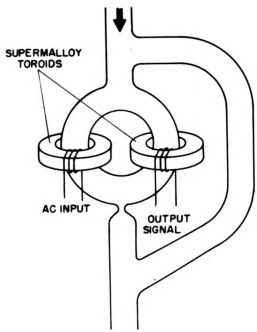
Optical density measurements were occasionally made with a Fisher Electrophotometer (antique) which was powered through a constant-voltage transformer. Only the 425 mμ filter was used.

The algae were examined in a scanning electron microscope (Advanced Metals Research, Cambridge, Mass.). Beam energy was 21Kv, sample current average 75 picoamps.

FIGURE 15.--The electrodeless conductivity cell. The signal conditioning unit is behind the cell and to the right.

FIGURE 16.--Scheme of the continuous flow electrodeless conductivity cell. The conducting fluid acts as a one turn secondary to the input transformer; it is also a one-turn primary for an output transformer.




SUPERMALLOY
TOROIDS
AC INPUT
OUTPUT
SIGNAL

Samples were prepared for the scanning electron microscope as follows: A drop of algal suspension was placed on a glass cover slip (12 mm diameter) and allowed to set for about 5 minutes. The sample was then submerged for 2 1/2 minutes each in aqueous solutions of 70% ethanol, 80% ethanol, 90% and 100%). The ethanol was replaced by extraction in a similar series of amylacetate-ethanol solutions, 70%, 80%, 90% and 100% amylacetate (3 methyl-butyl acetic acid ester). As much as 30 minutes elapsed with the algae in 100% amylacetate. At this point the sample was put in the pressure chamber of the critical point dryer. In the dryer, liquid carbon dioxide was admitted to the sample chamber (900 psi) until it displaced and amylacetate (endpoint detected by human nose). The chamber was sealed and heat (50°C water bath) was applied externally to drive the liquid CO_2 through its critical point (1000-1100 psi). At the critical point, fluid properties are strange: surface tension vanishes so the liquid can boil in a non-violent manner. After 5 minutes at the elevated pressure, pressure was slowly decreased in the chamber until ambient pressure was reached. The sample was removed and kept in a dessicator until the coating operation. In coating, the algae were first coated by vacuum sputtering a layer of carbon (0.005µm) followed by a layer of gold-palladium (0.005µm). The carbon is responsible for the generation of secondary electrons

which creates the image. The gold prevents local charging by providing a conducting path by which electrons can pass from the sample.

The measurement of cell size was by microscopic observation of cells using a calibrated eyepiece reticle. Random selection of cells was attempted by a random twiddling of the x and y microscope stage positioners and selecting the cell appearing nearest a zero point on the reticle.

Cell specific gravity was determined by a density gradient method (Pertoft, 1967) suggested to me by M. Jost (Michigan State University). A density gradient was established in a 10 ml glass centrifuge test tube by subjecting a 60% (approx.) aqueous dilution of colloidal silica (Ludox HS, Technical grade, E.I. DuPont, Wilmington, Delaware) to a gravitational field of 14000 x g to 27000 x g for 12 minutes (Sorvall centrifuge, SS34 head). The gradients were then transferred to a swinging bucket centrifuge (Precision Scientific Co., restored antique). Algal suspension - about one ml - was gently added on top of the gradients. After five minutes at about 200 x g, bands of algal cells can be observed in the gradient and compared with colored beads of known specific gravity which are also inserted into the gradient (Beads 4 mm in diameter from an automotive anti-freeze hydrometer, Meijer Thrifty Acres, calibration in a sucrose solution, Handbook of Chemistry

and Physics, Chemical Rubber Co., 59th edition). It can be shown that a sphere of constant specific gravity floats in a linear density gradient in a position such that its horizontal great circle is at its specific gravity value in the gradiant. Algal specific gravity was also checked occasionally by drawing the algal band into a tared 100µ liter or 200µ liter (λ) volumetric pipet (capillary constriction, S.G.A. Co.). The weight increment was compared with that of the same pipet full of water. The difference between 1.00 and the cell specific gravity was reproducible in this method to within five percent. The high-sodium colloidal silica gradient had an osmotic pressure of 373 milliosmols/kg. near where the algal band was located. (1 osmol/kg = 27 atm. approx.) In the case of Spirulina, Zarrouk medium was used instead of water to dilute the Ludox. Osmotic pressures were estimated by measuring the freezing point depression using an Advanced Osmometer (Advanced Instruments, Inc., Newton Highlands, Mass.) The 373 MOsm/kg osmotic pressure was considerably less than that of the Spirulina medium (440 mOsm/kg) in which the cells had been growing; pH was about the same - 10.0. If water moved due to the osmotic difference between gradient and growth milieu, it would have tended to move into the (gas-vacuole-less) cells, making them less dense. Since I was impressed with how much greater than 1.0 the density was, I accepted this possibility for error. Spirulina

was actively motile after 30 minutes in the colloidal silica and appeared healthy in all other microscopically observable aspects.

In the case of the green algae, the osmotic pressure of the medium was 17.5 mOsm/kg. Ludox HS gradients made with distilled water as diluent average 38 mOsm/kg, a condition that would tend to cause water movement out of the cells and give specific gravities that were higher than actual. Ludox LS (low sodium) has an osmotic pressure of 26 mOsm/kg and pH 8.5, still unacceptable. I dialyzed 164 g Ludox LS against distilled water. After dialysis there were 200 g of colloidal silica with an osmotic pressure of 7.5 mOsm/kg. This was used for gradient work with the algae other than Spirulina. The gradients are made with four ml dialyzed Ludox LS and three ml H_2O. Chlamydomonas was still motile in the gradients after 12 minutes at 200xg.

Other methods to arrive at cell specific gravity (cell sinking velocity by slow motion photography (Haddad and Lindegren, 1953), or a bulk method (Aiba et al., 1964)) pale before the relative simplicity of this one.

The balance was manufactured by Mettler Instruments Corp., Hightstown, N.J. (0 to 160.0000 g ± 0.0001 g). I have relied very much on a gravimetric filtration method.

Procedures

Measuring Techniques

The method I selected for measurement of culture density as well as rich and lean stream densities after separation is essentially the same as the suspended matter test for activated sludge (Standard Methods for the Examination of Water and Wastewater, 12th edition). A filter which will retain the algal material (but preferably not the associated mucilage or bacteria) is oven dried (103°C) and weighed. The filter is then placed in a filter holder and washed with a few milliliters of filtered distilled water. After a known volumetric quantity of algal suspension has been filtered (usually a vacuum filtration) and the filter cake rinsed with 10-15 ml distilled filtered water, the filter membrane, paper, or mat, is returned to the oven for drying and re-weighing. Culture density (Suspended algal matter) is computed by dividing the dry weight of the filter cake by the volume of fluid in which it was suspended. The values of culture density were quite reproducible (for Spirulina, 3.0μ filter, standard deviation was 1.6 percent of the mean). The selection of the proper filter medium was most important. I found that 3.0um membrane filters give the most consistent results. But less expensive fiberglass mats (Millipore glass prefilters) were used whenever possible since they are cheaper. Chlamydomonas and Chlorella penetrated the fiberglass

filters, however. In these cases, 0.45μ membrane filters were used. Whatman Nos. 1 and 41 papers retained Spirulina but the papers are very hygroscopic and can hardly be weighed accurately as they acquire moisture at about 1 mg. per minute. The dry algal matter might be 25 mg. in such a case. The glass prefilters were relatively non-hygroscopic as were the membrane filters.

A volatile solids (total solids (103°C) minus ash (600°C)) determination on the filter cake wash water indicated that the filter cake retained dissolved solids that were about 40% volatile and 60% nonvolatile. This was probably mucilage, possibly some bacteria and perhaps the debris liberated by osmotic rupture of some cells. The growth medium for Spirulina is more salty than other growth media (18 g. total solids per liter). Osmotic rupture of Spirulina cells which had spent twenty minutes in distilled water was evidenced by the obvious liberation of phycocyanin (fluoresced red in ultraviolet illumination). Filtrate bacterial dry weight amounted to a calculated 0.05% of the algal dry weight (5×10^6 cells per ml, and estimated 1μm radius, coccoid shape, therefore 0.005 g dry weight per liter). The bacteria which stick to the algae are likely the preponderant proportion of the bacterial population. Ward and Moyer (1966) estimate that in their unsterile algal cultures, the bacteria never amounted to more than one percent of the dry matter.

Washing of the filter cake was especially necessary in the case of Spirulina. An unwashed filter cake on a 3.0μ filter can trap so much of the total solids in the moist cake as to cause the culture density to appear 40 percent higher than if the cake had been washed (50 ml sample, 0.555 g/l culture density, 6 mg ash trapped unless washed out). When the filter cake was washed, the ash in the filter cake contributed only 3.4 percent to the culture density. In subsequent work this 3.4 percent was not taken into account since ash is usually accepted as part of the harvested crop, i.e. "volatile" crop is seldom considered.

Packed-cell-volume after centrifugation is a popular method for culture density determinations. I decided against using it because Spirulina possesses gas vacuoles and does not form a single pellet in the centrifuge. Optical methods were rejected for a similar reason: Spirulina gave anomalous results due to its gas vacuoles. The relative level of gas vacuolation in Anabaena flos-aquae was even proposed (Walsby, 1971) as measurable by the correlated differences observed in optical density. For example (my data), a culture of Spirulina (0.555 g/l) had an optical density (425 mμ) of 93. If the cells were sonicated for 10 seconds, the optical density was 68. Algal cell counting was rejected because of the great differences anticipated in cell size over the range of algae tested. So by a process of elimination, filtration

was selected as the single method most suitable for measurements using the seven algal species. Even so, filter medium changes had to be made to suit the species. And on occasion, a particularly rich and unfilterable sample had to be measured using an optical density method. The filterable suspended solids method was the primary indicator of the density of the ten-liter cultures of algae.

Management of the Culture

The simple mathematical treatment by Pipes and Koutsoyannis (1962) will be followed.

Most of my cultures were started as batch cultures in relatively sterile surroundings and with an inoculum of about 100 ml of algal suspension at about 0.5 g dry weight/liter.

In unlimited batch culture:

$$\frac{dX}{dt} = kX \qquad (1)$$

where X is the culture density in g dry wt/liter

t is time - in hours

k is a constant specific growth rate ($hours^{-1}$)

But with light being the "limiting nutrient" for culture density in excess of X_s where shading begins,

$$V \frac{dX}{dt} = K \quad \text{for } X << X_c \qquad (2)$$

where V is the culture volume,

X_c is that culture density at which the incoming light energy just compensates via photosynthesis

for the respiration losses,

and K is the constant growth rate permitted by the constant influx of light. (l.g/hr).

X will generally be much less than X_c in my experiments.

K is proportional to the illuminated area (A).

$$K = LA \tag{3}$$

where L is a constant proportional to the illumination level.

In a well-mixed continuous culture algae are removed at the rate X(F/V) where F is the flow rate of medium and harvest in liters per hour.

For dilute cultures

$$\frac{dX}{dt} = kX = X(F/V) = (k - 1/t_r)X \tag{4}$$

where t_r is the retention time in hours and is defined as V/F.

If the culture has grown beyond X_s where light becomes limiting,

$$\frac{dX}{dt} = K/V - X/t_r \tag{5}$$

Equation (1) integrates to $X = X_o e^{kt}$ (6)

where X_i is the concentration inoculated

Equation (2) integrates to $X = (K/V)t' + X_s$ (7)

where $t' = 0$ when $X = X_s$

Equation (4) integrates to $X = X_i e^{(k-1/t_r)t}$ (8)

and equation (5) integrates to

$$X = (K/V)t_r + (X_s - (K/V)t_r)e^{-t''/tr} \tag{9}$$

where $t'' = 0$ when $X = X_o$, the concentration when continuous operation begins.

In light-limited continuous culture, after a few retention times

$$X \rightarrow (K/V)t_r \tag{10}$$

and the culture productivity

$$kX = X(1/t_r) \text{ approaches } K/V \tag{11}$$

Recalling that $K = LA$, the productivity is

$$P = L\,(A/V) \tag{12}$$

Methods to increase culture productivity center on increasing the available light (in some cases already approaching the saturation value where further increase gains nothing) or designing culture vessels with large ratios of illuminated area to volume, usually a costly direction either in terms of land or energy. The fermentor which is used here has an illuminated area of 0.239 m^2 for its 10 liter volume. Thus my algae behaved as though growing in a well mixed pond 4.18 cm deep. a condition far too luxurious for an agricultural operation. Moreover the illumination at the surface was continuous in order to speed up the research. A simulated day-night cycle would have been more realistic and might even have suggested synchronized harvesting, but I declined investigating it. Emerson and Arnold (1932) discovered that short pulses of saturating light resulted in as much photosynthetic activity as continuous light of the same intensity. Mayer

et al. (1964) connected this fact to the turbulence in a stirred tank. Cells in such a tank have repeated short exposures to the incoming light. Thus, agitation has the effect of increasing productivity. It may be considered as increased L or effectively an A/V increase at the expense of agitation power. My fermentor was operated at 200 rpm (Power number 6, Reynolds number 4.8×10^4, 1.1 watts per liter) throughout. In light limited culture algal species can differ only by having different values for X_s or L. This suggests quite a different search (given an economical A/V ratio) than the search for a species with the greatest growth constant k under conditions not limited by any factor external to the cells.

Since productivity is theoretically independent of culture density for $X_s < X << X_c$, the culture density can be chosen (by operating at a certain retention time t_r) so as to make harvest more efficient or to obtain cells of a certain mean age. Physiological differences between young and old cells may be nutritionally important. These differences may also affect harvestability.

The concept of mean cell age is credited to Aiba et al. (1965). $\bar{\Lambda}$ is the average age of a population of cells. Age zero describes the newly divided cell, with age increasing thereafter until division occurs at an average age Λ_d. At division, the old mother cell aged Λ_d

divides into two daughter cells each having age zero. Λ_d is a random variable with a standard deviation σ. It is the distribution of Λ_d which is responsible for the loss of synchrony after four or five generations in cultures which start with an inoculum of cells all the same age. Were there no variation among cells in the value of Λ_d, all such synchronized cells would divide at precisely the same age and remain synchronized forever. The Markovian transition matrix corresponding to the case of a constant $\Lambda_d(\sigma = 0)$ is of the form

$$P_1 = \begin{vmatrix} 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \end{vmatrix}$$

where each matrix element (P_{jk}) is the probability that a group of cells in the j^{th} age group at time t will be in the k^{th} age group at time $t + \Delta t$ where t is the mean time between transitions. Six age groups are used for illustrative purposes, the sixth group being the oldest. Such a matrix operating on an initial age distribution, say $\Lambda = |0\ 0\ 0\ 1\ 0\ 0|$, will cause it to cycle with no loss in synchrony. After 600 transitions the age distribution will be the same as the initial age distribution.

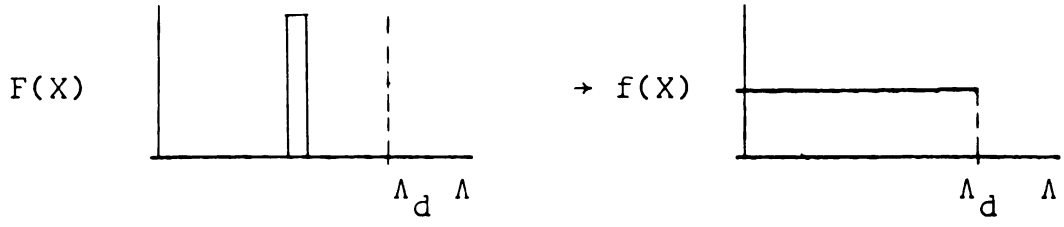
Λ_d being a distributed random variable, however produces a tendency for all inocula to gravitate towards a

uniform distribution in which all ages are equally represented. The Markovian transition matrix corresponding to this latter case might be

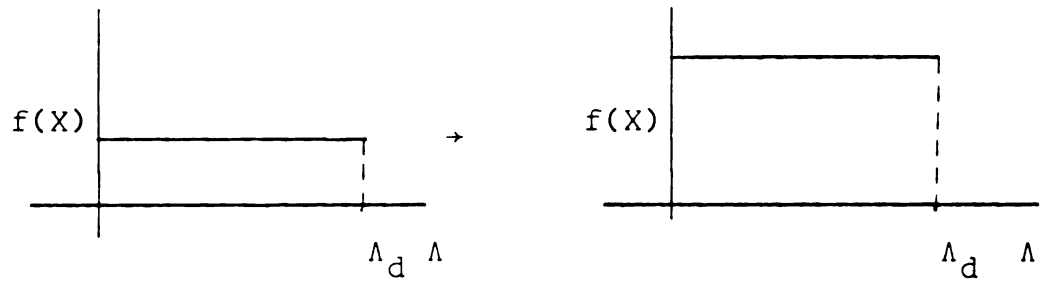
$$P_2 = \begin{vmatrix} 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ .2 & 0 & 0 & 0 & 0 & .8 \\ .8 & 0 & 0 & 0 & 0 & .2 \end{vmatrix}$$

P_2 operating on the initial age distribution $|0\ 0\ 0\ 1\ 0\ 0|$ results in an age distribution of $|0.129\ \ 0.0659\ \ 0.0898\ \ 0.188\ \ 0.306\ \ 0.221|$ after 25 transitions (approximately four generations). After 75 transitions (approximately twelve generations) the age distribution will be $|0.178\ \ 0.179\ \ 0.167\ \ 0.153\ \ 0.153\ \ 0.170|$, a nearly uniform distribution.

The frequency distribution is based on grams of cells per



liter, not cell numbers. For this reason, when the group of cells aged Λ_d returns to the $\Lambda = 0$ axis upon division, the height remains unaltered since total cell mass is unaffected. All age groups are assumed to grow at the same rate.



The growth may be exponential in time or linear in time depending on whether the culture density is greater than- or less than - X_s.

Λ_d is also known as the mean interdivision time. For our purposes it is equal to the doubling time. Painter and Marr (1967) conclude that mean interdivision time is not equal to doubling time, the former being

$$\Lambda_d \simeq \frac{\ln 2}{k} + \frac{k\sigma^2}{2} \qquad (13)$$

whereas the latter is

$$\Lambda_d = \frac{\ln 2}{k} \qquad (14)$$

for exponentially growing cells with a specific growth rate of k. While $\sigma > 0$ is required for damping of synchrony, a ratio of $\sigma/\Lambda_d = 0.5$ produces an error of only ten percent. Therefore equation (14) will suffice for this discussion.

For the uniform age distribution, $\bar{\Lambda} = \Lambda_d/2$. In the light-limited batch culture growth is linear with time. Linear growth may be considered exponential growth with a non-constant specific growth rate. From equations (2) and (7)

$$\frac{dX}{dt} = \frac{K}{V} \tag{2}$$

and

$$X = \frac{K}{V} t' + X_s$$

$$\frac{dX}{dt} = \kappa X \tag{15}$$

(where κ is a variable version of the specific growth rate k)

$$\kappa = \frac{1}{t' + \frac{V}{K} X_s} \tag{16}$$

and

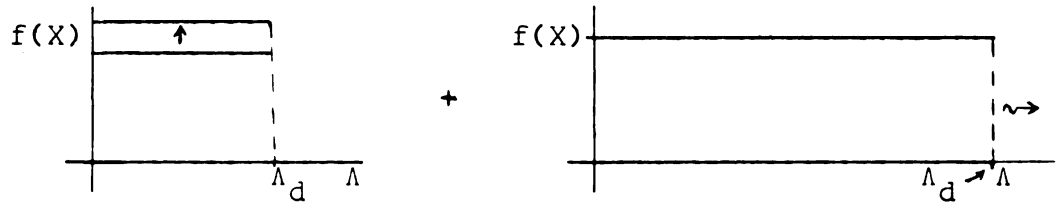
$$\Lambda_d = \frac{\ln 2}{\kappa} = \ln 2 (t' + \frac{V}{K} X_s) \tag{17}$$

In light-limited batch culture, Λ_d increases with time as the culture grows. For a culture which grows exponentially up to a density of X_s and linearly thereafter, one must consider the transition between growth modes. My data show continuity between the exponential growth region and the linear growth region. Since the onset of shadowing is probably not a sudden phenomenon there is no theoretical reason to expect a discontinuity either in the function or its derovatove

Therefore at the transition time t_s,

$$X_s = \frac{K}{V} \frac{1}{k} \tag{18}$$

In equation (17) $t' = 0$ at time t_s, and equations (17) and (14) are the same at that instant. Evidently, during linear batch growth, Λ_d is moving to the right on the Λ axis.



The distribution may be temporarily short of newborn cells and a transient dent in the distribution could arise and persist until damped. The Λ_d boundary moves to the right at the rate (ln2)t while the age groups progress along their cyclic course at rate t, thus some damping is assured.

Proceeding to the light-limited continuous culture, the condition maintained in my experiments, the mathematical treatment is similar.

Equations (5), (9) and (15) yield

$$\kappa = \frac{1}{\frac{X_o V}{K} e^{-t''/t_r} - t_r(1 - e^{-t''/t_r})} \qquad (19)$$

$$\Lambda_d = \frac{\ln 2}{\kappa} = (\ln 2)\left[\frac{X_o V}{K} e^{-t''/tr} + (1 - e^{-t''/t_r})t_r\right] \qquad (20)$$

where t'' is the time measured from the start of continuous operation - at which time ($t'' = 0$), the culture density is X_o grams per liter. Since my cultures were batch grown to the desired culture density and the retention time t_r was set to maintain that culture density, equation (10) states

$$t_r \to \frac{X_o V}{K} \quad \text{in steady state operation}$$

Consequently

$$\Lambda_d = (\ln 2)t_r \text{ for all } t'' \text{ and there is}$$

apparently no time lag involved.

In sum, Λ_d is a fixed value (ln2/k) during exponential growth; it increases linearly with time during light-limited growth ($\ln 2(t' + \frac{V}{\bar{X}} X_s)$); and it is constant again ($t_r \ln 2$) once continuous operation (light limited) is begun. Meanwhile the mean cell age $\bar{\Lambda}$ follows at $\Lambda_d/2$ plus or minus small, damped transient deviations caused by the changes from exponential to light-limited to continuous light-limited cultivation.

My routine for establishing a culture (data from Scenedesmus) was to inoculate at about X_i = 0.005 g/l (t = 0) then to batch-grow to the desired operating density, say X = 1.0 g/l. Exponential growth would proceed at k = 0.07 hr^{-1} for about 60 hours (equation 6) at which point (X_s = 0.333 g/l, t_s = 60 hrs, t′ = 0) shading limited growth. Thereafter growth followed equation 7. K/V is 0.033 g/l/hr for Scenedesmus (See Results, Table 11). So growth to 1.0 g/l took an additional 20.2 hours (t′ = 20.2 or t = 80.2 hrs). Then t_r is set at 30.3 hours to maintain the culture density at 1.0 g/l.

In the exponential growth stage,

$$\Lambda_d = 9.90 \text{ hours.}$$

In the light-limited batch stage,

$$\Lambda_d = 7.0 + 0.693t' \text{ for } 0 \leq t' < 20.2$$

i.e. Λ_d is increasing from 7.0 hours to 21.0 hours.

In the light-limited continuous culture

$$\Lambda_d = 21.0 \text{ hours}$$

Even though there is no change in Λ_d (or steady state $\bar{\Lambda}$) caused by the last transition, no data were collected until at least one retention time (30.3 hours) after the start of continuous operation. This gave any cell age distribution transients fifty hours to be smoothed. It is fairly certain that $\bar{\Lambda}$ would have settled down by this time; my data suggest nothing to the contrary.

Differences in physiological characteristics of the algal cells are important when considering the possible application of algae as a nutrient trap for polluted water. In a properly designed system, the algae will be starved for the nutrient they are supposed to trap. Since light limited growth is probably the best that can be provided when the nutrient is in luxurious supply, the nutrient-starved growth rate will certainly be less than the light limited growth rate with ample nutrient. This is equivalent in my cultures to reducing the flow of nutrient solution (F) until the culture density responds to it in other than the hyperbolic manner expressed in equation (11). In general t_r becomes very large and the cells become, on the average, very old. Cell division is prevented due to lack of sufficient nutrient. The effluent solution, its algae removed, is also very low in that nutrient--as

planned. To wait one of these very long retention times beyond adjustment to the desired culture density would have cost too much research time. Furthermore, operation in the continuous culture mode would have little point since I was prepared at that point to stop cultivation of the algae and sacrifice the culture to science. Very dense cultures (old cells) were harvested as if in a batch culture. The major point to be made is the inherent differences in cell age between the fast growing cultures designed for feed production and the slow growing cultures designed for wastewater treatment.

A mathematical statement of the dichotomy between cellular productivity and substrate (nutrient) removal is found in Aiba et al. (1965) p. 114. Their work is for an exponential growth situation, but the conclusion carries over to our case.

The idea of recycling algal biomass to achieve a high population of starved cells (desirable for wastewater treatment) has probably never received serious thought due to the reputed difficulty of harvesting the algae. If recycling is considered, a mass balance on a single culture vessel in the case of unlimited growth is

$$\frac{dX}{dt} = \frac{F}{V}(rX) - \frac{F}{V}X + kX \qquad (21)$$

where r is the fraction of the harvested algae recycled.

In the case of light limited growth

$$\frac{dX}{dt} = \frac{F}{V}(rX) - \frac{F}{V}(X) + \frac{K}{V} \qquad (22)$$

In the steady state continuous culture dX/dt = 0.

and

$$\frac{F}{V} = \frac{1}{t_r} = \frac{K}{V} \quad \frac{1}{X(1-r)} \qquad (23)$$

Obviously the recycled cells are not getting any younger in the process. The result is that with shorter retention times the desired level of nutrient removal may be accomplished. But the cells are still aged.

Before leaving this topic it must be mentioned that Nooney (1968) approached the mean cell age concept using a stochastic formulation rather than the deterministic form used here. With the stochastic formulation (in which standard deviation is computed as well as the mean), the standard deviation of the mean age of an exponentially growing population is found to increase with time while the mean value agrees with that found deterministically. The stochastic formulation should be applied to the case of light-limited growth.

Procedures Followed in Harvesting the Algae

Sedimentation. This was done in batches of 500 ml of culture. The culture was drawn from the mixed fermentor at a level 5 cm from the bottom of the vessel. It was collected in a 0.5 liter or a 1.0 liter separatory funnel, sampled immediately and let stand at room temperature and

illumination. After a measured time interval (when some separation was apparent) the lower cell-rich fraction was carefully withdrawn, its end point being judged by eye. In retrospect, it is here that the greatest scattering of data occurred. Rich and lean fractions were both mixed and sampled using in most cases the filterable suspended solids method. Total rich and lean volumes were also measured so that a material balance could be determined.

In the case of Spirulina, pre-treatment by brief exposure (10 sec.) to high pressure (5.2×10^5 n/m^2 ≃ 5 atmospheres) made settling possible. Unless their gas vacuoles were collapsed by pressurizing or sonication (Lehmann and Jost, 1971) the algae sedimented only very slowly. Neither did Spirulina float especially well of its own accord.

Flocculation. This is a variation on the sedimentation technique whereby the algae are electrochemically flocculated prior to settling. This is accomplished by placing aluminum electrodes into the separatory funnel, then passing through the liquid a direct electric current (500 ma.) for 30, 60 or 90 seconds. Voltage was noted. Settling followed usually for about 60 minutes. Although I agreed not to try additive chemical flocculants, I felt this method deserving of attention. Alum ($AlK(SO_4)_2 \cdot 12H_2O$) produces an unpalatable product, and the toxicity limits of the sulfate ion are set at 250 mg/l. The aluminum ion

toxicity level is not set, nor does it produce the taste characteristic of alum.

Centrifugation. About 5 liters of algal culture were placed above the semi-continuous centrifugal bowl and sampled. The head was adjusted to about 70 cm. before flow was started. The centrifuge was brought to a constant speed, flow was started and measured. After about a minute when an equilibrium of sorts was established, a sample of the effluent was taken and compared optically (425mμ) with the sample of feed stream. Centrifuge power consumption was recorded during flow. Flow was then stopped and the centrifuge brought to a new speed. The hydrostatic head was re-set and the entire process repeated. The centrifuge speed range was selected to show the region of poor separation.

Filtration. Vacuum (10 cm. Hg abs. pressure) was applied under a wetted 3μm pore-size membrane filter. A sample of algal culture was then poured on top of the filter at time zero. Filtrate was collected in a graduated cylinder inside the vacuum flask. Filtrate accumulation was measured at ten-second time intervals after starting.

Electrodecantation. The apparatus is shown schematically in Figure 14. Electrolyte circulation was started. The filtrate chamber was filled with distilled water just to overflowing into a graduated cylinder. A 500 ml sample of the culture was drawn. Algal culture

was let into its side of the cell. Pressure head was maintained constant by adjusting culture flow just to the point where there was no flow out of the rich-stream exit port. Thus all the exit flow from the cell was clear filtrate. No measurements were made until the current through the cell stabilized (i.e. the distilled water was replaced by filtrate of higher conductivity). Periodically the rich algal accumulation was flushed out. Voltage was set and maintained constant. Filtrate accumulation was measured as a function of time.

Surface attachment. Tared samples of glass, polyethylene, teflon, polycarbonate, and stainless steel were slowly withdrawn (1 cm/hr) from a stirred beaker of algal culture. The polymers were roughened; one sample of glass was pre-treated by exposure to concentrated HCl; another sample to NaOH; a third was simply allowed to accumulate whatever organic scum it would retain. All glass samples were rinsed in distilled H_2O before the test was begun.

Phototaxis. This applies only to Chlamydomonas. Light (10000 lux) was applied from above a one liter graduated cylinder filled with culture. A second cylinder was illuminated from beneath. Samples were taken from top and bottom of both cylinders after 15, 30 and 60 minutes.

Predation. Daphnia magna cultivated by Mrs. B. Burk (Michigan State University) were kept in a starved state. Twenty organisms were placed into bottles

containing 20 ml of algal culture of known density on day zero. The bottles were kept in the light to provide the crustaceans their necessary oxygen. After 48 hours, the Daphnia were collected by straining the culture through 80-mesh brass cloth. The filterable suspended solids test was run on the algal culture. Controls without Daphnia were also monitored for growth.

Fresh material from the fermentor was harvested whenever possible. Sometimes in the centrifugation tests, accumulated harvested material was used even though it may have been as old as 12 hours. There was definitely a difference in, for example, filterability of freshly harvested Chlamydomonas and filterability of the cumulative continuous harvest (average age 7.2 hours). The differences suggested that fresh material is more harvestable than old material. There was no provision for refrigerating the cumulative harvest. Simulation of an industrial process would most likely involve the continuous delivery of fresh culture to the harvesting device.

Methods 6, 7 and 8 were tested more for success or failure than for the quantitative results sought with the other methods where some degree of success was relatively assured.

Measurement of Success

Since "harvest" has been more or less defined, so also has a quantity which I will call enrichment:

$$E = C_R/C_F \qquad (24)$$

where C_R is the concentration of dry algal material in the rich harvest stream,

C_F is the corresponding concentration in the feed or input stream to the process.

Acceptable enrichment will be on the order of 20-40.

Since this is bracketed, the criterion for successful harvest weighs more heavily on a factor known as recovery, R, the fraction of dry algal material in the feed stream which is recovered in the rich (or harvest) stream.

$$R = v_R C_R / v_F C_F \qquad (25)$$

where v refers to the volume of the fraction indicated by the subscript.

R can be rewritten in terms of concentrations alone. Letting $v_f = 1$

material balances can be written down for

algal mass: $C_R v_R + C_L v_L = C_F$ (26)

and liquid volume: $v_R + v_L = 1$ (27)

where the subscripts R, L and F refer to rich fraction, lean fraction and feed.

Solving simultaneously for v_R and substituting in equation (24),

$$R = \frac{C_R (C_F - C_L)}{C_F (C_R - C_L)} \qquad (28)$$

In some cases (centrifugation, for example) $C_L << C_R$ and

$$R \approx (C_F - C_L)/C_F \qquad (29)$$

When the material balance on a process was in order (<5 percent error) the recovery as calculated by equation (25) agreed with recovery as calculated by equation (28). Data were rejected if the materials did not balance within this limit. But computation of the material balance was not possible in all cases: e.g., the rich fraction was not continuously discharged from the centrifuge, and the weight of algae collected by the predators could not be measured directly.

By itself, the recovery in a harvest does not describe the process completely. For example, two possibilities for the separation of one liter of culture at 1 g/l dry algal matter are:

$C_R = 7$ g/l $v_R = 0.1$ liter
$C_L = 0.333$ g/l $v_L = 0.9$ liter
} $R = 0.70$

and

$C_R = 3.5$ g/l $v_R = 0.2$ liter
$C_L = 0.375$ $v_L = 0.8$ liter
} $R = 0.70$

The first is obviously a more successful process, even though both have the same R. Clearly, without another parameter, say E, information is missing.

Prediction of the Behavior of R with Time in a Batch Settling Operation

The plane separating a settled vessel into rich and lean fractions will be the horizontal plan which passes through an algal concentration equal to C_F where $C_L < C_F < C_R$. Time (T) will be set equal to zero at the first instant that this becomes possible. The only trapping boundary (or absorbing state in the Markov sense) is at the bottom (for sinkers). In a brownian movement situation, the only discontinuity in algal concentration will develop starting next to the first few algae to be trapped. The volume which must be withdrawn to harvest the rich stream is very small at first.

$$\text{At } T = 0+,\ E = C_R/C_F \to 1+$$

and
$$R = C_R v_R / C_F v_F \to 0+ \text{ since } v_R/v_F \to 0+ \qquad (30)$$

By making different assumptions about the situation at T=0+ and the definition of the boundary, one can also derive initial conditions (E,R) = (1,1), (>>1,0), (>>1,1), (1+,1/2) and (1, indeterminate). These last five initial conditions are hard to reconcile with the experimental results and this lends credence to the surviving set (30) which I have selected as reasonable and real.

The arrival rate of algal cells on the surface discontinuity that separates rich and lean fractions is stochastic. Interarrival times depend on the population density (C_L) above the surface and the terminal sinking

velocity. The time course of settling will also depend on the area presented for settling a given volume of culture. The algal accumulation at the bottom may be thought of as a number of parallel unserviced queues (unserviced if there is no flow out of the bottom). The arrival of cells at the end of the queues occurs at the rate at which cells drop-out (die) from the lean fraction above. The death or drop-out rate (λ) is a function of the population size (N_L); the probability of a cell dropping out between an arbitrary time t and Δt later is expressed as

$$P_r \{N_L(t+\Delta t) = N_L(t) - 1\} = \lambda N_L(t)\Delta t \qquad (31)$$

Growth during the settling phase will be neglected

$$P_r \{N_L(t+\Delta t) = N_L(t) + 1\} = 0 \qquad (32)$$

and changes of more than one cell will be improbable.

$$\text{Then } P_r \{N_L(t+\Delta t) = N_L(t)\} = 1 - \lambda N_L(t)\Delta t \qquad (33)$$

The outcome of this classic model for simple stochastic death is a binomially distributed temporal population. (Bailey, 1964, p. 91; see also Hillier & Lieberman's (1967) limited source queuing model). The probability of there being N_L cells remaining in the lean fraction at time t is:

$$P_r \{n = N_L \text{ at time } t\} = \frac{N_F!}{N_L!\ (N_F - N_L)!}(e^{-N_F\lambda t})\ (1 - e^{-\lambda t})^{N_F - N_L} \qquad (34)$$

(notation, mine)

The probability that the rich fraction contains $(N_F-N_L) = N_R$ cells at time t is the complement:

$$P_r \{n' = N_R \text{ at time } t\} = \frac{N_F!}{N_R!(N_F-N_R)!} (1 - e^{-\lambda t})^{N_F} (e^{-\lambda t})^{N_F-N_R} \quad (35)$$

The equations imply that complete settling can be realized after an infinite wait.

The average cell population decreases exponentially in time, as expected.

$$N_L = N_F e^{- t} \quad (36)$$

while its complement N_R increases on a saturation curve.

$$N_R = N_F(1-e^{-\lambda t}) \quad (37)$$

The standard deviation is also time dependent.

$$\sigma N_L \text{ or } N_R = (N_F e^{-\lambda t}(1 - e^{-\lambda t}))^{1/2} \quad (38)$$

N_L, N_F and N_R can be numerical populations or the number of cells per unit volume. In the latter case they are easily transformed into concentrations of dry algal matter per liter, assuming an average cell dimension and specific gravity. To be dangerously specific, I have found that a rich stream density of sixty grams per liter is probably seldom achieved by the inexpensive techniques I have tried, certainly not by settling. A centrifuged pellet might contain 200 g/l but it no longer behaves as a liquid.

Dussart (1966) estimates 5.5 μμg dry weight for a Chlorella cell of $20\mu^3$ volume. A reasonable model for 3.36μm diameter algal cells attaining a maximum density of say 70 g/l is that each cell is in the middle of a 4.3μm cube of medium and all other cells are excluded from an occupied cube. Closest packing of these cubes gives a C_{max} of 70 grams of dry algae per liter, there being 12.6×10^{12} cubes per liter. So a culture density of C = 1 g/l has 180×10^9 occupied cubes per liter or a population per unit volume (N) of 180×10^9 cells per liter.

Since N is proportional to C,

$$C_L = C_F e^{-\lambda t} \tag{39}$$

or

$$C_L/C_F = e^{-\lambda t} \tag{40}$$

Another fact can be gleaned from the mathematical analysis. The standard deviation on the average number of cells which have left the lean fraction applies as well to the same cells as they arrive at the end of close-packed unserviced queues at the bottom of the vessel. The number of queues is the bottom area divided by the area of a 4.3μm cube or 49×10^9 queues per m^2, a large number. In a vessel containing separated fractions, the boundary moves upward as more algae settle. The reduction in volume of the lean phase was ignored in equation (31)ff, it being usually a very small percentage of v_L, the volume

of the lean fraction. It is, however, a considerable factor when considering v_R which is small early in the process. In fact the interface defines v_R. The separation level has been set somewhat arbitrarily as that level at which the algal concentration is C_F. It is a plane which cuts across the ends of the queues of close packed cells such that $(C_F/C_{max}) \times (49 \times 10^9)A$ cubes are occupied. Since $C_F < C_{max}$ there will be some lean phase also in the plane filling C_L/C_{max} of the cubes. If the separation plane intersects the queues h cubes from the bottom, then the difference between C_F/C_{max} and C_L/C_{max} must be made up by queues that are longer than or equal to h units long. There are $(C_F - C_L)/C_{max} \times (49 \times 10^9)A$ of them. That is to say, h is located where the probability of a particular queue being longer than or equal to h is $(C_F - C_L)/C_{max}$.

$$P_r \{N_{RQ} \geq h\} = (C_F - C_L)/C_{max} = (C_F/C_{max}) (1 - e^{-\lambda t}) \quad (41)$$

in a queue where N_{RQ} is the number of close packed cubes in a single queue.

The probability of there being h or more units in the queue at time t is the same as the probability of there being fewer than N_F-h units in the lean phase directly above (and destined to arrive at) a particular queue. Modifying equation (35):

$$\Pr\{N_{RQ} \geq h\} = \sum_{N_{RQ}=0}^{h} \frac{N_{FQ}!}{N_{FQ}!(N_{FQ}-N_{RQ})!} (1-e^{-\lambda t})^{N_{FQ}} (e^{-\lambda t})^{N_{FQ}-N_{RQ}} \tag{42}$$

where the subscripts FQ, RQ and LQ refer the population N to the number of cells in a single queue or its special extension into the lean region. This expression is difficult to evaluate for large values of N_{FQ}.

The binomial probability distribution for large values of $N_{FQ}(e^{-\lambda t})$ $(1 - e^{\lambda t})$ is approximately the same as the normal distribution (Papoulis, 1965) with mean $\bar{N}_{RQ} = N_{FQ}(1 - e^{-\lambda t})$ and standard deviation

$$\sigma = (N_{FQ} e^{-\lambda t}(1 - e^{-\lambda t}))^{1/2} \tag{43}$$

Figure 17 is a visual display of the situation for large λt.

The question to be answered is, how fuzzy is the boundary between rich and lean streams? More importantly, is the quantity of lean fraction which is separated along with the rich fraction going to reduce C_{Rmax} by dilution and impart to it a time dependence? The area under the normal curve for values of $N_{RQ} > h$ represents the probability that a queue is longer than h. Over a large number of queues, assumed equal at $t = \infty$, this is the percentage of queues longer than h and is, of course, computed by the error function. If 60 percent of the queues are longer than say 0.5h, then the concentration measured at a plane cutting through the vessel at this

level be 0.6 C_{max} + 0.4 C_L. The first fraction usually predominates. The cumulative distribution function, then, gives a pictorial representation of the boundary between the two fractions (Figure 17). Since it represents C, and the numbers in the queues represent depth, the area under the curve represents total quantity of each component contained beneath a boundary, say h:

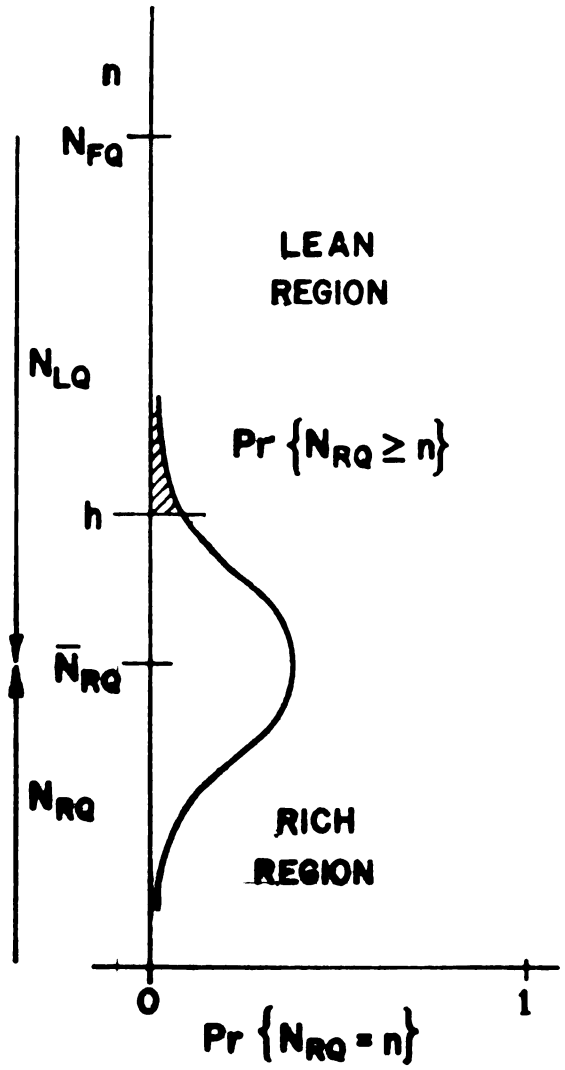
$$A \int_o^h \frac{C}{C_F} dy \qquad \text{and} \quad C_R = \frac{\int_o^h C dy}{\int_o^h dy} \qquad (44,45)$$

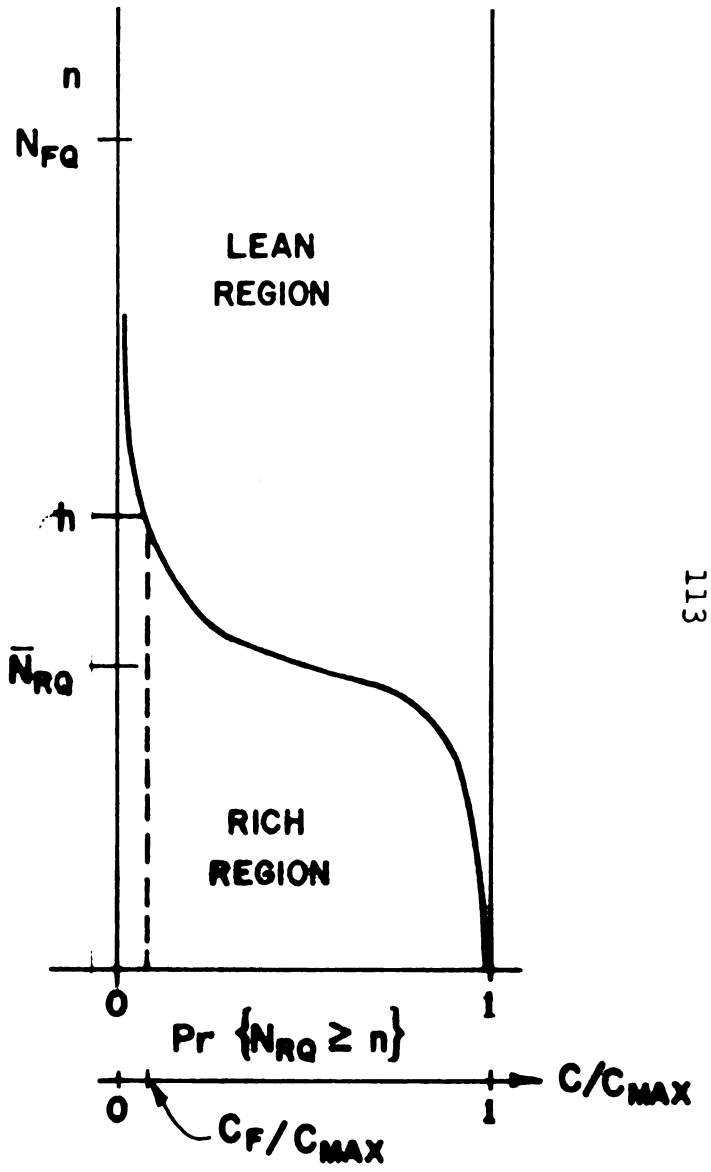
where C/C_F is measured from the Pr = 0 axis and y is the vertical dimension in a settling vessel of constant cross sectional area A.

The area under the cumulative distribution function of the normal curve is computed numerically (in Abramowitz and Stegun, 1964). The area further than one standard deviation from the mean constitutes only 4.6 percent of the total area outward from the mean itself; beyond 2σ it is 0.085 percent. Little error will be introduced by assuming that lean fraction penetrates below h as far as the mean, and the entire volume below the mean is occupied by packed cells at a density C_{max}. For $(h - \bar{N}_{RQ}) > 1\sigma$, the error in C_R can be disregarded.

When λt, the dimensionless time, is small, the normal approximation to the binomial distribution cannot be used. This is logical since when the mean queue length is small, the symmetric normal distribution would

FIGURE 17.--Imprecision at the rich-lean interface: An interpretation.


n
N_{FQ}
LEAN
REGION
N_{LQ}
$Pr\{N_{RQ} \geq n\}$
h
$\bar{N}_{RQ}$
N_{RQ}
RICH
REGION
0
1
$Pr\{N_{RQ} = n\}$


n
N_{FQ}
LEAN
REGION
h
$\bar{N}_{RQ}$
RICH
REGION
0
1
$Pr\{N_{RQ} \geq n\}$
0
1
C/C_{MAX}
C_F/C_{MAX}

indicate some negative (impossible) queue lengths. Use of the binomial distribution is awkward for large values of N_F, especially since the tail probability is known and the parameter h is the unknown. Tchebyshoff's approximation will serve.

$$\Pr\{|N_{RQ} - \bar{N}_{RQ}| \geq a\sigma\} \leq a^{-2} \quad \text{for } a>0 \tag{46}$$

Since this will only be applied in cases where $a\sigma > \bar{N}_{RQ}$ (i.e. close to the bottom of the vessel and early in time) the case $N_{RQ} < \left(\bar{N}_{RQ} - a\sigma\right)$ has zero probability and the only way N_{RQ} can be outside the interval is to be greater than $(\bar{N}_{RQ} + a\sigma)$.

If $$h = \bar{N}_{RQ} + a\sigma, \tag{47}$$

then $$\Pr\{N_{RQ} \geq (\bar{N}_{RQ} + a\sigma)\} = \Pr\{N_{RQ} \geq h\} \tag{48}$$

$$a^{-2} \geq C_F/C_{max}\ (1 - e^{-\lambda t}) \tag{49}$$

From equations (37), (43), (47) and (49), h and $a\sigma$ can be numerically determined as functions of time. Corresponding values of C_R are computed as above, assuming, as before, that having defined h, the level of mean queue length in the rich domain does a pretty good job of equally dividing the volumes of packed cells and intruding lean phase, i.e.

$$C_R = (\bar{N}_{RQ}C_{max} + a\sigma\ C_L)/(\bar{N}_{RQ} + a\sigma) \tag{50}$$

Use of the Tchebysheff theorem will cause C_R to be conservatively low in the region where it is used. The

normal distribution can probably be trusted for $\bar{N}_{RQ} \geq \sigma$.

In Table 9 are tabulated some computational results. The model is applied to a one-liter settling vessel 40 cm^2 in horizontal area, 25 cm deep. C_F = 1 g/l and N_{FQ} = 832. C_{max} is 70 g/l.

Other Separation Indices

Separation indices other than R and E include an interesting one by Rony (1968). It is called the extent of separation and was designed to describe chromatographic separations.

$$\varepsilon = \text{abs det} \begin{vmatrix} Y_{11} & Y_{12} \\ Y_{21} & Y_{22} \end{vmatrix} \text{ for binary separations} \quad (51)$$

where Y_{ij} is the number of moles of phase i in region j divided by the number of moles of i initially present in the system and ε is the "extent" index. Its alleged universality is appealing but for typical algal or sludge separations it can be simplified. Grams substance per liter is analogous to the molar quantities prescribed. Consequently if material 1 is water and region 1 is the lean region,

$$Y_{11} = \frac{(1000 - C_L)v_L}{(1000 - C_F)v_F} \simeq 1 \quad \text{for most algal harvests.} \quad (52)$$

TABLE 9.--Computation Scheme for Deriving the Enrichment E as a Function of Dimensionless Time, λt.
Cubes 4.29μ on a side
$C_{max} = 70$, $C_F = 1$ cells 3.36μdia, 20 ^{3}vol.
$N_{FQ} = 58275$, 832 will be full at $C_F = 1$

λt	$C_F/C_M(1-e^{-\lambda t})$	$\bar{N}_{RQ}$	σ	a	h	$E = C_R/C_F$	
.0005	.00000714	.416	.64	<374	<240	>1.12	Tcheby
.001	.0000143	.831	.91	<264	<241	>1.24	Tcheby
.002	.0000285	1.66	1.29	4.02	6.85	17.7	Normal
.005	.0000713	4.15	2.03	3.80	11.86	25.1	"
.01	.000142	8.28	2.86	3.63	18.66	31.6	"
.02	.000283	16.5	4.02	3.45	30.3	38.5	"
.05	.000697	40.6	6.22	3.20	60.5	47.3	"
.1	.00136	79.2	8.46	3.00	104.6	53.2	"
.2	.00259	151	11.1	2.79	181.8	58.2	"
.5	.00562	328	14.1	2.54	363	63.2	"
1	.00903	526	13.9	2.36	559	65.9	"
2	.0124	720	9.99	2.24	742	67.9	"
5	.0142	827	2.36	2.19	832	69.6	"
10	.0143	832	.195	2.19	832	70	"

Similarly $$Y_{12} = \frac{C_L v_L}{C_F v_F} = (1 - R) \tag{53}$$

$$Y_{21} = \frac{(1000 - C_R)v_R}{(1000 - C_F)v_F} \cong \frac{R}{E} \tag{54}$$

$$Y_{22} = \frac{C_R v_R}{C_F v_F} = R \tag{55}$$

Then $$\varepsilon = \text{abs det} \begin{vmatrix} 1 & 1-R \\ R/E & R \end{vmatrix} \tag{56}$$

will be close enough. Some more manipulation yields

$$1 - \varepsilon = \left(\frac{1-R}{1-R/E}\right)(1 - (R/E)^2) \quad 0 \leq R \leq 1 \tag{57}$$

In all but the worst harvesting situations, $E > 5$.

So $$1 - \varepsilon \approx \frac{1 - R}{1 - R/E} \tag{58}$$

Substituting for R and E using equations (28) and (51), one finds that

$$\frac{1 - R}{1 - R/E} = \frac{C_L}{C_F} \tag{59}$$

In working with C_L/C_F, we are using something close enough to ε to be assured of its generality.

DeClerk and Cloete (1971) deduce a relationship for the intermediate local entropy level and derive from it an approximate local quantity called the purity index of the separated region.

$$I_j \cong - \log_{10} \frac{n_{ij}}{n_{jj}} \tag{60}$$

where n_{ij} is the number of moles of component i in the j^{th} separated fraction and I_j is the purity index defined for the region

$$0 < \frac{n_{ij}}{n_{jj}} < 0.1.$$

In our case the concentration of algae in the lean fraction is certainly small enough so that I_j would be defined for the lean fraction. The fraction of water in the rich stream, however, is greater than 10%, so the index would not be meaningful.

Said (1964) had the right idea in keeping the recovery index as a complex number.

RI(ν,ϕ) where RI represents the recovery indes and (ν,ϕ) are respectively the fractional impurity and size of the fraction.

$$\nu = \frac{\text{moles of impurity}}{\text{moles of main component}} \text{ in a fraction} \quad (61)$$

$$\phi = \frac{\text{moles of main component recovered}}{\text{moles of main component at the start of the process}} \quad (62)$$

A New Separation Parameter Based on Entropy

Using the entropy of a completely separated culture as reference, the ratio of the total entropy of a partially harvested system to the entropy of a perfectly harvested system is a new parameter which has some interesting properties. I dub it G. Its definition is based on the entropy-of-mixing concept which can in turn be

derived from information theory (see Fast (1962), p. 129, for example). The validity of my parameter depends on Shannon's proof (Shannon, 1964) that entropy is additive. (I am indebted to Prof. M. Krzywoblocki, Michigan State University, for raising this point.) In brief, the entropy of a mixture of $N_o x$ particles of type A with $N_o(1-x)$ particles of type B is related to the number of ways (m) in which the particles can be distributed over N_o sites. (N_o is the number of sites in a given volume and is also equal to the total number of particles; x is the fraction which is type A).

$$m = \frac{N_o!}{(N_o x)!(N_o(1-x))!} \tag{63}$$

Appealing to the cornerstone of information theory,

$$S = k_B \ln m \tag{64}$$

where S is the entropy above a reference level and k is the Boltzmann constant, 1.38×10^{-23} j/K°

Using Stirling's approximation.

$$\ln N_o! = N_o \ln N_o - N_o \tag{65}$$

we can derive:

$$S = - N_o k_B\{ x \ln x + (1-x) \ln (1-x)\} \tag{66}$$

Thinking of the particles as the occupied or unoccupied cubes, x is analagous to the volume fraction of occupied cubes, viz.

$x = \frac{C}{C_{max}}$ and N_o is now the number of cubes per liter. (67)

Figure 18 depicts the derivation of G:

$$G \equiv (S_L + S_R)/S_F \tag{68}$$

G is a measure of the completeness of mixing, the complement to the completeness of harvesting. It is normalized on <0,1> .

Table 10 presents a comparison of several parameters which might be used to measure the success of a harvest operation. It can be argued that C_R and C_L are really all that is required and their orthogonality is guaranteed. On the other hand they are dimensioned quantities and neither is normalized. R is well known in the literature but E is not. Neither is E normalized. A normalized version of E must involve the maximum value that E can attain. Let H_{Cmax} be the normalized version of 1/E:

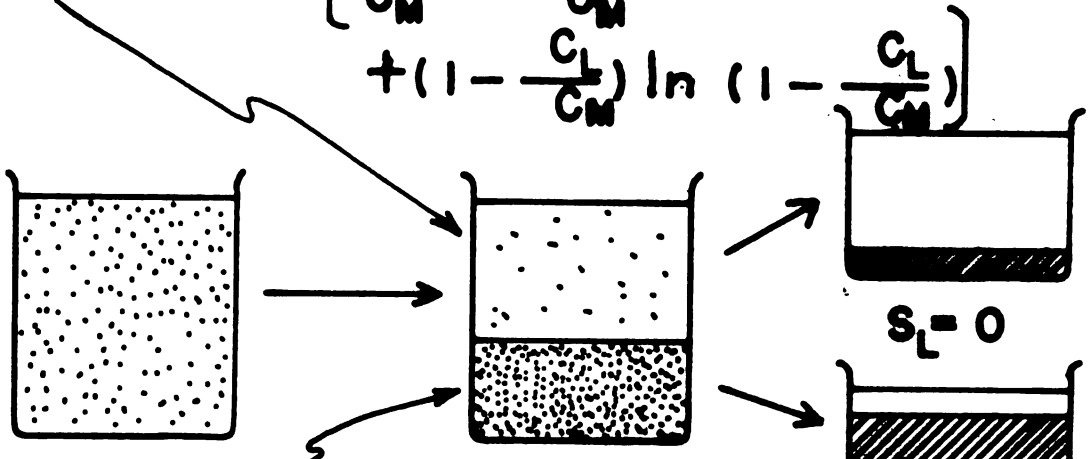
$$H_{Cmax} \equiv \frac{1}{E}\frac{(E_{max}-E)}{(E_{max}-1)} = \frac{C_F(C_{max}-C_R)}{C_R(C_{max}-C_F)} \tag{69}$$

The choice of C_{max} is guided by the data. The case for keeping a two dimensional vector is strong; of the three pairs shown, (C_L/C_F, H_{70}) is the only one which is normalized. Furthermore it will be found to behave well in the model described. Notice (Table 10) that if the parameters are taken singly, they can all (except G) be made to misrepresent the quality of the harvest; only the double combinations and G survive the test.

FIGURE 18.--Rationale behind the parameter G.

$$S_F = -v_F N_0 K_B \left[\frac{C_F}{C_M} \ln \frac{C_F}{C_M} + \left(1 - \frac{C_F}{C_M}\right) \ln \left(1 - \frac{C_F}{C_M}\right) \right]$$

$$S_L = -v_L N_0 K_B \left[\frac{C_L}{C_M} \ln \frac{C_L}{C_M} + \left(1 - \frac{C_L}{C_M}\right) \ln \left(1 - \frac{C_L}{C_M}\right) \right]$$



$$S_R = -v_R N_0 K_B \left[\frac{C_R}{C_M} \ln \frac{C_R}{C_M} + \left(1 - \frac{C_R}{C_M}\right) \ln \left(1 - \frac{C_R}{C_M}\right) \right]$$

FIGURE 18.--Rationale behind the parameter G.

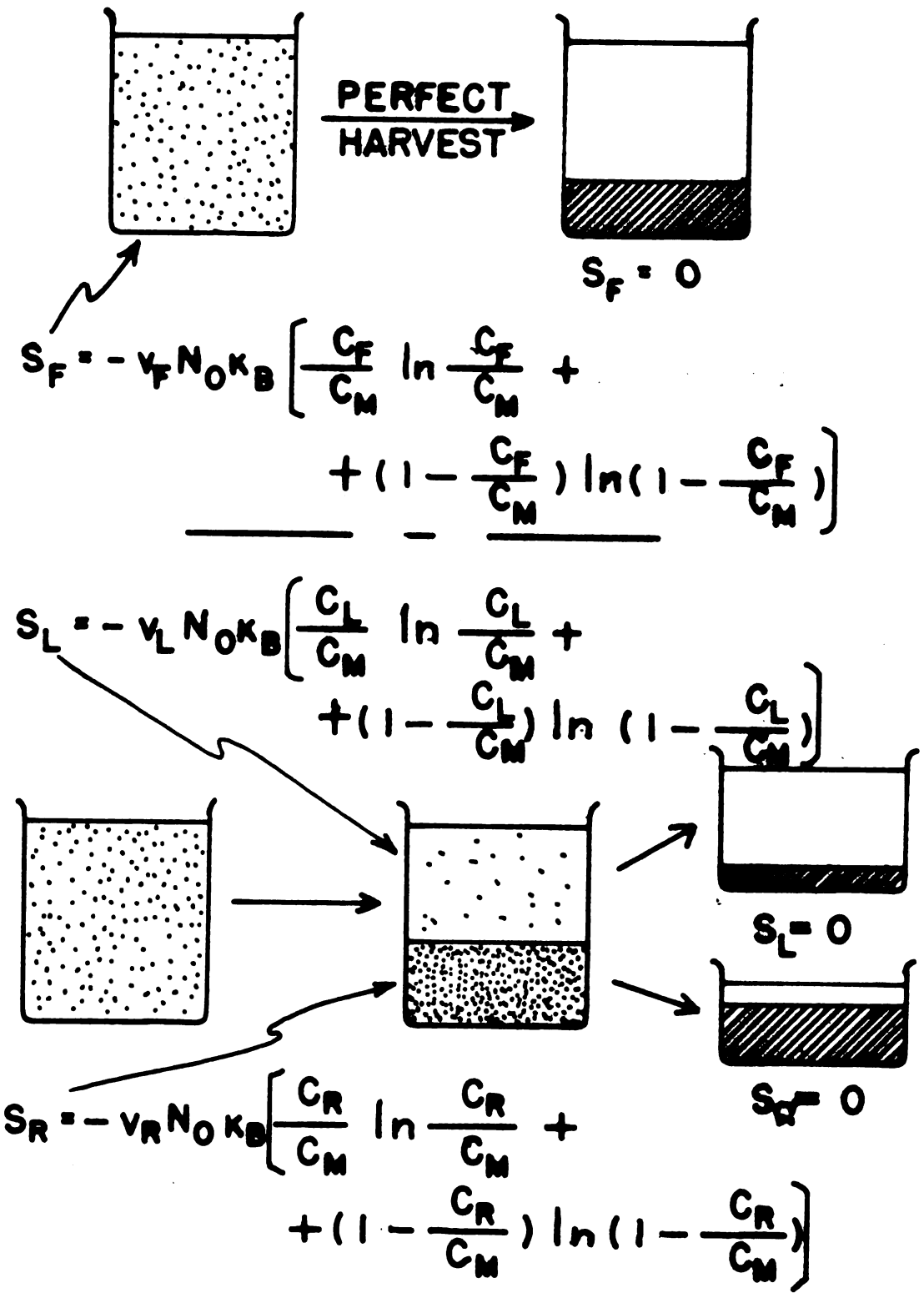

PERFECT
HARVEST
$S_F = 0$
$S_F = -v_F N_0 K_B \left[\frac{C_F}{C_M} \ln \frac{C_F}{C_M} + \left(1 - \frac{C_F}{C_M}\right) \ln \left(1 - \frac{C_F}{C_M}\right) \right]$
$S_L = -v_L N_0 K_B \left[\frac{C_L}{C_M} \ln \frac{C_L}{C_M} + \left(1 - \frac{C_L}{C_M}\right) \ln \left(1 - \frac{C_L}{C_M}\right) \right]$
$S_L = 0$
$S_R = -v_R N_0 K_B \left[\frac{C_R}{C_M} \ln \frac{C_R}{C_M} + \left(1 - \frac{C_R}{C_M}\right) \ln \left(1 - \frac{C_R}{C_M}\right) \right]$
$S_R = 0$

TABLE 10.--A Comparison of Candidate Harvest Parameters for a "Good" Harvest and a "Poor" Cne.

	C_F	C_R	v_R	C_L	v_L	R	E	C_L/C_F	H_{70}	ϕR	νR	Rony ε	G
Perfect	(1 liter)	70	.0143	0	.986	1	70	0	0	1	0	1	0.000
Good	1	7	0.1	0.333	0.9	0.7	7	0.333	0.130	.7	9	0.67	0.797
Good	1	19	.05	.0526	.95	0.95	19	.0526	0.0389	0.95	2.68	.9475	0.4686
Bad	1	1.83	.528	.0741	.472	.965	1.83	.0741	0.54	0.965	37.3	.9475	0.906
Bad	1	1.4	0.5	0.6	0.5	0.7	1.4	0.600	0.71	0.7	49	0.55	0.9840
Bad	1	7	.05	0.722	.95	0.35	7	0.722	0.130	0.35	9	0.3175	.9458
No harvest	1	1	0+	1	1	0	1	1	1	0	69	0	1

C_{max} is taken to be 70 g/l.

A harvest process can be described as the trajectory of a point on the C_L/C_F, H_{70} plane; "good" harvests (i.e. harvests where most of the algal biomass is recovered in fairly high concentration) are located near the origin. "No harvest" is at the point 1, 1 where all time based harvests begin. Examination of Figure 19 will suggest why G is so much better a parameter than ε. A low value of G means a harvest that was almost certainly acceptable. G is even better than the hyperbolae of constant $(C_L/C_F)(H_{70})$ since the lines of constant G don't "leak" along the axes. In summary if a single figure of merit is desired, G seems most suitable in that it embodies "a reasonable" compromise between high recovery and high enrichment (Recall that $C_L/C_F = (1-R)/(1-R/E) \approx (1-R)$ and $H_{70} \cong 1/E$).

Batch filtration can be thought of as a rapid excursion from (1,1) to near (1,0) followed by decelerating progress toward the origin along the x-axis. Batch centrifugation speeds the trajectory of a point toward the origin. A continuous process has no trajectory; it is simply an operating point in the plane.

The time required to accomplish separation of the algal matter from solution has a penalty associated with it. If the algae are kept at room temperature for six or eight hours, and the pH is on the order of 6, the unmistakable odor of hydrogen sulfide can be detected. This is

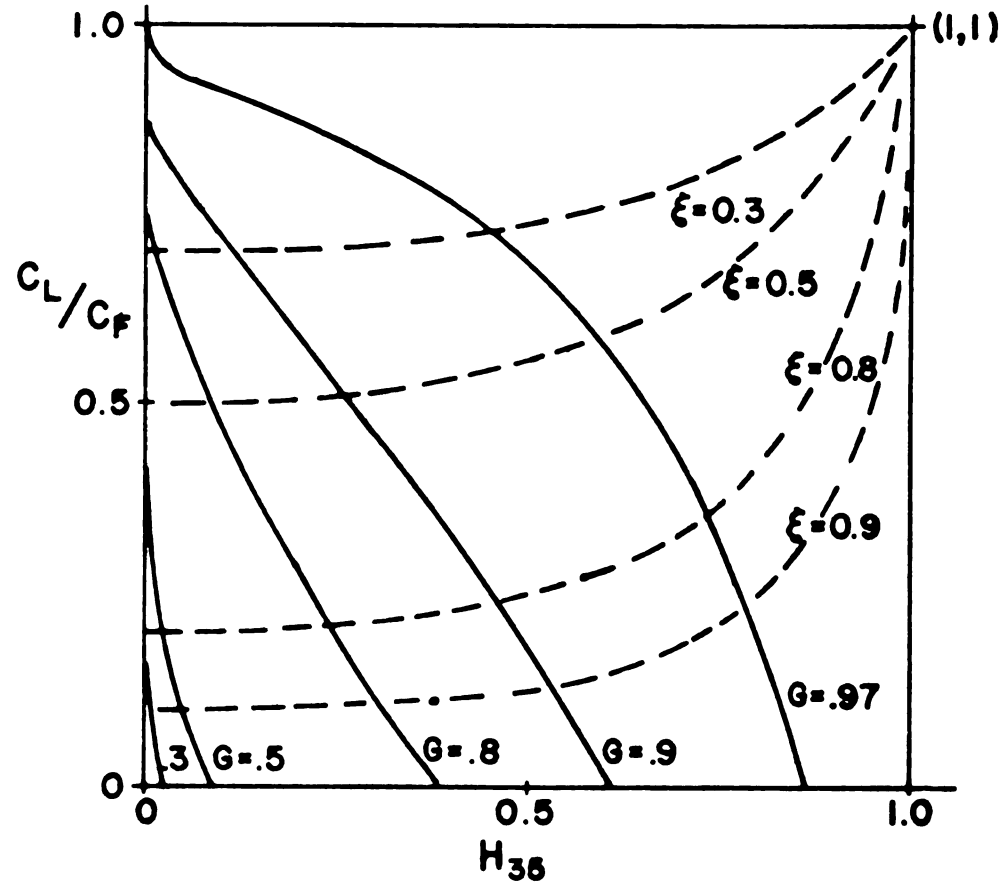


FIGURE 19.--Lines of Constant ε and Constant G.

indicative of anaerobic growth which may signal the proliferation of putrifiers and pathogens as well under similar conditions but at pH 8. The result is the loss of food/feed value of the recovered algae. A process which requires holding the algae at room temperature in the dark for more than 2-3 hours is probably inconsistent with any plans to recover the algae as feed. A little light would not penetrate the thickening algal sludge to prevent anaerobiosis in the dark interior of the sludge layer. Another cost for a harvest that takes too long comes in the figure called retention time; for a given flow rate, a longer retention time means a larger tank, more land area, and therefore more money.

A problem with accelerated harvest (centrifuge, electrodecantation, flocculation) is the associated cost. Not only must one account for the energy added to each liter of culture (or gram of dry algae) but there is capital investment as well. The capital investment can be over-simply represented in energy terms as that energy required to convert pig iron into say, a centrifuge and then return it to pig iron form. Properly done, this demands more time than I can afford.

The Thermodynamic Limit for Separation Energy

An illuminating exercise is the computation of the thermodynamic energy per unit volume based on the expression

for complete mixing (Equation 66). In a situation were C_{max} = 70 g/l and C_F = 1 g/l, one liter of culture contains 12.8×10^{12} close packed cubes 4.29μm on a side. Of these, one cube in seventy is occupied by an algal cell.

$$S = (1.635 \times 10^{-23} j/K^\circ)(12.8 \times 10^{12})(\tfrac{1}{70} \ln \tfrac{1}{70} + \tfrac{69}{70} \ln \tfrac{69}{70})$$

$$S = 1.07 \times 10^{-11} j/K^\circ/liter$$

creation of this required an energy exchange of

3.2×10^{-9} joules/liter at 300°K, a number ten or eleven orders of magnitude smaller than any harvest-accelerating device provides. Clearly, there is not a statistical thermodynamic limit against which we are working. Rather, the large energy requirements for accelerated harvest arise from the physical properties of the algae and water.

CHAPTER V

RESULTS AND DISCUSSION

Algal Growth

Culture density during batch culture of the algae was monitored as a function of time; the data appear in graphical display in Figure 20. To make the graph, times are adjusted so that the cultures are at 0.2 g/l at relative time zero. The linearity predicted by equations 2 and 7 is convincing. One can come by a linear growth rate either due to carbon dioxide limitation or light limitation, since both enter the system at a constant rate. The lack of growth response to intentional fluctuations in CO_2 supply argues well for light being the growth limiting nutrient. Equation 18 depends on assumed continuity in both the growth curve and its time derivative; my data, though scanty in the critical region, do not suggest any error in that assumption. The point at which exponential growth converts over to shaded growth is difficult to estimate. Based on the several algae, the culture density at which shading begins (X_s) is between 0.3 and 0.4 g/l. Values for the slopes of the lines appear in Table 11. The relative advantage Scenedesmus

FIGURE 20.--Batch history of the several algae in the fermentor. Data were adjusted by a time shift so that all cultures had a density of 0.2 g/l at time zero. Full light was applied (10,000 lux, entire surface).

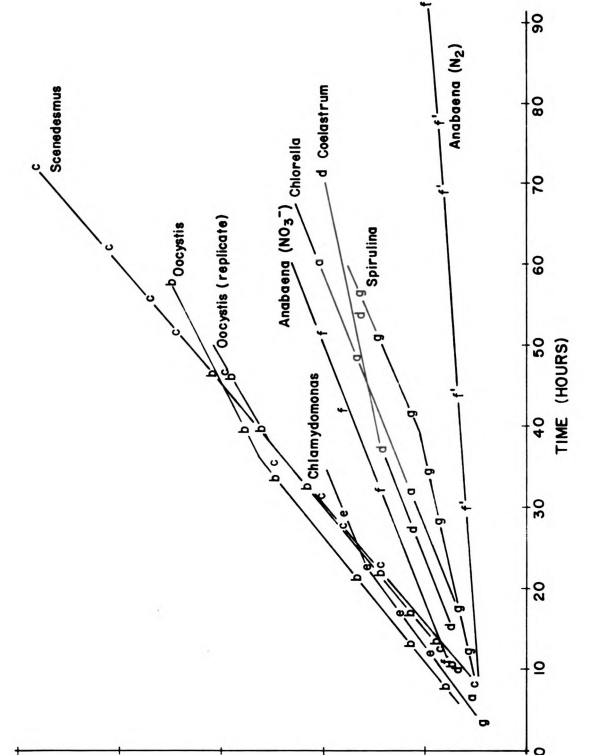

Scenedesmus
Oocystis
Oocystis (replicate)
Anabaena (NO_3^-)
Chlorella
Coelastrum
Chlamydomonas
Spirulina
Anabaena (N_2)
X (g/l)
TIME (HOURS)
0
0.5
1.0
1.5
2.0
2.5
10
20
30
40
50
60
70
80
90

TABLE 11.--Productivities of the Light-limited Batch Cultures

Alga	Slope K/V g/l-hr.	Estimated Xs (g/l)
Chlorella	.01585	0.35
Oocystis	.0326	0.35
Scenedesmus	.0337	0.32
Coelastrum	.0160	0.38
Chlamydomonas	.0278	0.30
Anabaena (NO_3^-)	.01565	<0.2?
Anabaena (N_2)	.00304	<0.3*
Spirulina	.0178	0.25

Xs is the culture density (under my conditions) above which the culture is light limited. Slopes are taken at 0.6 g/l. The slope K/V is also the productivity attained in steady state in continuous culture (Equation 11).

*Insufficent data.

seems to hold over the other algae, especially the green algae, may stem from the fact that the Schultz medium on which all the green algae were grown was designed for Scenedesmus cultivation. I have no explanation for the decreased slopes that some of the cultures exhibit late in growth. Possibilities include burnt out fluorescent lamps which always went out three-at-a-time (one eighth of the light), a change in pH which went unnoticed, a change in viscosity which might have affected the degree of turbulence (and exposure of individual cells to the light) or production of a growth-inhibiting substance by the algae themselves (Pratt et alia multa, 1944).

In the Spirulina cultures, one must be careful not to exceed the CO_2 demand of the algae by too much; at the high pH of the medium (pH~10); enough CO_2 can be scrubbed from the atmosphere so that no enrichment is necessary. But even the air flow rate must be kept low until the algal biomass has grown up - otherwise a precipitate appears in the culture ($CaCO_3$?).

The constant growth rate and productivity of light limited cultures means theoretically that several species can be grown in the same culture and one will not necessarily take over. Of course, this reasoning assumes no antagonistic behavior beyond the over-growth threat. As evidence for mutual growth, I found that my nitrate-grown Anabaena culture had a constant level of Chlorella

contamination for a week of continuous (not batch) operation. (1 g/l algal biomass, Chlorella estimated at 20%, pH 7.5, 1/8x Allen and Arnon Medium). Chlorella's reputation for overrunning other cultures it contaminates has probably been built on experience with batch cultures; it may tolerate a high culture density (shading?) better than other algae, but in continuous culture, the culture density can be kept low enough to offset any advantage Chlorella might find in a high culture density.

Harvesting

The various harvesting methods will be considered as topics; the algal species are a parameter.

Sedimentation

As concluded earlier, two variables are needed to describe fully a harvest's relative success. Having selected C_L/C_F and H as suitable, the data were processed into that form and plotted on graphs. (The hypothesized model which was presented earlier was derived only after consideration of these experimental results. Hence these data, being the basis for the model, cannot be said to validate the model, rather the theoretical approach was taken to instill confidence in the trends deduced from the scattered data.) C_L/C_F versus time (not shown) confirmed the suspected exponential decay of the population in the lean fraction. The exponential time constants λ for the

algal species and subpopulations were computed using a least squares linear regression analysis (on log-transformed data) which suppressed the constant term and ensured passage of the curve through the point (0,1). $\lambda$is set in tabular form (Table 12) along with a predicted value of C_L/C_F one hour after sedimentation has begun. Subpopulations were identified by studying a graph like Figure 21, "picking off the wild ones" and checking back to see if for some (any) reason they could be grouped. Identification was "confirmed" by ascertaining statistically significant difference in their mean time constants (λ) at the ninety percent confidence level. In this way a behavioral difference between young and old cultures of both Oocystis and Chlamydomonas was noted. This is an elaborate method for checking out a "hunch" based on the experience with the algae.

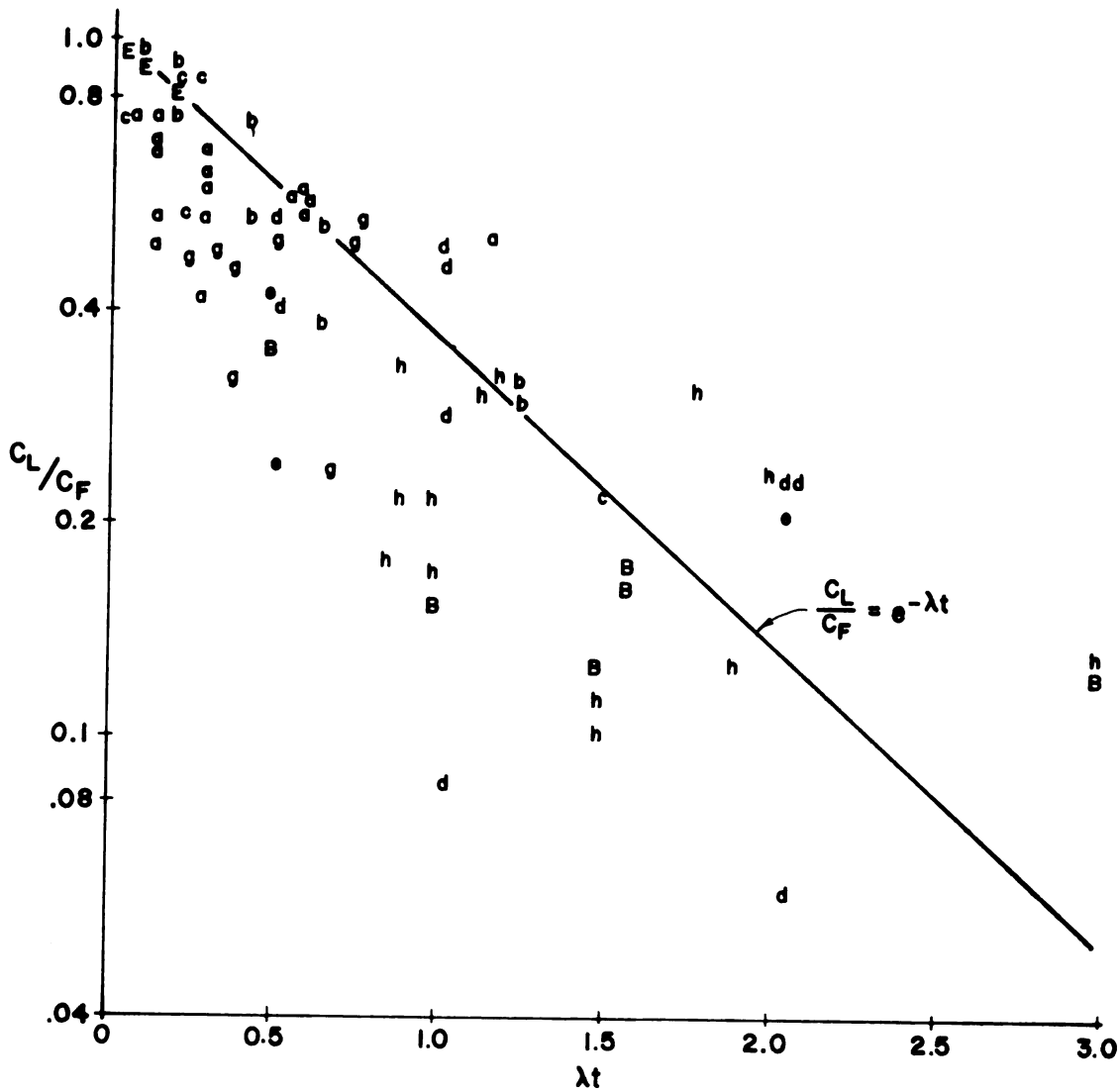
In truth, one cannot at this point differentiate between cell age and culture density. It may well be that $C_L/C_F = f(C_F)$ in which case the model is incorrect and C_L/C_F is not the well behaved parameter (independent of C_R) that is desired. Other experimental evidence will bear on this point, generally in favor of cell age as the distinguishing feature.

A quick appraisal of the tabulated values of λ indicates that treated Spirulina and Coelastrum are the fastest settlers while young Chlamydomonas and Anabaena

TABLE 12.--Recovery Time Constants.

	λ min^{-1}	90% confidence interval on λ	C_L/C_r at t=60min. (90% confidence interval in parentheses)
Chlorella	-.009806	-.00685 to -.0128	.555,(.465 to .663)
Oocystis (young)	-.001815	-.00157 to -.00205	.897,(.884 to .909)
Oocystis (old)	-.00828	-.00556 to -.0110	.609,(.517 to .716)
Scenedesmus	-.002263	-.00170 to -.00283	.873,(.844 to .903)
Coelastrum	-.0343	-.0242 to -.0444	.127,(.070 to .234)
Chlamydomonas (young)	-.00097	-.000642 to -.00119	.946,(.931 to .961)
Chlamydomonas (old)	-.00853	-.00349 to -.0136	.599,(.443 to .811)
Spirulina (untreated)	-.006408	-.00451 to -.0109	.681,(.607 to .763)
Spirulina (gas vacuoles collapsed)	-.03159	-.0205 to -.0521	.150, (.077 to .293)
Anabaena		Settling is imperceptibly slow.	

FIGURE 21.--Scatter in the data when compared with the exponential decay model. Table 13 is the code for data point identification.


1.0
0.8
0.4
0.2
0.1
.08
.04
C_L/C_F
0
0.5
1.0
1.5
2.0
2.5
3.0
λt
$\frac{C_L}{C_F} = e^{-\lambda t}$

are almost hopeless. The range of λ for the species is reassuring since the algae were chosen in order to obtain just such variety. Scanning the third column of numbers will give a feel for the concentration of algae remaining in the lean fraction after one hour. As can be imagined, the lean fraction is still quite green. The traditional method of presenting this type of data is to present a time series of photographs of 1 liter cylinders in which settling is transpiring. That method of display is impressive and photogenic when the upper fraction is almost clear ($i.e. \ \frac{C_L}{C_F} = 0$). But in algal sedimentation, after a reasonable settling time (one hour), the upper fraction is far from clear and the numerical data convey more information than would a photograph.

Figure 21 is a graph intended to display the scatter remaining in the data after the sub-populations have been identified. In one case a batch (5 points) of bad data was detected and rejected. The legend of symbols identifying the points on the graphs is as follows:

TABLE 13.--A Glossary of Symbols used in Graphical Presentation of Data

Chlorella	a
Oocystis	b
Scenedesmus	c
Coelastrum	d
Chlamydomonas	e
Anabaena	f
Spirulina (untreated)	g
Spirulina (treated)	h

(Old cells are indicated by an upper case letter.)

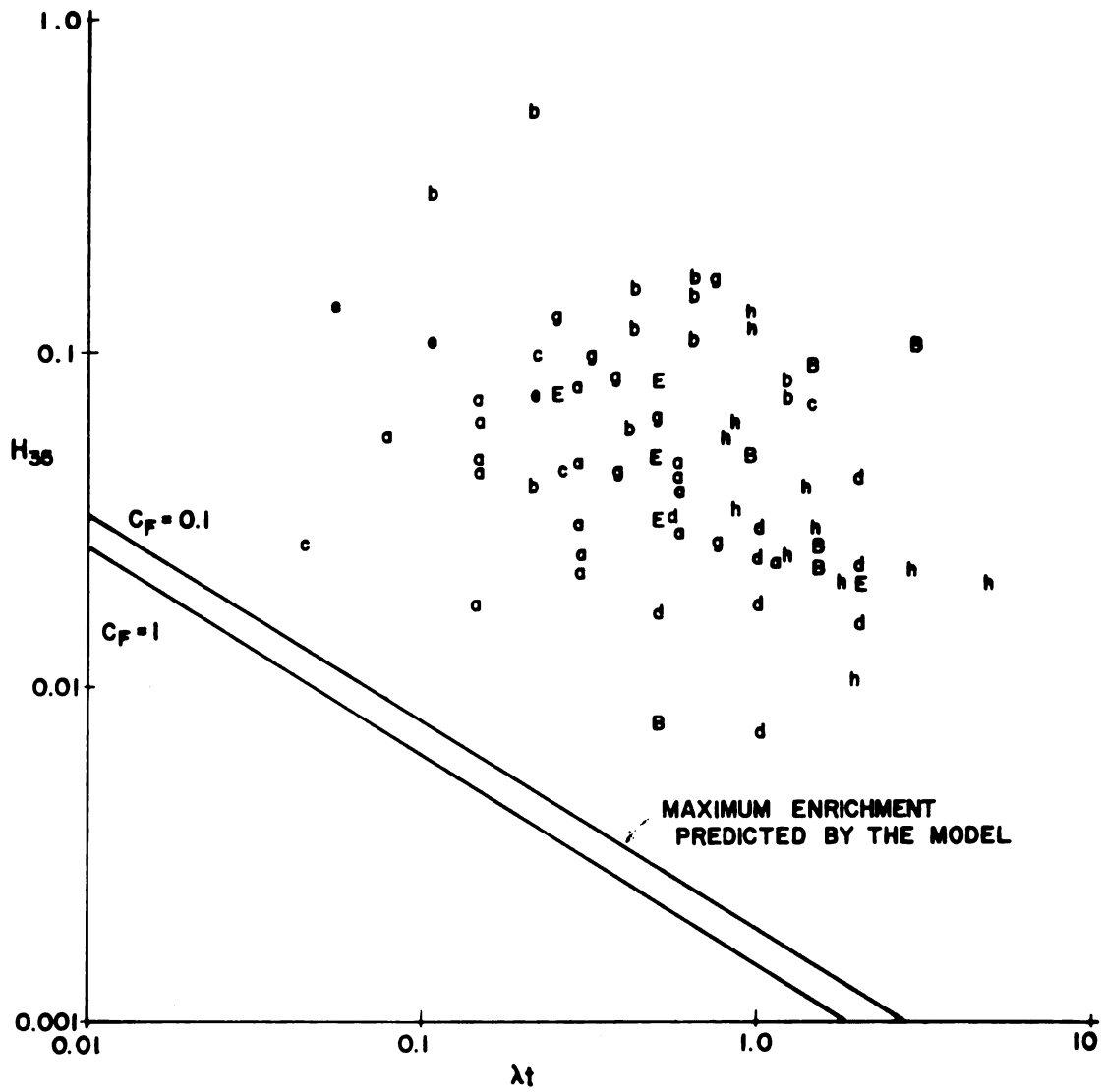
In studying Figure 21, observe that a deviation downward, one unit from the line $C_L/C_F = e^{-\lambda t}$ is larger to the eye than an upward deviation of one unit. One expects the data to appear further (by eye) below the line that it is above the line. But the number of points above and below the line should be better balanced. The algae are settling faster than the model predicts. But this should not be since the λ values were derived from this same data. The answer lies in a deficiency in the least squares method applied - as I have applied it - to the logarithm of the data. A point far out on the x-axis (i.e. a batch which was separated several hours after it was drawn) has a strong influence over the value of the least squares slope. For example, λ for the earliest fifteen

Chlorella separations (0 to 60 minutes) is-0.0128. When the last point (at 120 minutes) is added, the value of λ shifts to-0.00981 min^{-1}.

I tried the "old eyeball method" on a piece of log paper and including the last point, I chose a line which coincidentally gave λ as -0.0098 min^{-1}. Eyeball values for the other algae were in fairly close agreement with the least squares computed values. I decided the improvement in the scatter symmetry possible with other methods did not justify the search for those methods until the model was further examined. If all the data were "adjusted" to be more symmetric to my eye, all the λ's would increase by about twenty percent.

Figure 22 is a plot of the parameter H_{35} against dimensionless time. A trend can be discerned: the later the separation, the richer will be the rich phase - and roughly at the rate of increase predicted by the model. The problem is that the stochastic model predicts the attainment of higher densities earlier in the course of λt. A twenty percent change in λt will do little and a one milliliter overshoot in collecting the rich fraction moves most points vertically downward just a few percent. The difference suggests that the algal sludge is much fluffier than the close-packed arrangement I have envisioned. Reducing the value of C_{max} is possible only to the point where it is less than the density actually achieved in one

FIGURE 22.--H_{35} as a function of λt. H_{35} is a normalized reciprocal of the enrichment E. It is based on a maximum attainable concentration of 35g/l.


1.0
0.1
0.01
0.001
H_{36}
$C_F = 0.1$
$C_F = 1$
MAXIMUM ENRICHMENT
PREDICTED BY THE MODEL
0.01
0.1
1.0
10
λt

of the separations. The case for sludge compression is weakened by the occurrence several times of very high densities soon after settling has begun (i.e. those points which are near the operating region of the model). So it appears that the poorly defined interface between rich and lean is not the entire reason for rich fraction densities being less than their theoretical maximum value. If flocculation were a dominant factor, this would explain the fluffiness of the sludge since settled floc particles would have a high porosity. The density would probably have a time dependence something on the order of the trend shown in Figure 22. Notice that young Chlamydomonas and young Oocystis never get very rich (H_{35} small). Figure 21 suggests that they may be approaching a minimum value of C_L/C_F as well. Chlamydomonas's swimming is possibly the reason for its approach to a minimum C_L. Only Scenedesmus and Oocystis exhibit the classic hindered settling phenomenon. In the case of Scenedesmus the uppermost boundary (clear fluid above) moves down 4 cm in 2 hours (one-liter conical separatory funnel, 1/2 liter of culture). For Oocystis the interface moved downward at 5 mm per hour, with no further progress after 4 hours.

The conical geometry may be another reason for disagreement between the model and the data. Bridging of algal floc was observed on only one occasion. The model is derived for a cylindrical settling vessel; the

problems of removing the rich fraction are most easily solved using a separatory funnel. Note (Figure 22) that treated Spirulina starts to enrich after $\lambda t=1$. This is very likely an effect of the clumping phenomenon which will be discussed shortly.

It is scattered results such as these that are enough to discourage a designer from planning on sedimentation as a harvest method. Add to the scatter for an individual species the variables pH (not well controlled in my case, but occasionally observed), light (in outdoor culture), temperature, and nutritional variables and the sedimentation process in open culture becomes an unpredictable object.

Figures 23, 24, 25 are a time sequence, again in λt, of the batch sedimentation process on the C_L/C_F, H_{35} plane, a display suggested in an earlier section
The data do behave fairly well (i.e. in a group) which is surprising in view of the diversity present. Figure 26 showed the trajectory of the idealized alga obeying the stochastic model I set forth. (A good trick for bringing the trajectories out of the corner is to use $H_{35}^{1/2}$ or $H_{35}^{1/4}$ as the variable instead of H_{35}.) The important things to note are that recovery is the bottleneck in both the idealized process and the real process.

Recall that $C_L/C_F = (1-R)/(1-R/E) \approx (1-R)$ and as $C_L/C_F \to 0$, $R \to 1$.

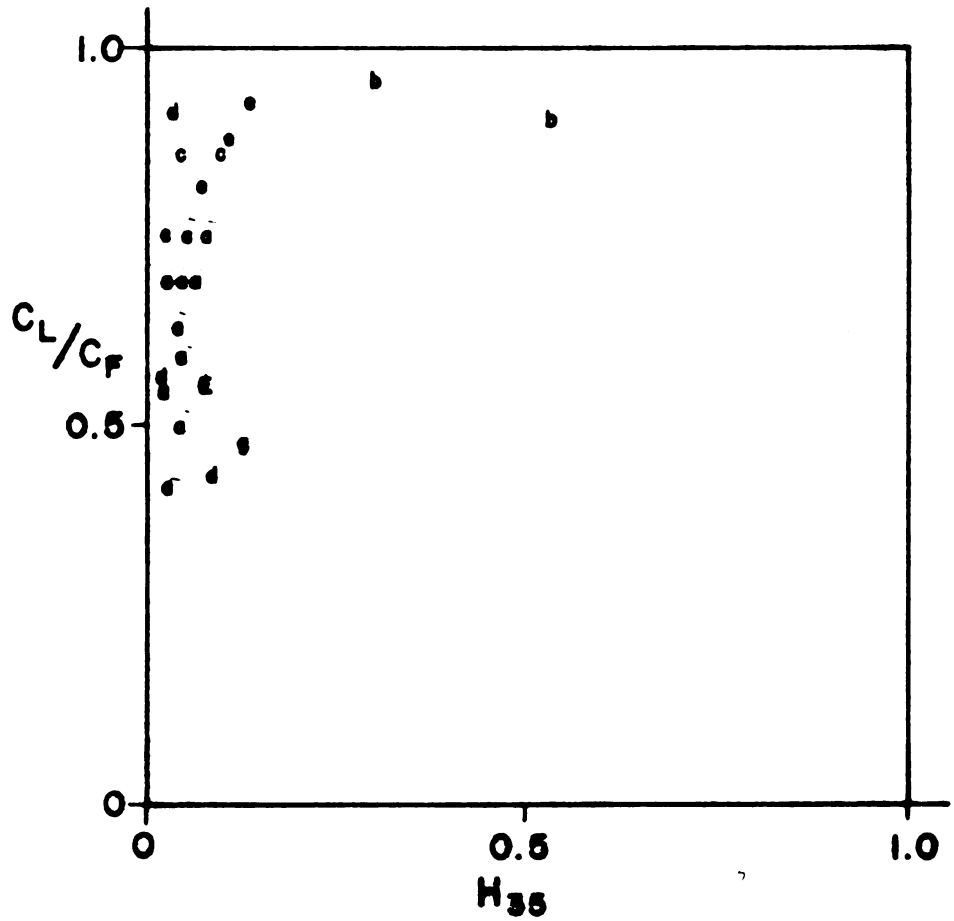


FIGURE 23.--A two-dimensional display of harvesting success. Sedimentation during the interval $0<\lambda t<0.3$. First of a time sequence.

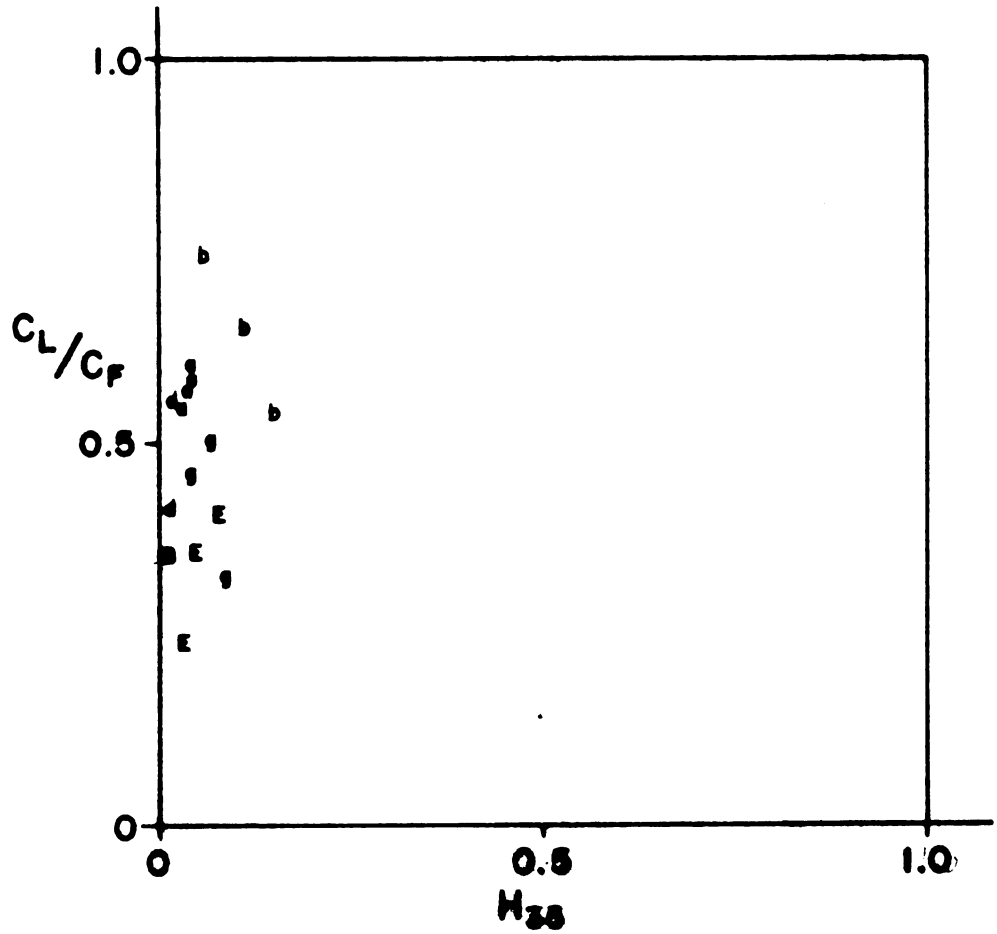


FIGURE 24.--Second of a time sequence. $0.3<\lambda t<0.6$

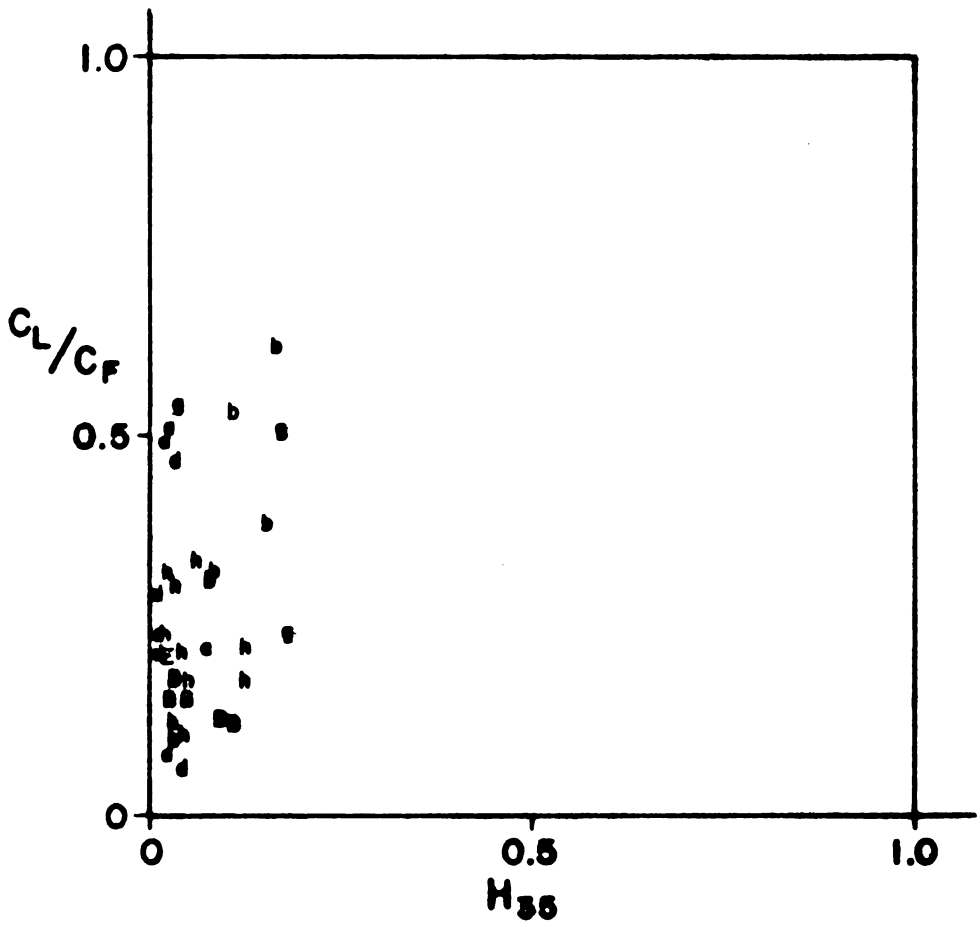


FIGURE 25.--Third and last of a time sequence. $0.6<\lambda t$

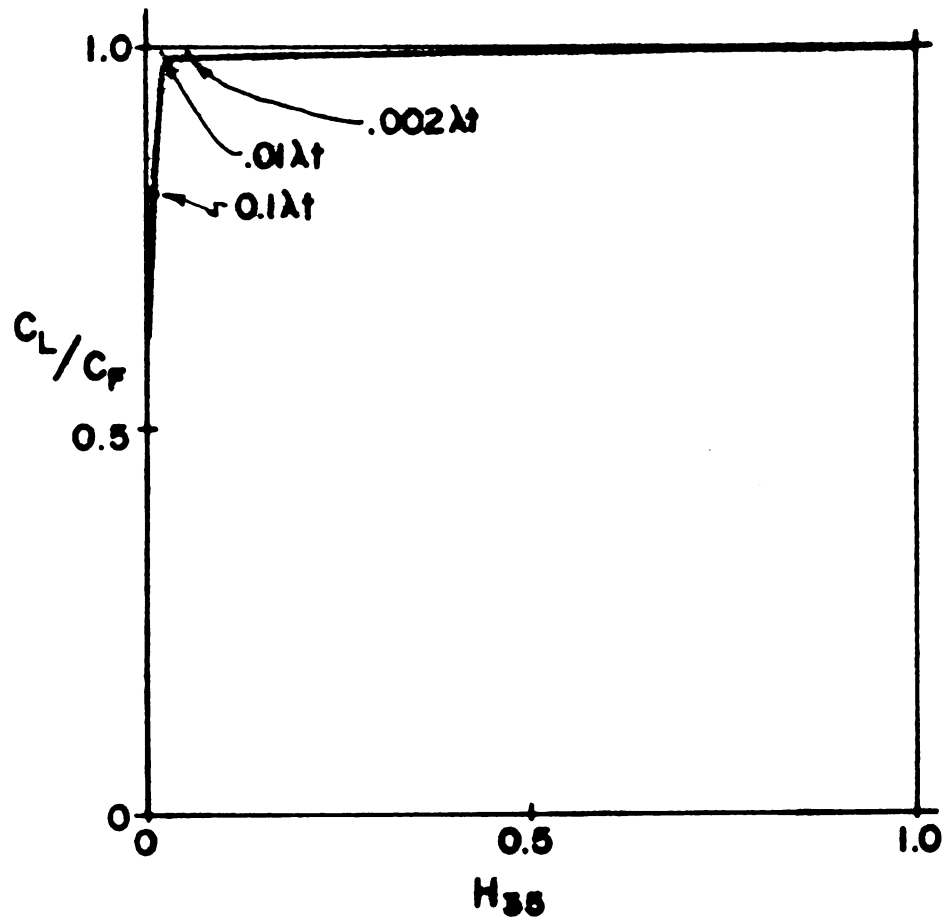


FIGURE 26.--Trajectory of an idealized alga which obeys the proposed model for sedimentation.

Enrichment takes place quite rapidly so that if sludge compression is operative it is perhaps not a dominant process until the recovery has come to a halt. The fact that enrichment takes place before recovery is fully underway is a fact which the model predicted.

The basic properties of the algae which would determine their non-flocculating behavior are the cell size and specific gravity. These are tabulated in Tables 14 and 15. The salient point in these tables is the difference in the individual cell properties for cells which come from different continuous culture densities. One still cannot claim that it is cell age and not some other factor responsible (say, surviving long dark periods in the dense shaded culture). But variation in sedimentation rates for a given species is almost certainly not an interfering effect of C_F (where C_F=X) on poorly chosen parameters (i.e. not independent). So the case is quite strong for explaining the different λ's (Table 12) on the basis of different types of cell within the same species. Carbon to nitrogen ratios were not the spectacular bit of added evidence I had hoped for: old Coelastrum ($\bar{\lambda}$=>36 hours), 7.7 versus 6.15 for young Coelastrum ($\bar{\lambda}$=10.4 hours). This change in C/N may be the phenomenon responsible for increased specific gravity with increased mean cell age (or whatever).

TABLE 14.--Size of the Algae

	culture condition	D_H µm	σµm	N	
Chlorella	0.60 g/l	2.95	2.02	120	} The mean values are different at 87% confidence level
	1.54 g/l	3.25	0.62	100	
Oocystis	3.0 g/l	16.8	2.9	25	Culture "old"
Scenedesmus	2.5 g/l	6.04		32	Measurements made on quadricellular colonies; adjusted to represent dimensions of unicells
Chlamydomonas	0.80 g/l	8.12	3.8	32	
Coelastrum (colonies)	0.96 g/l	36.7	12.9	98	} Means differ (99% conf.)
	2.5 g/l	50.8	7.6	100	
Coelastrum settling test	control	53.3	8.6	100	Specific gravity around 1.084
	first settlers	57.7	7.4	113	} Means differ (99% conf.)
	last settlers	49.5	11.7	117	

D_H is the hydraulic diameter defined as $(abc)^{1/3}$ where a, b, and c are the three orthogonal diameters of a spheroidal body. σ is the standard deviation and N is the number of observations.

Anabaena filaments range from 5µm to 1mm long, approximately exponentially distributed with mean 297µm and std. dev. 273µm. Filament diameter is 2.5-3.5µm.

Spirulina can also attain 1mm length. Filament diameter is 5µm; coil diameter is 15µm and the pitch is 1. The last two figures are quite variable in different growth media.

TABLE 15.--Specific Gravity of the Algae

	culture density X g/l	mean cell age (hours)	specific gravity	pH* med.	pH* grad.	π med.	π grad.
Chlorella	~1.0	16.5	(1.075-1.085)	6.5	8	8.5	2.0
	.8, floc	16.5	(1.090-1.100 floc.) (1.070-1.082 unicells)	-	-	-	-
	1.5	36.9	(1.095-1.107 floc.) (1.090-1.096 unicells)	7	8	12	6.8
Oocystis	.636	6.75	(1.095-1.100)	7	8	13	6.0
	2.635		(1.13-1.145)		8	11.45	6.2
	1.06	34.6	(1.105-1.120)	6	8	~14	6.2
	3.0	41.5	(1.102-1.138)	7	8	15.2	7
Scenedesmus	1.85	16.6	1.099	-	-	-	-
	0.96		1.099	5	10.2	~15	37
Coelastrum	2.5	<40	1.085-1.087	6.5	10.2	~15	37
	0.6	6.3	(1.072-1.080)	6	10.2	~15	37
	0.7	batch (culture)	(1.090-1.100)	-	-	-	-
		batch(after standing 4 hours)	<1.087	-	-	-	-
	0.95	11.6	(1.085-1.090) <(1.087 after standing 6 hrs.)	6	8	14	8.0

TABLE 15.--Continued

	culture density X g/l	Λ mass cell age (hours)	specific gravity	pH* med.	pH* grad.	π med.	π grad.
Chlamydomonas	.8	15.7	1.048	6	8	14.8	12.2
	.98	19.7	1.036				
	0.58	6.55	(1.035-1.040)	6.5	8	14.4	10.6
Spirulina (pressurized)	.743	24 hr. (batch)	(1.061-1.068)	11	11	439	373
	1 g/l batch (shaker cult.)		(1.050-1.065)	11	11	439	373
Scenedesmus	1.624	36	(1.131-1.149)	-	-	-	-
	0.792	7.15	1.100	-	-	-	-
	1.736	36 "senescent"	1.170	-	-	-	-
Anabaena			1.036	8	8	12.2	9.2

*pH is measured near the algal band in the density gradient. There is also a pH gradient with pH7 at the upper surface and running as high as pH10 at the bottom of the tubes.

A spherical cell of 10μm diameter must take on one-ninth its volume of pure water to change its specific gravity from 1.1 to 1.09. This amounts to a barely noticable three percent change in cell diameter. The last two columns in Table 15 show that the osmotic pressure in the silica density gradients was in all cases but one such that if the cell "remembered" the former osmotic pressure (in the growth medium), water would tend to be taken into the cell under the new conditions. The specific gravity measured in the gradients, then, was slightly lower than the specific gravity of the cell in the medium from which it was to be harvested. Thus I claim that the specific gravity of the cells is at least that value shown in Table 15.

Other features of Table 15 include the difference in specific gravity between Coelastrum that has been drawn fresh from the culture and Coelastrum that has been standing for 4-6 hours. The poor settling characteristics of the latter may be partially explained by the reduced specific gravity. Notice also that when Chlorella is in a flocculating mood (it was sporadic) the cells are more dense. This suggests a pH relationship with cell specific gravity, something that is known to occur in chloroplasts. (There is also a light induced size change). My attempts at amplifying the pH effect were only partially successful. To lower the Ludox pH from 8 required enough

acid to run the osmotic pressure up to 33 mOsm/Kg. I was unable to get a gradient with matched pH and matched osmotic pressure. Table 16 shows the effect which density gradient osmotic pressure had on the measured specific gravity of Oocystis cells grown in a medium of pH7.1 and osmotic pressure 14mOsm/Kg. The pH effect causes a slight reduction in specific gravity for osmotic pressures that tend to move water out of a cell acclimatized to lower osmotic pressure. A tentative conclusion is that a decrease of the same order of magnitude is expected at lower osmotic pressures which tend to drive water the other way. Consequently I must temper my statement guaranteeing that the specific gravity is at least the tabled value; it may be slightly less, depending on the as-yet-uncertain size of the pH effect. The effect might be studied by acclimatizing a culture to a more saline medium and then repeating all tests indicated in Table 16. One cannot be certain that the cells really have the same specific gravity in saline solution that they have in the medium with lower osmotic pressure. It may be possible to set up a density gradient using colloidal gold.

Sedimentation of Spirulina was much improved by first collapsing the gas vacuoles either by a pressure pulse or by exposure to ultrasonic action. The energy involved is nominal: with a 0.1% headspace over a batch (total volume, 100%), 36 joules per liter will

TABLE 16.--The Effect of Gradient Osmotic Pressure and pH on the Specific Gravity of Oocystis

π pH	pH<7.1	pH>7.1
<14		1.076 1.076 1.067
=14		1.086
>14	1.091	1.098

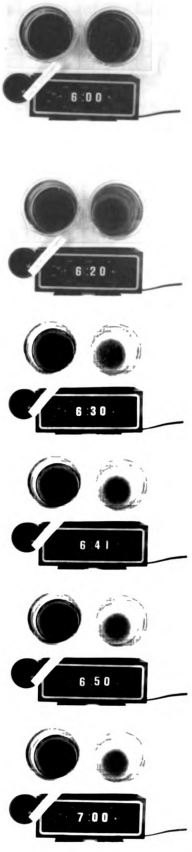
Oocystis was cultivated at pH 7.1, 14 mOsm/Kg.

collapse most (90%) of the gas vacuoles (Figure 27). I used tank CO_2 and ran the static pressure up to about 5 atmospheres, gauge. A dramatic color change occurred at this pressure which could not be produced at a lower pressure. For sonication, 22 watt-seconds was sufficient to treat 100 ml at 2.21 g/l dry Spirulina - or 220 joules/ liter.

Coelastrum behaves very well as a settler as long as there is no scouring (turbulence near the rich-lean interface). This alga can be resuspended with very little energy expenditure per unit volume. An important consideration in cultivating algae which settle well is the agitation power required to prevent unwanted harvesting in the growth chamber. At an agitator power input of about 1.1 watts per liter, for example, a Coelastrum culture-

FIGURE 28.--A one hour time sequence illustrating the clumping effect in Spirulina. To the beaker on the left has been added 5 mM 2,4-dinitrophenol.

FIGURE 27.--Spirulina platensis, light microphotograph. x100:
Right: with gas vacuoles
Left: gas vacuoles collapsed.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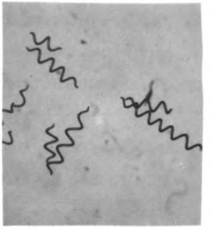

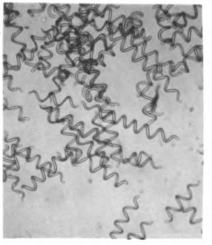

density gradient could be measured between the top and bottom of the fermentor vessel - 0.884 g/l at the top compared with 0.923 g/l at the bottom. When the speed was doubled, 400 rpm, power 8.8 watts per liter, the gradient was a little less but still pronounced - top 0.908 g/l, bottom .935 g/l. The bottom sample was taken 5 cm above the bottom of the jar; the top about 30 cm from the bottom. Evidently there was a rich layer below the five cm level which was sufficiently mixed at 400 rpm to increase the culture density of the 5 cm level from 0.923 to 0.935 g/l. The measurements were made within minutes of each other.

Using empirical relationships presented in Fair and Geyer (1954, p. 600) one can estimate the conditions under which Coelastrum must be cultivated if harvesting is to be prevented at the cultivation site. Using their equation 15-6:

$$v = \sqrt{(8k/f)g(\frac{\rho p}{\rho w} - 1)d} \qquad (70)$$

where v is the minimum velocity to insure scouring

k is a sediment constant which runs about 0.8 for thorough scouring

f is the Darcy-Weisbach friction factor (0.01 is a non-committal value)

d is the particle size

g is gravitational acceleration

ρp and ρw are the mean densities of particle and water respectively.

Taking 50μm as the diameter of the typical Coelastrum colony and taking its specific gravity as 1.08, then good scouring will take place at velocities greater than 0.158 m/sec. The fermentor agitation power and requirement of 1.1 watts per liter of culture in the growth vessel will maintain scouring velocity along 730 meters of travel in a sloped shallow channel (1m x 0.1m deep) such as might be designed for photosynthesis. Rapid settling can be a disadvantageous characteristic.

In the case of the other six algae, velocities less than 0.158 m/sec will be satisfactory since none of the others settles as fast as Coelastrum. The energy to prevent premature harvesting might best be considered a part of the harvesting cost in that it amounts to the cost of transporting the cells to the harvest site.

Spirulina, the other good settler, has gas vacuoles in culture. Only the pressurized or sonicated cells were good settlers; untreated Spirulina was not. Therefore harvest-preventing energy was not required.

Centrifugation

The sedimentation principle operates in the centrifuge, the difference being the shrunk time scale and the energy expended.

The terminal velocity of a particle settling in a laminar flow situation is

$$v = \frac{(\rho p - \rho m)\, D^2 \omega^2 r}{18\mu} \quad \text{or} \quad \frac{(\rho p - \rho m) D^2 g}{18\mu}$$

where ω is the angular velocity in radians per second and r is the radius at which the particle in its medium is being spun.

g is the acceleration due to gravity, μ is the fluid viscosity and ρp and ρm are respectively the particle and medium bulk densities.

Times taken to travel a distance x are, respectively,

$$t_1 = x \left(\frac{18\mu}{(\rho p - \rho m)\, D^2}\right) \frac{1}{g}$$

$$t_2 = x \left(\frac{18\mu}{(\rho p - \rho m) D^2}\right) \frac{1}{\omega^2 r}$$

and consequently $t_2 = t_1 \frac{g}{\omega^2 r}$

Now the centrifuge measurements can be compared with measurements made at 1xg (sedimentation).

The product of relative centrifugal force ($g/\omega^2 r$) and detention time (flow rate through the 400 ml volume centrifuge was measured) becomes the t value; (1-R) is equal to C_L/C_F since E is very large (and difficult to measure). The least squares routine for determining the exponential decay constant is then applied and the resulting information appears in Table 17. This table shows that the centrifugation process finds the algae all behaving more or less alike. This is perhaps why centrifugation is the most popular solution to the problem: it harvests everything equally well (or badly if

TABLE 17.--Recovery Time Constants Determined in Semi-continuous Centrifugation

	x g/l	λmin^{-1}	λrange (90% conf.)	λmin^{-1} at 1x g (Table 12)	t_r'**	$\frac{W}{V}j/l$
Chlorella, old	1.85 g/l	-.000965	(-.00069 to -.00124	-.009806	610	8398
Chlorella, young	.563 g/l	-.00265	(-.00155 to -.00374	-.009806	222	6386
Oocystis*	.592 g/l to 2.6 g/l	-.00326	(-.00270 to -.00382)	-.001815	333	5408
Scenedesmus	2.7 and .810	-.00309	(-.00252 to -.00365)	-.002263	44	5463
Coelastrum		not done		-.0343		
Chlamydomonas	1.08 g/l	-.000867	(-.000636 to -.00110)	-.000917 young -.00853 old	64	5568
Anabaena		separated Chlorella				
Spirulina	1.06 and 1.125	-.00254	(-.00204 to -.00307)	-.03159	747†	9109

** $t_r' = t_r g/w^2 r$

* failed to differentiate between young and old cultures

† pressurized at 5 atm. prior to centrifugation

expense is considered). The centrifuge values of λ did not differentiate between old and young cells of Oocystis. But old and young Chlorella were found to behave differently in the centrifuge - suggesting that old cells might be more difficult to harvest than young cells. The energy required for a retention time of one minute in the centrifuge to accomplish a one hour's separation at one-times' gravity is an interesting figure of merit. My data suggest that,

$$W/V = 5235 + 5.184\ t_r' \qquad (71)$$

(correlation coefficient .959)

where W/V is the energy requirement per unit volume (j/l), and t_r' is the scaled time parameter mentioned above ($t_r' = t_r g/\omega^2 r$)

The limitation on Equation 71 is $0.8 < t_r < 1.2$ minutes.

For a given value of recovery or its complement (C_L/C_F), one can determine the time t_r' required using the constants from Table 17 and equation (39); equation (71) then permits calculation of the energy per liter. The last column in Table 17 gives the energy required to do a one hour's free settling in one minute of centrifuge time. $g/\omega^2 r$ is assumed to be adjustable so that t_r can be set equal to one minute. This expression (71) was derived from data where t_r was always about 1 minute; it should not be used to determine energies where t_r will be greatly different from one minute.

Equation (71) suggests that a lowering of the energy requirement per liter of culture can be achieved through increasing r or ω or both. This would mean a larger centrifuge bowl or a higher speed machine - greater capital investment in either case. The larger machines would be more efficient, but the capital investment increases faster with centrifuge detention time than the decreased running costs can compensate.

Electroflocculation

Electroflocculation also has an accelerating effect which can be described in a manner similar to that used for describing centrifugation. Sparse and scattered data make the energy prediction somewhat uncertain. Figure 29 displays the values of C_L/C_F after treatment by the aluminum electrode flocculant and after having settled 60 minutes. The trend is some sort of law of diminishing return as energy is increased.

It can be argued that ampere-seconds is the proper variable instead of electrical energy. Constant current was applied, the dosage being adjusted by varying the length of time the current was on. The electrode spacing was relatively constant as was the solution conductivity. Therefore the ampere seconds and joules are very nearly proportional.

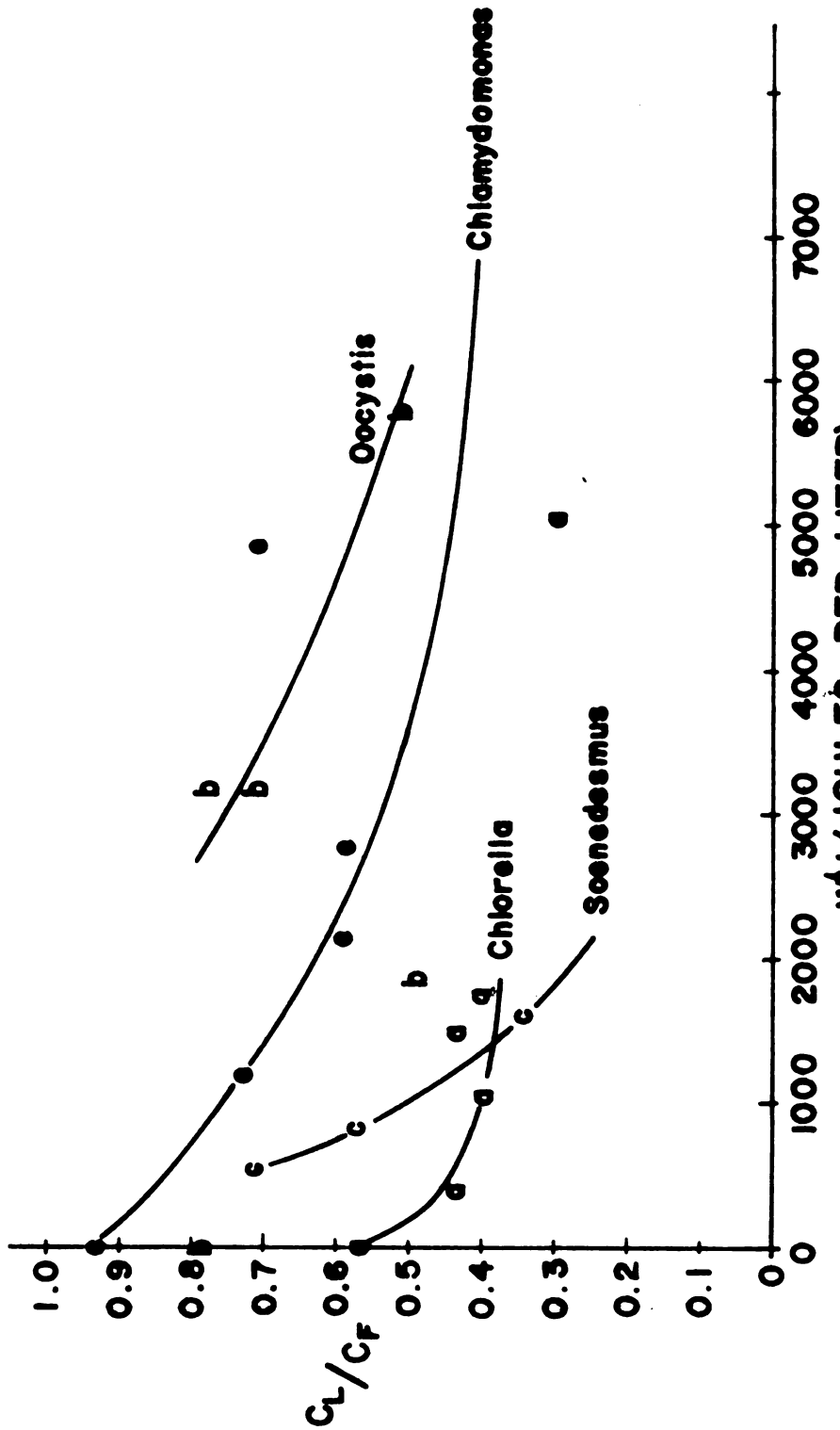

FIGURE 29.--Sedimentation enhancement due to electroflocculation.

If an exponential curve is allowed to represent the data of Figure 29 we have

$$C_L/C_{F_{60}} = e^{-60}\ e^{-\alpha(W/V)\ 60} \qquad (72)$$

where α is a constant experimentally determined for each alga.

I assume that the expression is valid for times other than just where α is evaluated.

$$C_L/C_F = e^{-(1\ +\alpha(W/V))\lambda t} = e^{-\lambda' t} \qquad (73)$$

To achieve the one-hour recovery (or C_L/C_F, its complement) in, say, 1/4 of the time,

$$(1 + \alpha(W/V))\lambda\frac{t}{4} = \lambda t$$

or $$1 + \alpha(W/V) = 4$$

$$(W/V) = 3/\alpha$$

Examples are tabulated in Table 18 along with computed values for the algae examined. The model does fairly well in predicting C_L/C_F values at 120 minutes which compare favorably with the data (± 15%). From Table 18 one can conclude that electroflocculation is not a very good method for harvesting Oocystis; Scenedesmus and Chlamydomonas seem to be helped by the treatment and Chlorella seems to be "difficult". The settled material appears to be on the average neither richer nor leaner than settled material from an untreated batch.

TABLE 18.--The Acceleration of Settling Caused by Electroflocculation

	α(l/j)	$(\frac{W}{V})^{*}_{60}^{80\%}$	$(\frac{W}{V})^{\dagger}_{30}^{60}$ joules/l	Comments
Chlorella	+.0000373	10200	26800	
Oocystis	.0000265	16900	37700	Old cells
Scenedesmus	.000556	2960	1800	
Coelastrum				No differences between flocculated settling and normal settling, i.e. Coelastrum did not respond.
Chlamydomonas	.000151	26900	6620	Young cells
Anabaena				Separated but did not settle; no basis for comparison.
Spirulina				insufficient data

†Energy input per unit volume to achieve a 60 minute equivalent (C_L/C_F basis) settling in 30 minutes.

*Energy input per unit volume to get C_L/C_F 60 down to 0.8 of its value-without-treatment in the same hour's time.

Filtration

The classical mathematical descriptions of filtration so well describe my data that there is little point in dabbling here in the mathematics. The method of Ruth (1933) is to measure filtrate volume (V) as a function of time (t). t/V is then plotted against V and the data describe a straight iine which extends from (near) the origin outward with a positive slope. At a point not too far out, the algae will start to clog the filter and the slope of the line increases abruptly. The slope of the first part of the line is related to the resistance of the filter cake to flow through it. Eckenfelder and O'Connor (1961, p. 276) give it as specific resistance

$$r = \frac{2bPA^2}{\mu c} \text{ (cm/g)} \qquad (74)$$

where P is the pressure drop across the filter, A is the filter area, b is the slope of the graph, μis the viscosity of water and c is the weight of solids per unit volume of filtrate.

(For most typical algal harvests, c works out to be approximately $C_f/1000$ where C_f is the grams dry algae per liter in the incoming suspension). Table 19 lists the specific resistances of the algae. Coelastrum is by far the most filterable. The specific resistance says nothing about how long it will be until the filter clogs. Anabaena was notorious in this respect; it would filter slowly, and then suddenly no more filtrate could be drawn

TABLE 19.--Filterability Assessment of the Several Algae

	C_F(g/l)	specific resistence* (cm/g) x 10^9
Chlorella	~.5 g/l	38300
Chlorella	~ 1 g/l	10700
Oocystis (y)	< 1 g/l	466
Oocystis (old)	> 1 g/l	266
Scenedesmus		928
Coelastrum	.5 g/l	12.6
Coelastrum	> 1 g/l	4.0
Chlamydomonas	~.5 g/l	190000
Chlamydomonas	> 1 g/l	310000
Chlamydomonas ("Palmella")	.58 g/l	23800
Anabaena NO_3 (y)	.5 g/l	55600
Anabaena NO_3 (old)	1 g/l	29400

All tests were performed at 76 mm Hg absolute pressure in the vacuum flask, 3μm pore size membrane filter.

*Single measurements only. The differences among the species are the significant factor to note.

through - a matter of five minutes on the small filters I used. Coelastrum was the opposite; filter cake an inch thick was not uncommon. The difference in filtration specific resistance between the motile swimming Chlamydomonas cells and the sedentary Palmella-stage cells is quite dramatic. A mixture of the two cell types filters quite rapidly for a few seconds but then the filter clogs quite abruptly. Undoubtedly an interesting mathematical analysis could be undertaken on the subject of filtration of two species, one a high resistance species, the other a low resistance species. The reader is spared such an analysis in favor of the common sense hypothesis that in the mixed population, the number of filter clogging motile cells did not accumulate enough to clog the filter for the first ten seconds. Proof that the Palmella stage was indeed a stage and not a contaminant species was undertaken. A single 5-cell clone of Palmella type cells was withdrawn from very dilute suspension using a homemade micromanipulator. This was then cultivated in a shake culture and produced a good population of motile cells. I also observed (once) a Palmelloid clone break up into four motile cells which then swam away. The reddish eyespot characteristic of Chlamydomonas was also visible in the non-motile form. Note here the couple of instances in which old cells filtered more easily than young cells (Chlamydomonas and Anabaena). Note also that they are

the two worst filterers. The more usual case is represented by Chlorella, Oocystis and Coelastrum and I submit that old cells filter more easily than young cells.

By way of comparison of numbers, Trubnick and Mueller (1958, referenced in Eckenfelder and O'Connor, 1961) reported 264×10^9 cm/g (units changed, r adjusted for compressibility) for digested and activated conditioned bacterial sludge from a wastewater treatment plant. If that is any indication of an economic limit for vacuum filtration, it means that only Coelastrum and possibly Oocystis (old) would be eligible for this method. Here again the range represented by the algae is impressive and it certainly makes a case for unseating Chlorella as the typical alga.

The energy requirements for filtration are nominal. At constant pressure (the manner in which my tests were conducted).

$$\frac{\int P dV}{\int dV} = P = \text{Energy per unit volume}$$

The pressure just happens to equal numerically the energy required per unit volume. This comes to 0.9×10^5 n/m^2 (or nm/m^3)

W/V = 90 joules/liter and is independent of the filter cake resistance. The pump inefficiency is another matter. If maximum power is being transferred, then the maximum power theorem puts the pump dissipation

at another 90 j/l. So the job gets done for around 200 j/l exclusive of backwashings and investment.

Electrodecantation

The shape of the filter clogging curve is shown in Figure 30. Electrodecantation data are plotted to the same scale. The straight line continued past 70 minutes before it was found necessary to flush out the enriched algae from the collection chamber. Longer filtration runs is the advantage claimed for this system. Chlamydomonas and Chlorella turned yellow-brown in the chamber - quite possibly because of the excessive heating. Current densities of 300 ma/cm^2 at field strength 33 v/cm give a power density of 10 watts/cm^3. Although the principle of operation is supposedly removal of the negatively charged algal cells from the filter membrane surface, this is only part of what occurs. Figure 11 shows the algal harvest slumped down (gravity) and some little bit attached to the electrode chamber membrane. Filter cake formation is effectively prevented. The extent of migration to this last mentioned membrane is evidence for the electrophoretic effect expected. Movement of filtrate through the filter and filter cake is driven by the electric field. Electrokinetic pumping is the major effect: if the electric field is shut off, flow virtually stops (even with the 0.15 m hydrostatic head forcing fluid through the filter). Reversed polarity causes reversed

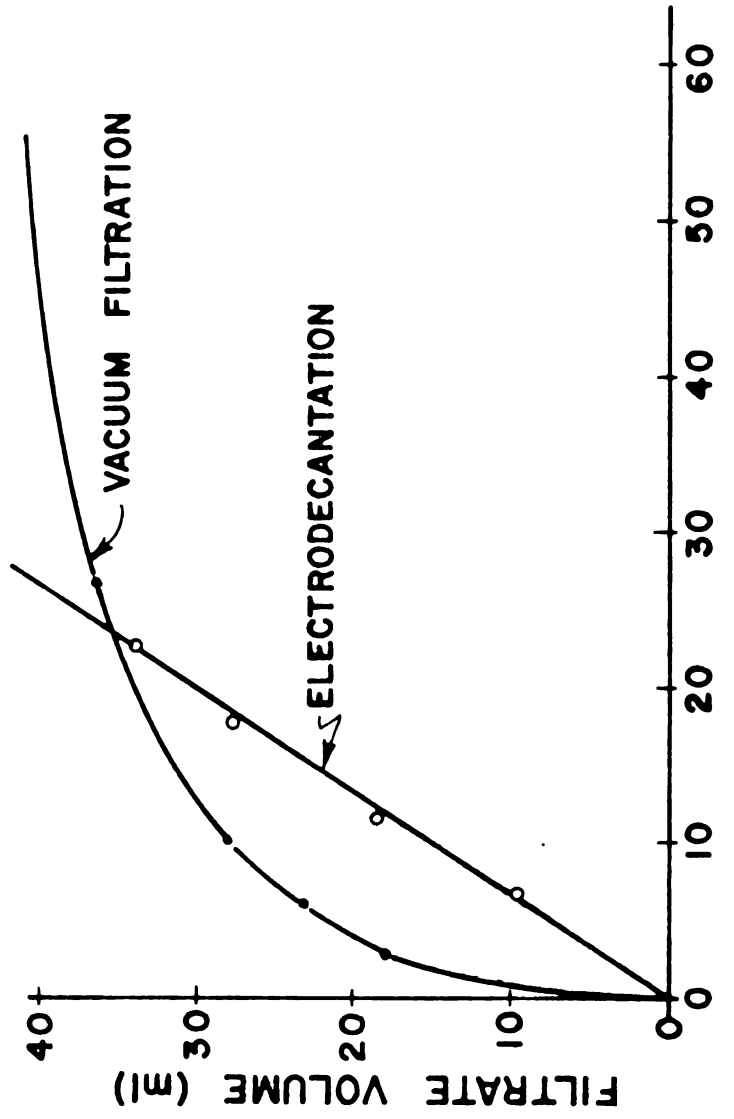

FIGURE 30.--Vacuum filtration and electrodecantation of Scenedesmus (1.6 g/l). Filter areas are approximately equal, 3.0µm membrane filters were used.

pumping. I.e., after operation for one half hour, the apparatus is essentially electrokinetically pumping fluid through the clogged filter. Table 20 presents the results of my experience with this apparatus. The dismal energy consumption was difficult to accept. Electrolyte was changed to Na_2SO_4 so that O_2 would be evolved (rather than, say Cl_2 from NaCl) in the hopes of recovering energy. The amounts of H_2 and O_2 generated are small; the recoverable energy from 9650 joules spent amounts to only 214 joules.

In both the filtration and electrodecantation process, recovery is complete (i.e. $C_L/C_F = 0$). E (and H) varies depending on the frequency and extent of backwashing.

Cooper et al. (1965) worked out a relationship for the energy requirements of electrokinetic filtration/pumping. They conclude that electrical conductivity of the water is the single most important factor in determining power requirements. As an example they predict an energy requirement of 10000 joules per liter for a water having a conductivity of 300 μmho/cm in a unit with a flow rate of 0.07 ml/min per square centimenter of filter area. The figure is directly proportional to conductivity and flow rate. They claim, moreover, that this is the upper limit for an economically interesting water purification scheme (21¢/1000 gal., U.S. 1972).

TABLE 20.--Electrodecantation

Filter area 7.5 cm^2; Electric field ~33 v/cm.

	filtrate rate ml/min	feed concentration C_F (g/l)	conductivity µmho/cm.	joules/liter feed stream	rich rate ml/min	joules/liter adjusted**
Chlorella	3.13	.757	1450	665000	0.8*	11700
Chlorella	1.32	.757	1450	113400		4750
Oocystis	2.99	2.51	1046	323000		27800
Oocystis	0.50	2.51	1046	0		0
Scenedesmus	1.52	1.62	†	570000		49400
	0.71	1.62	†	0		0
	1.53	1.02	386	173000		46400
Coelastrum	not done, filters too easily		†			
Chlamydomonas	3.11	0.98	†	407000		9680
	0.40	0.98	†	135000		24900
Anabaena (NO_3)	4.0	1.00	2750	540000		7730††
Spirulina	σtoo high. Works on washed & resuspended algae in tap water.					

†All Schultz medium. Probably 1000-1400. †† mobility value questionable on Table 19. Use 1.5.

*To flush the enriched fraction out and keep the algae and apparatus cool, a volume of about 25% of the filtrate was found to be a minimum. Thus $C_F/C_F = 5$.

**Adjusted for Q = 0.07 ml/min/cm^2, µ= 1.5µm/sec/v/cm and σ = 300µmho/cm.

It should be apparent that by reducing the flow rate - either by slowing the flow or by investing in larger filter areas - one can reduce this cost still further for a water of the same conductivity. For comparison purposes the last column in Table 20 gives the joules-per-liter figure adjusted for a flow rate of 0.07 ml/min-cm^2. Evidently my cell is less efficient than the one used by Cooper et al. My chamber thickness was one centimeter whereas theirs was 3.5 mm giving them a higher electric field for the same applied voltage. Reducing the chamber thickness also increases the current density for a given fluid conductivity. Too narrow an aperture would be a source of trouble should particulate matter become lodged. In terms of energy use there seems to be little sense in reducing chamber thickness. A source of variation among the energy figures for the various algae might arise from the electrophoretic mobilities of the cells. Electrophoretic mobility appears in the expression for energy given by Cooper et al. Table 21 gives the electrophoretic mobility of the several algae in their growth media, but it is difficult to concoct a connection with Table 20.

Other Methods

Coagulation of Anabaena. The high electrophoretic motility for Anabaena may be related to the high viscosity of the mucilagenous slurry which seems to be one of the hazards of Anabaena cultivation. The filaments moved

TABLE 21.--Electrophoretic Mobility

	culture density	electrical conductivity of the algal culture σ	pH	M (μ/sec/v/cm)	standard deviation	
Chlorella	0.7	2600	6.5	-2.138	.369	(flocculating)
	0.8 g/l		5.5	-2.415	.420	(unflocculating)
	1.5 g/l		7	-0.978	.191	(flocculating)
Oocystis	0.9		6.5	-1.281	.196	
	1.0		6.6	-1.359	.254	
	3.0	1040	6.5	-1.154	.373	
Scenedesmus		386				
Coelastrum*						
Chlamydomonas	.5		6.5	-1.930	.979	sonicated to break flagella
	.8			-2.095	.693	
Anabaena (NO_3)	2.0	2750	7.7	-3.530	.374	
Spirulina		20900	10	σ too high		
Red blood cells			7.4	-1.8		norm is -1.31

*Sank in cell, no measurements possible

electrophoretically with almost uniform velocity - the standard deviation being such a low percentage of the mean illustrates this point.

The behavior of mucilage without algae is strange. The aluminum-electrode treatment seemed to stimulate coagulation of Anabaena but no subsequent settling occured. Instead, the mass of cells shrank into the center of the separatory funnel leaving a clear yellowish solution above and below. Boundaries were well defined and not disturbed by slight movement. Upon investigating this electric-current effect in a centrifuged supernatant (5000 x g for 10 minutes), a sheath was found surrounding both electrodes. Around the cathode, after 500 ma for 20 sec, there was a very thin skin formed which did not cling to the electrode but remained in the liquid after the electrodes were removed. Around the anode was a large coagulum of about 1 cm radius. This was also left in solution but some of the clear coagulated material stuck to the electrode. The cylindrical coagulum in the beaker could be moved around and even partially lifted out of the liquid before it would break. These same phenomena evidently took place in the harvested Anabaena but could not be seen in such detail. It is significant that the yellowish fluid which was separated from coagulated Anabaena would not produce the electrode sheaths when current was applied. This suggests that the mucilage is responsible for the coagulation of

Anabaena and that it remains with the cell-rich mass. A recovery of 1 after 24 hours was easily obtained but the rich fraction was quite dilute (E ≈1.5).

Uncoagulated Anabaena supernatant exhibited pseudoplastic behavior. Viscosities are as follows:

For Anabaena culture (1/8 Allen and Arnon, 1 g/l KNO_3, 0.91 g dry algae/liter) the viscosity ranged from 140 to 25 centipoises as viscosimeter speed varied from 6 rmp to 60 rpm.

For the centrifuge supernatant, the viscosity ranged from 35 cp. to 7.2 cp. over the same viscosimeter speed range. After electro-coagulation, the viscosity measurement was not meaningful becuase of the inhomogeneity of the solution.

Tube-settling, Anabaena. Anabaena was a particularly uncooperative alga to try to harvest, having the lowest specific gravity and a high viscosity, too. It settled only when it was grown in dilute medium (1/8 A & A) and only then when the culture density was around 0.6 g/l - 0.8 g/l. and the algae were grown on a KNO_3 nitrogen source. Because of the low growth rate, I had insufficient material to examine N_2-fixing Anabaena properly. This should be done since the possibilities for unialgal open culture are best under those conditions. The NO_3^- cultures eventually were shared with Chlorella. At one point in the Anabaena cultivation (continuous, 0.9 g/l,

dilution rate 0.007 liter/liter/hour), I noticed the "formation of excellent floc", as the literature would phrase it, in the tygon tubing leading out from the harvest pump. The tubing is 10 mm inside diameter, 2 meters long and leads upward at 45 degrees to the collecting vessel. The algal culture was in the tube for two hours, the tube was agitated once each hour. Apparently the detention time and culture density were just matched for effective operation of the harvest line as a tube settler. This was the most promising result with Anabaena. At a higher tube dilution rate (and both lower culture density and higher culture density) floc formation did not take place in the tube and there was no settling in the collection vessel. So a promising method does exist but its design and operation may be critical. Perhaps a combination of the electrical current (or is it Al+++) coagulation and the tube settling effect would be a good solution to this problem.

Predation by Daphnia seems possible only with five of the species tried (all green algae - or is it all non-filamentous?). Table 22 shows the grazing rates by starved Daphnia over a 48 hour period. The initial grazing rate might have been higher but that experiment is left to the future. In brief, Scenedesmus and Chlamydomonas appear to be the most suitable algae for Daphnia consumption. What is surprising is that Coelastrum was eaten at all since the colonies get so large (almost none smaller

TABLE 22.--Grazing by Starved Daphnia

	Consumption per daphnid
Chlorella	.392 µg/hr.
Oocystis	.729 µg/hr.
Scenedesmus	1.460 µg/hr.
Coelastrum	.417 µg/hr.
Chlamydomonas	1.140 µg/hr.
Anabaena	avoided*
Spirulina	medium too saline †

*Daphnia and algae could not be separated. They were caught in the mucilagenous mass.

†Daphnia dies immediately.

than 25µm). The Daphnia must have found some loose single cells or else they have the ability to chew the colonies up. The low grazing rate for Chlorella may be connected with cell age (2-week-old shake culture); Mullin (1963) found that Daphnia would not accept old Chlorella cells. An interesting search for more suitable grazers might turn up one which could eat more than 1 µg per hour or could compress the algae into large fecal pellets in a short time.

Clumping in Spirulina. In addition to the passive behavior expected of the algae in sedimentation, filtration and the like, certain of the algae can be induced to participate actively in the harvesting process. Spirulina

is an example. Spirulina, being motile only when in contact with a solid substrate must first be brought into contact either with a surface or with other algae. Walsby reported a clumping effect (Walsby, 1968b) in Anabaena which I noticed occurring in Spirulina. I noticed it only in cases in which I had enhanced settling by collapsing the gas vacuoles (by sonication or pressurization as described earlier). The algae promptly settled and then the rich algal material in the bottom of the beaker withdrew from the sides at an impressive rate. The pellet formed and reduced its volume to about 1/8 of its settled volume in 1 hour. Walsby related this to movement and reported some mucilage rings which I was unable to find on Spirulina. Walsby stopped the clumping by adding an unspecified amount of a detergent called Teepol. I found that an American detergent (Alconox, .1 g/l) stopped the clumping. I also found that 0.005 Molar 2, 4-dinitrophenol (DNP) poisons the clumping phenomenon at room illumination levels (probably "dark" throughout most of the pellet). Since gliding motility has been convincingly linked to oxidative phosphorylation in Oscillatoria princeps by Halfen and Castenholz (1971), poisoning of the clumping effect by DNP in the "dark" connects the clumping effect to gliding motility. Figure 28 shows a time sequence of the clumping phenomenon. The resulting pellet is coherent enough to be caught and held

in the hand when the beaker is emptied. The clumping phenomenon apparently takes place only at culture densities greater than about 0.2 g/l (Walsby, 1968b) (my estimate based on Walsby's cell counts). In the harvesting method I propose, the pressurization of Spirulina and subsequent rapid settling has the effect of rapidly exceeding whatever critical density might be needed (I did not attempt to find the critical density). Then clumping proceeds for the next half hour and the two fractions are separated. I suggest that this method is a simple and effective alternative to the vacuum filtration now employed in harvesting Spirulina (Clément, et al., 1968). The lean stream from a clumped separation might still contain some filaments. These would perhaps be returned with the liquid to the culture with make-up nutrient added. If filtration of the lean stream seems desirable, then the ultrasonic method of collapsing the gas vacuoles is not as good as the pressure method since the lean-stream from the sonicated process consistently clogs the filters I have used. The energy used for pressurization is also less per liter than ultrasonic energy which would accomplish the same task.

Clumping may be the pehnomenon which, in nature, would be termed "mat formation". Moss (1968) describes a 2 mm thick mat of Arthrospira jenneri. He also mentions a "surface aggregation" for the "planktonic" Arthrospira platensis. Clumps forming on the bottom of ponds could

be borne afloat by trapped O_2 gas bubbles if any photosynthesis were taking place. It is probably the clumped algae that were netted by the Aztecs; I cannot imagine a net being effective on any other planktonic (free floating) form.

Phototaxis in Chlamydomonas. Chlamydomonas is also motile in a different way - it swims. I attempted to get Chlamydomonas to swim phototactically into a region where the algae would be harvested in a rich stream. I consider this to have failed perhaps because I expected too much. The experiment was inspired by discovery of a rich culture of Chlamydomonas sp. in a puddle in a pig pasture. The algae seemed to be exclusively on the surface of the muddy (!) water. I later (in the laboratory) got an impressive phototactic response which might have amounted to a recovery of 0.7 and an enrichment of perhaps 1.5 after 2 hours. The cultivated Chlamydomonas however, were variously motile and in their Palmella stage. Because of this only a fraction of the cells could have been swimming. At any rate the enriched fraction which collected never appeared worth separating. It was at most a few mm deep at the top of the illuminated cylinder. Perhaps continuous removal would have permitted continuous replacement from the population below. So much more algae settled than swam to the surface that I did not pursue the phototactic effect further. Healey and Myers

(1971) did, however, actually use this principle to enrich Chlamydomonas in the non-motile cells which they wanted.

Surface attachment. Chlamydomonas uses its flagella effectively in attaching to surfaces. Of all the algae tried Chlamydomonas attached best. Sonicated Chlamydomonas cells did not adhere to the surfaces as well as unsonicated cells. Sonication increased the proportion of non-motile cells in the suspension. None of the other algae stuck really well to the surfaces at the withdrawal rate used. Coelastrum stuck to stainless steel, glass and polycarbonate but gentle rinsing removed it so that a layer never accumulated. Anabaena and Spirulina did slightly better than the others (save Chlamydomonas); this is primarily because of the mucilage. Spirulina mucilage sticks to other Spirulina mucilage better than it sticks to anything else tried. Spirulina in shake cultures will grow to what may be a critical density at which point it clumps. A day later the entire blob of algae has spread itself thinly around the glass at the water surface where it is constantly re-wetted by the sloshing action of the shaken flask. The medium is virtually clear of algae and a second growth beings. It is this phenomenon which I had hoped to exploit; it apparently depends on clumping which in turn may cause or be caused by the secretion of mucilage.

Chlamydomonas adhered to polyethylene better than to other materials tried. The Chlamydomonas which I found in the puddle was in a surface layer which behaved like an oil film; the Chlamydomonas could not be drawn into an eyedropper, but remained in the surface film on the outside the eyedropper. I harvested several milliliters of very rich culture in this way. I can only assign that experience to the presence on the puddle surface of a layer of stearic acid or some such substance common in animal wastes. Whatever the substance, it behaved like an oil film and the algae seemed to prefer living in it or attached to it. Chlamydomonas is known to thrive heterotrophically on acetate among other things. The point to be made is the possibility for growing algae in an aqueous dispersion of a non-aqueous liquid in much the same way the yeast Candida lipolytica is grown in diesel fuel, then taking advantage of the surface tension differences to harvest the cells. But this is as close as I can come to seeing a future for the economic exploitation of surface attaching properties of the algae I tried. The method of Sălageanu (1970) is one of avoiding the harvest but it is appropriate that he chose to work with Chlamydomonas on polyethylene bands. I can confirm his good judgement in selecting that combination; I have little else to offer but negative results.

CHAPTER VI

SUMMARY

Surveying the situation, some harvesting methods are energetically expensive compared with others. In joules per liter of culture processed, the methods are (from least to most expensive, feasible only) sedimentation, filtration, centrifugation, electro-flocculation, electro-decantation.

The other factor to consider is the capital investment in acquired equipment. This can be roughly conveyed by stating the detention time, which for a given flow rate determines the volume held by the apparatus. These are (from least to most expensive, i.e. shortest retention time to longest) centrifugation, electrofiltration, filtration, electroflocculation, sedimentation. The centrifuge costs more per minute of detention time than a filter so the sequence may not represent dollar costs. The reasons for excluding other methods such as predation is that they fall too low on one or the other of these lists. This is not to say they should be excluded from the list. They should perhaps be included as another category. Feasibility implies a satisfactory state of

the vector (R,E) or (C_F/C_L, H). This can also be expressed as the single parameter G. The trajectory of a filtration harvest approaches the origin from the wrong direction making Rony's ε an unsatisfactory single parametric description of the quality of the harvest. The index G, by the way, may be extended to other harvesting or sorting operations commonly performed in agricultural operations. If a term can be added to account for crop damaged by the harvest machine, it would have wide application.

The difference in filtration speed between Chlorella and Coelastrum gives an indication of the improvement in algal harvesting to be realized in selecting the proper species. Compare this improvement with that of increasing the energy efficiency of a centrifuge from fifty percent to ninety percent. The orders of magnitude filtration resistance change is very impressive and points toward the type of breakthrough needed to inspire agricultural acceptance of algal cultivation. Unless a totally new concept for separating micro-algae from solution is discovered, the advantage lies with those investigators who would examine new species.

If an alga were presented for evaluation of its harvestability, there seems to be no single method which would give a certain answer. For example, a measurement of Chlamydomonas size and filtration resistance could be made in a matter of minutes. But only the person who

lived with a Chlamydomonas culture would have the inspiration to also examine the palmelloid stage of this creature (Motile Chlamydomonas has a filtration specific resistance of around 200000 x 10^9 cm/g. The Palmella stage is 24000 x 10^9). Size is the single most important factor in determining terminal velocity for an estimate of sedimentation behavior. Based on size and specific gravity, however, one would think the recovery time constant for Oocystis would be much better (i.e. larger) than for Chlorella -which it is not. Add to such facts the uncertainties of nutritional conditions, continuous cultivation and cell age, more than a nodding acquaintance with the species in question is needed before its potential as a harvestable crop can be assessed. It seems, too, that the more familiar one becomes with the various species, the less inclined he is to make statements like my conclusions which follow.

Perhaps that is why the literature is so evasive of the engineer's search for a number.

CHAPTER VII

CONCLUSION

1. Variation between easy-harvesting and difficult-harvesting is well represented by the seven species chosen.

2. The relative success of a given harvesting method, although a vector, can be represented satisfactorily by a single factor based on entropy.

3. A stochastic model for the accumulation of settling algal material only partially explains the experimentally observed phenomena; it predicts a harvest that is too concentrated although it does properly represent the recovery as a function of time.

4. In sedimentation of algae, recovery proceeds more slowly toward completion than does enrichment.

5. Algae grown at high culture density in continuous, light-limited culture are physiologically different from algae grown at low culture density but under otherwise similar conditions. These differences tend to support the theory that the former type of cell harvests more easily than the latter where "ease" is determined by the time or energy required for a given amount of "success". This has important implications in the use of algae as a nutrient trap in polluted waters.

6. No single criterion for the selection of promising algae seems satisfactory (promising from the harvesting point of view). A complete array of passive cell physical properties would have missed the potentially important clumping of Spirulina.

7. Centrifugation is a brute-force method of separation which does not take advantage of the differences among species or types. It is a reliable but energetically prodigal method.

8. Electrodecantation is an energetically expensive method for separation of an alga from its high conductivity growth medium.

9. Coelastrum filters exceptionally well. It also settles rapidly.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

1. Explore the energy costs such as the true cost of the centrifuge, the cost of land, the cost of a tank. Then approach the problem of whether Coelastrum is better settled or filtered.

2. Apply the flocculation models of Vold (1959, 1960, 1963) to the case of settling algae. Will these explain the enrichment phenomenon and predict the rich fraction concentration?

3. A continuous separation operated in conjunction with a continuous culture should be tried. This should be a sedimentation tank at first. The tube settler should be examined since there are indications that it might be effective.

4. Of the genera I have come across, two stand out as additional candidates for further efforts - Pediastrum and Oscillatoria. Pediastrum is large and in several respects it is like Coelastrum. Oscillatoria is heterotrophic and grows well in sewage; it may be nutritionally as good as Spirulina. The larger desmids may also be interesting since they can tolerate nutrient starvation and would therefore be good nutrient traps in a final stage.

5. A stochastic treatment of mean cell age would be a straightforward task. Some data would be needed.

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APPENDICES

Appendix A

Assorted observations on the algae as cultivated and harvested.

1. Taste. Spirulina was quite acceptable - cooked and in small quantities (10 g dry wt.). Chlorella (raw) and Coelastrum (raw) were rather tasteless. The bitter taste reported for Chlorella was only present in the unwashed cells which suggests that the added expense of washing the cells may bring returns. The filter cake of Coelastrum is particularly easy to wash. Spirulina loses pigment to the wash water (tap water) probably due to osmotic cell rupture. Washing is necessary to reduce the nitrate concentration to a safe level. EDTA may also be a problem if not diluted down. I used a 1:5 dilution with tap water where unity represents the pellet volume collected from the semicontinuous centrifuge. The resulting nitrate concentrations are safely within the U.S.P.H. Drinking Water Standards provided the tap water used for dilution is free of nitrate or nearly so. I consumed 5 g dry weight each day for a week with no apparent short-term ill effects. I did not eat the other algae. Spirulina had a slimy texture and Coelastrum had a texture like the yolk of a hard-boiled egg.

2. Odor. The Chlamydomonas culture had predominantly a fishy smell about it. But when the cells were in the Palmella stage the fishy smell was no longer present. Chlorella and Coelastrum had a musty smell. The other cultures smelled like fresh algae, an unobjectionable odor. Spirulina and Anabaena did not develop the hydrogen sulfide smell as did the other algae when let stand 4-6 hours at room temperature. This is probably due to the higher pH of the media for the blue-green algae. Coelastrum was slowest of the green algae to develop an unpleasant odor.

3. Acceptability in the diet. Roachus americanus eats all seven algal species quite readily. A turtle of uncertain pedigree preferred Spirulina cake (washed) to raw hamburger, cheese and lettuce. My four children ate, without complaint, gram quantities of cooked Spirulina mixed with rice and chicken.

APPENDIX B

Representative data.

Sedimentation.

Scenedesmus	Date	X(g/l)	D(hr^{-1})	C_F(g/l)	C_R(g/l)	C_L(g/l)	Settling time(min.)	Material balance(%)
	17Jan72	1.564	0.037	1.44	10.67	1.24	100	99.7
	"	"	"	1.44	13.1	0.308	660	98.9
	19Jan72	1.578	"	1.57	19.8	1.18	20	99.8
Oocystis								
	21Feb72	1.824	0.019	1.82	13.8	0.948	373	98.2
	"	"	"	1.81	21.5	1.33	240	98.5
	3Mar72	2.62	0.004	2.62	42.2	0.444	190	99.1
	"	"	"	2.62	43.7	0.420	190	98.8
	4Mar72	0.884	0.051	0.884	10.0	0.244	690	94.4
	"	"	"	"	9.89	0.248	690	94.1
	11Mar72	0.963	0.031	0.963	3.11	0.922	60	101.7
	"	"	"	"	18.3	0.728	120	97.1
	"	0.950	"	0.950	5.79	0.514	240	100.3
	"	"	"	"	5.60	0.349	360	97.7
	18Mar72	1.33	0.01	0.987	4.77	0.923	120	98.3
	"	"	"	0.962	5.34	0.590	360	100.0
	20Mar72	1.20	"	1.20	9.13	0.772	240	100.1
	23Mar72	2.41	batch	2.46	57.96	0.917	60	103.9
	"	"	"	2.44	29.35	0.372	120	99.8
	"	"	"	2.43	19.4	0.324	180	101.2
	"	"	"	2.45	17.7	0.272	360	98.0
Chlorella								
	11May72	0.402	0.021	0.723	10.7	0.481	15	95.8
	"	"	"	0.584	12.5	0.410	30	102.6
	"	"	"	0.596	13.7	0.213	30	96.3
	"	"	"	0.587	10.2	0.340	60	96.6
	"	"	"	0.592	14.5	0.299	120	98.8
	15May72	1.00	0.015	1.00	17.6	0.570	60	104.8
	"	"	"	"	19.7	0.558	30	102.7
	16May72	0.924	"	0.941	11.5	0.694	8	97.5

Sedimentation (continued)

	Date	X(g/l)	D(hr^{-1})	C_F (g/l)	C_R(g/l)	C_L(g/l)	Settling time(min.)	Material balance(%)
Chlorella (cont'd)	16May72	0.924	0.015	0.930	10.5	0.627	15	96.9
	"	"	"	0.952	14.1	0.541	30	95.4
	"	"	"	0.940	13.5	0.526	60	98.8
	23May72	1.54	batch	1.31	12.1	0.984	15	100.2
	"	"	"	1.34	15.6	0.816	30	95.8
	"	"	"	"	15.9	0.766	60	98.4
Chlamydomonas	29Mar72	0.379	0.022	0.402	2.74	0.376	60	102.0
	"	"	"	0.385	3.24	0.338	120	100.3
	"	"	"	0.378	4.46	0.318	240	104.9
	7Apr72	0.620	0.053	0.619	6.65	0.329	30	96.6
	"	"	"	"	16.4	0.245	60	97.5
	1Apr72	1.02	0.055	0.836	18.86	0.159	240	96.0
Coelastrum (day-old harvest)	21Apr72	0.899	0.027	0.799	7.79	0.722	15	98.6
	"	"	"	0.797	13.7	0.485	60	97.4
	"	"	"	0.792	13.8	0.623	30	100.7
	"	"	"	0.412	9.4	0.192	30	96.4
Coelastrum (fresh-harvested)	22Apr72	0.952	0.027	0.920	21.8	0.383	15	99.2
	"	"	"	"	27.5	0.260	30	101.1
	"	"	"	"	22.3	0.212	60	103.2
	24Apr72	0.563	0.054	0.527	11.7	0.289	15	97.1
	"	"	"	0.499	13.4	0.114	60	96.0
	"	"	"	0.535	12.1	0.251	30	98.3
	28Apr72	2.7	batch	2.63	28.8	0.223	30	99.8
	"	"	"	2.67	23.1	0.161	60	97.9

Sedimentation (continued)

	Date	X(g/l)	D(hr^{-1})	C_F(g/l)	C_R(g/l)	C_L(g/l)	Settling time(min.)	Material balance(%)
Spirulina (untreated)	12Dec71			1.23	6.19	0.564	120	
				1.33	6.08	0.344	105	103.0
				0.800	6.62	0.392	50	100.8
				0.842	12.43	0.392	60	101.6
				0.842	16.7	0.450	120	98.9
Spirulina (pressurized/ sonicated)				1.23	22.2	0.125	120	-
				1.33	8.2	0.312	20	103.5
				0.697	13.0	0.216	18	95.2
				"	17.2	0.208	36	98.7
				1.33	8.0	0.226	20	95.6
				1.15	12.3	0.230	18	95.6
				0.895	13.7	0.101	30	100.0
				"	18.6	0.108	60	99.0
				0.800	15.3	0.099	34	104.4
				"	10.11	0.148	17	101.5

Anabaena: Clumped, but didn't settle in the separatory funnels.

Filterability

30Mar72; Chlamydomonas at 0.712 g/l; filter pore 3 microns. vacuum 125 mmHg abs.

Time(seconds)	Filtrate volume (ml)	T/V
0	0	0
10	5	2.0
20	6	3.33
30	6.5	4.62
40	6.75	5.93
50	6.9	7.25
60	7.0	8.57
90	7.75	11.6
120	8.0	15.0
180	8.75	20.57
240	9.25	25.95
300	9.75	30.77
600	11.25	53.33
900	12.50	72.0
1200	13.50	88.89
1500	14.25	105.3
1800	15.0	120.0

The points from 10 seconds to 180 seconds give a straight line of slope 7.90. The correlation coefficient is 0.994. Filterability or specific resistance is 1.97×10^{14} cm/g.

$$\text{Specific resistance } r = 49.8 \times 10^{10} \times \frac{\text{slope} \times \text{pressure drop}}{\text{culture density}}$$

where pressure drop is in inches Hg vacuum and culture density is in grams dry algae per liter.

For comparison,

23Apr72; Coelastrum at 0.95 g/l; filter pore 3 microns. vacuum 125 mm Hg abs.

	Time(seconds)	Filtrate volume	T/V
	0	0	0
	10	140	0.0173
	20	203	0.0985
	30	254	0.118
	40	296	0.135
	50	335	0.149
Replicate:	0	0	0
	10	149	0.0671
	20	220	0.0909
	30	275	0.109
	40	323	0.1238
	50	354	0.141

Centrifugation

	Date	C_F(g/l)	Acceleration	C_L(g/l)	Energy(j/l)
Chlorella	12May72	0.563	2786 x g	0	20,000
	"	"	1935	0.036	15,400
	"	"	1238	0	14,100
	"	"	697	0.165	9,370
	"	"	310	0.204	7,780
	"	"	77.4	0.521	5,570
	25May72	1.853	3792	0.141	27,100
	"	"	2786	0.154	19,300
	"	"	1935	0.206	15,100
	"	"	1567	0.245	13,100
	"	"	1238	0.245	11,500
	"	"	"	0.157	12,400
	"	"	697	0.420	9,350
	"	"	310	0.664	7,790
Spirulina (pressurized)	15Dec71	1.06	77.4	0.782	9,750
	"	"	309	0.267	10,600
	"	"	445	0.186	11,300
	"	"	606	0.270	12,900
	"	"	697	0.016	12,200
	"	"	792	0.032	12,800
	19Dec71	1.125	309	0.340	9,540
	"	"	309	0	37,688
	"	"	309	0.190	37,700
	"	"	309	0.578	11,400
	"	"	484	0.245	10,400
	"	"	697	0.215	11,900
	"	"	948	0.260	14,100
	"	"	1238	0	16,900
Oocystis	23Mar72	2.44	948	0.0717	8,970
	"	"	697	0.832	7,450
	"	"	948	0.752	8,680
	"	"	1088	0.007	9,340
	"	"	1238	0.076	9,230
	"	"	1238	0	11,800
	"	"	1238	0.059	-

Centrifugation (continued)

	Date	C_F(g/l)	Acceleration	C_L(g/l)	Energy(j/l)
Oocystis (cont'd.)	3Mar72	2.635	1935	0	13,750
	"	"	1567	0.127	11,450
	"	"	1238	0.163	10,125
	"	"	948	0.173	8,380
	"	"	697	0	7,980
	"	"	484	0	9,170
	"	"	310	0.958	-
	11Mar72	0.956	948	0.04	8673
	"	"	697	0.04	8800
	"	"	484	0.067	9072
	"	"	310	0.125	8851
	"	"	174	0.375	9412
	"	"	310	0.330	9063
	"	"	484	0.083	8964
	"	"	697	0.059	7920
Chlamydomonas					
	2Apr72	1.08	2786	0.152	16,770
	"	"	"	0.173	16,250
	"	"	3792	0.105	21,250
	"	"	4954	0.058	26,750
	"	"	6269	0.017	33,220
	"	"	1935	0.039	13,060
	"	"	1238	0.067	9,103
	"	"	697	0.298	7,703
	"	"	310	0.491	6,534

Electro-flocculation

	Date	X(g/l)	D(hr^{-1})	C_F(g/l)	C_R(g/l)	C_L(g/l)	Settling time(min.)	Energy (j/l)
Scenedesmus	16Jan72	0.976	0.006	0.964	20.72	0.572	90	826
	17Jan72	1.334	"	1.334	41.63	0.452	60	1612
	"	"	"	1.334	27.45	0.756	60	830
	"	"	"	1.334	23.06	0.944	60	564
	19Jan72	1.916	0.037	1.916	42.0	1.348	75	1602
	"	"	"	"	33.3	1.304	55	1560
	"	"	"	1.304	32.9	0.908	55	1740
	"	"	"	1.916	26.5	1.572	35	1600
Coelastrum	21Apr72	0.899	0.027	0.811	7.926	0.739	15	1917
	28Apr72	2.0	batch	0.230	5.67	0.131	30	1846
Chlamydomonas	29Mar72	0.379	0.0314	0.356	3.80	0.261	60	1243
	2Apr72	1.0	0.0154	1.097	15.95	0.476	60	1489
	3Apr72	0.518	"	0.449	4.43	0.257	60	2776
	"	"	"	0.456	5.18	0.226	60	5810
	7Apr72	0.8	"	0.619	9.68	0.180	60	5024
Chlorella	10May72	0.698	0.021	0.591	4.03	0.581	60	2942
	"	"	"	0.687	1.858	0.685	60	1119
	"	"	"	0.700	8.77	0.675	60	5069
	24May72	1.538	0.017	1.588	25.47	0.692	60	396
	"	"	"	1.742	19.68	0.699	60	1744
	"	"	"	1.761	18.92	0.690	60	1025
	23May72	"	"	1.341	15.88	0.766	60	0
Oocystis	27Feb72	1.892	0.044	1.892	2.22	0.924	120	3562
	"	"	"	"	15.21	1.548	120	3337
	"	"	"	"	8.09	1.556	120	3474
	12Mar72	0.946	0.031	0.904	9.16	0.650	120	2268
	"	"	"	"	9.43	0.594	120	1603
	24Mar72	2.76	batch	2.656	45.18	1.275	60	1875
	"	"	"	2.903	44.13	1.477	60	5777
Anabaena	Flocculates well but does not settle.							
Spirulina	16Dec72	0.842	0.021	0.842	12.82	0.444	60	405