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MICROALGAE CONCENTRATION BY FOAM FRACTIONATION

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We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of science in Biosystems Engineering.

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ABSTRACT

A technique using foam fractionation has been investigated for concentrating marine microalgae *Chaetoceros sp.*, a unicellular diatom, from mass cultures. Foam fractionation is a water treatment technology that is useful in removing dissolved and suspended solids from water. The removal of dissolved and suspended solids is accomplished by bubbling air, or some other gas, through water to concentrate the dissolved and suspended solids in a foam which is then removed from the water. The amount of biosurfactant, which was produced by microalgae, was found to be the major factor affecting microalgae removal by foam fractionation. A logarithmic relationship between biosurfactant activity in the microalgae culture and algae removal efficiency by foam fractionation was found. A two step microalgae harvesting technique was developed which used foam fractionation as the first step to concentrate microalgae from fairly diluted microalgae cultures into foam condensates and used centrifugation as the second step to produce a thick algae paste. The natural occurring biosurfactant was reused in this two step microalgae harvesting technique. More than fifty percent of microalgae removal efficiency was achieved during the experiments.

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Chapter 1. INTRODUCTION

A. Background

There is increasing scientific and commercial interest in the production of microalgae species as sources of human food, animal feed, biochemicals and energy (Soeder 1980). However, microalgae production is currently limited to approximately 2000 tons per year, used primarily as health foods (*Spirulina* and *Chlorella*) and for the extraction of β-carotene (*Dunaliella*) (Benemann, 1992).

Half a century after the medical breakthrough of penicillin, antibiotics are losing their miraculous power to heal pneumonia, meningitis, tuberculosis and other dangerous infections. The medications are being thwarted by superbugs--bacteria with the ability to resist antibiotics. An estimated 13,000 Americans die each year from drug-resistant bacteria, while others who survive face lengthy hospitalization and treatment with more expensive and more toxic drugs (Cheevers, 1995).

Marine microalgae is potentially a good source for new antibiotics against drug-resistant bacteria. This is because the antibacterial properties of marine microalgae may have their origin in the microalgae's need to possess active defenses against bacteria and to combat competitors (Wang, 1995). A research project entitled "Naturally Occurring Antibacterial and Antifungal Substances from Marine Algae *Chaetoceros* sp.", founded by the National Defense Center of Excellence for Research in Ocean Sciences (CEROS), is based on these simple assumptions.

Microalgae are normally in a dispersed state in cultures and are extremely difficult and costly to separate from their liquid environment. This is because the cells of most microalgae species are extremely small in size and have a density not greatly different from water. Harvesting has become one of the major problems to hamper the advancement of the microalgae industry.

Several harvesting techniques have been explored. The most feasible approach, from a purely technical standpoint, is centrifugation. However, the high capital and power costs associated with this process prevent its commercial application. Techniques which utilize large microstrainers and more conventional filtration methods (e.g., rotary drum filters) have proven unfeasible due to the clogging of filters by the microalgae. Flocculation, with the subsequent precipitation of the flocs, has been investigated. However, the settling rate of these flocs is slow, and either long detention times or relatively high amounts of flocculants is required. A similar approach has been the use of flocculants to promote flocculation, followed by air flotation to lift the flocs to the surface where a froth is formed and skimmed off. A major drawback to this process is the high concentration of inorganic flocculant which remains in the harvested microalgae and greatly limits its subsequent use. A more serious limitation is that this technique is not economical for microalgae concentrations of less than about 250 mg dry cell mass per liter (Honeycutt et al., 1983).

Another method for harvesting microalgae, although very few reports on it have been found, is foam fractionation. Foam fractionation is widely used to remove dissolved and suspended solids in the water. It is a gas/water interfacial phenomenon, which is

accomplished by bubbling air or another gas through water to produce a foam which can then be removed from the system. Foam fractionation is widely used in mineral refining processes (Prud'homme and Warr, 1996), enhancing oil recovery (Rossen, 1996) and personal care products (Rieger, 1996). Foam fractionation has been used with some success to remove fine suspended solids and excessive nutrient concentrations from aquaculture systems (Dwivedy, 1973; Lomax, 1976; Chen et al, 1993B) and to harvest microalgae (Raymond, 1978; Honeycutt et al.,1983).

The key component needed for fractionation to occur is the surfactant which can reduce the surface tension and be concentrated on gas/water interfaces. Once an air bubble provides the gas/water interface during fractionation, surfactants will concentrate on the bubble surface.

Foam fractionation can be used in microalgae harvesting which relies on the charged nature of the microalgae cell surface. This charge appears to cause the cells to be attracted to bubbles that carry an opposite charge which is controlled by the surfactants. Bubbles whose surfaces have attached surfactants and algae cells are collected and removed later as foam once they have emerged from the surface of a foam fractionator.

It is well known that algae release biosurfactants during their stationary growth phase, and there is increasing evidence to support that algae also release biosurfactants during their exponential growth phase (Mopper et al., 1995). Raymond (1978) reported that foam fractionation can be used for microalgae harvesting with no additional surfactants added. The advantages of using naturally occurring biosurfactants are their biodegradability and the absence of toxicity. Kosaric (1993) pointed out that although

initial interest and application of biosurfactants were primarily in the area of petroleum engineering and enhanced oil recovery, new applications in medicine and industry have evolved because of the advantages of biosurfactants over synthetic surfactants. Biosurfactants are also of increasing interest in cosmetics, food, environmental control and abatement, and any industry where surface-active phenomena play a role in processing and product formulation.

Because of the differences in biosurfactant production among microalgae cultures, different performances of foam fractionation for microalgae harvesting are expected. However, no research publication was found on the relationship between biosurfactant production by microalgae and foam fractionator performance. The importance of such a relationship is obvious and testing such a relationship is technically possible.

Foam fractionation is not yet well understood. In the limited research up to date, most efforts have been devoted to the removal of dissolved solids. In the few papers which deal with the removal of suspended solids, the results varied greatly. There is no data that can be used either to describe or to assess the performance of foam fractionation for microalgae harvesting.

B. Objectives

The objectives of this research are:

1. to provide data and information on the performance of foam fractionation for concentrating the marine diatom *Chaetoceros*;
2. to quantify the biosurfactant production by microalgae at different growth stages, and

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to test the relationship between the amount of biosurfactant produced by microalgae and foam fractionation performance;

3. to develop a pilot microalgae culturing and harvesting system to produce algae paste for extracting antibiotics.

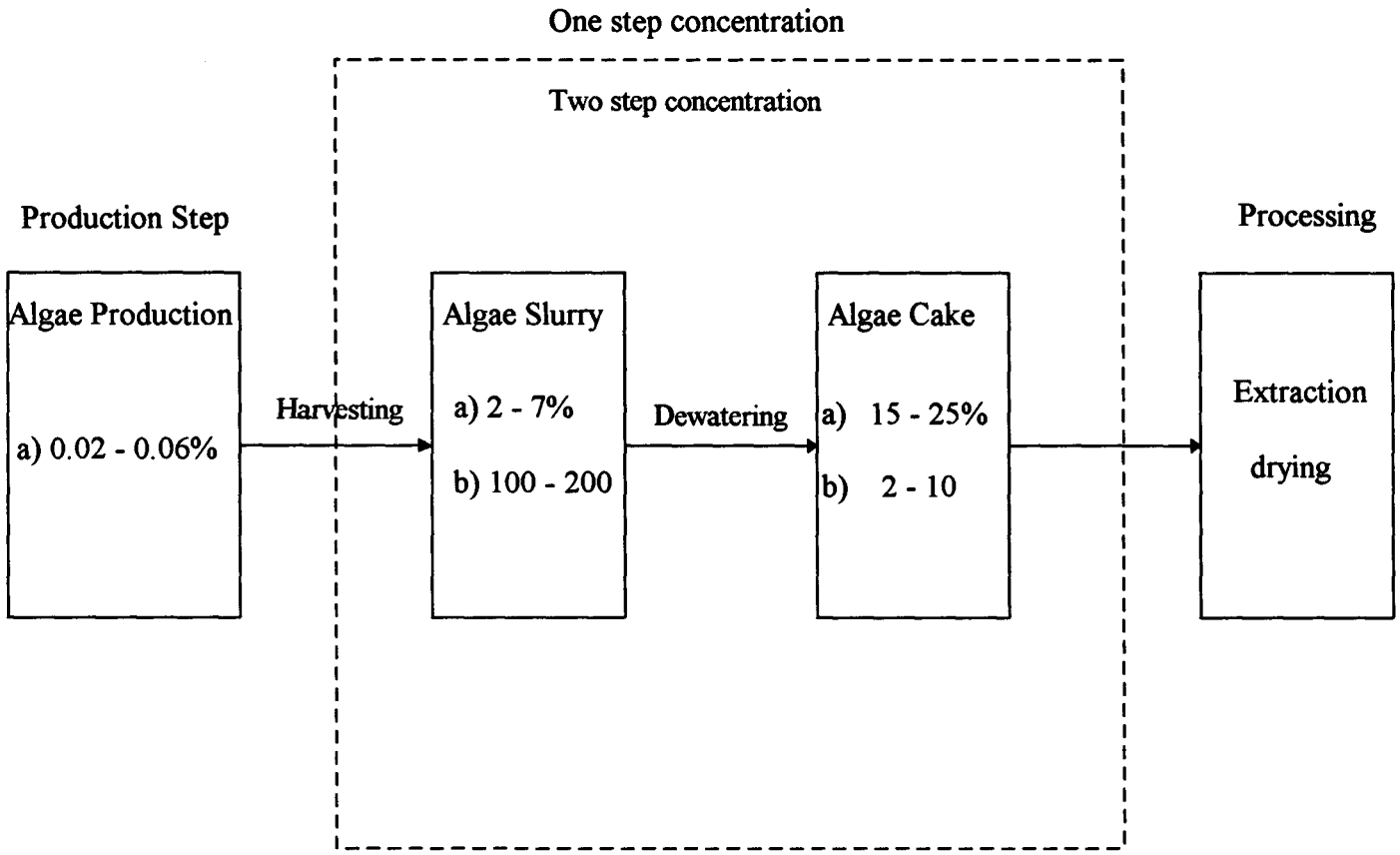
Chapter 2. LITERATURE REVIEW

A. Microalgae Concentration techniques

The term microalgae concentration refers to the process of concentrating microalgae suspension to a slurry or paste containing 5-25% TSS or higher (Shelef et al., 1984). Microalgae solids concentration in the harvestable suspensions of large scale outdoor facilities usually ranges between 0.015% TSS (150 mg/L) and 0.06% TSS (600 mg/L) (Sukenik et al., 1988). Thus, the solids concentrating factor from microalgae culture to the final product is in the range of three orders of magnitude. The microalgae production and processing can be summarized in Figure 1 (Shelef et al., 1984).

The initial step, concentrating the biomass from a suspension of microalgae to a slurry, is a prerequisite to any commercial scale processing (usually involving dewatering and drying). Skipping this initial step will otherwise involve dealing with a large volume of material that requires a high energy investment.

The methods and devices which are suitable for microalgae concentration depend on the algae species, the production system and the objectives of the final product (Mohn, 1980; Dodd, 1980). Common industrial techniques for microalgae concentration are summarized in Figure 2 (Shelef et al., 1984).



a) algae concentration % TSS
b) concentration factor

Figure 1. Schematic presentation of algae production and processing

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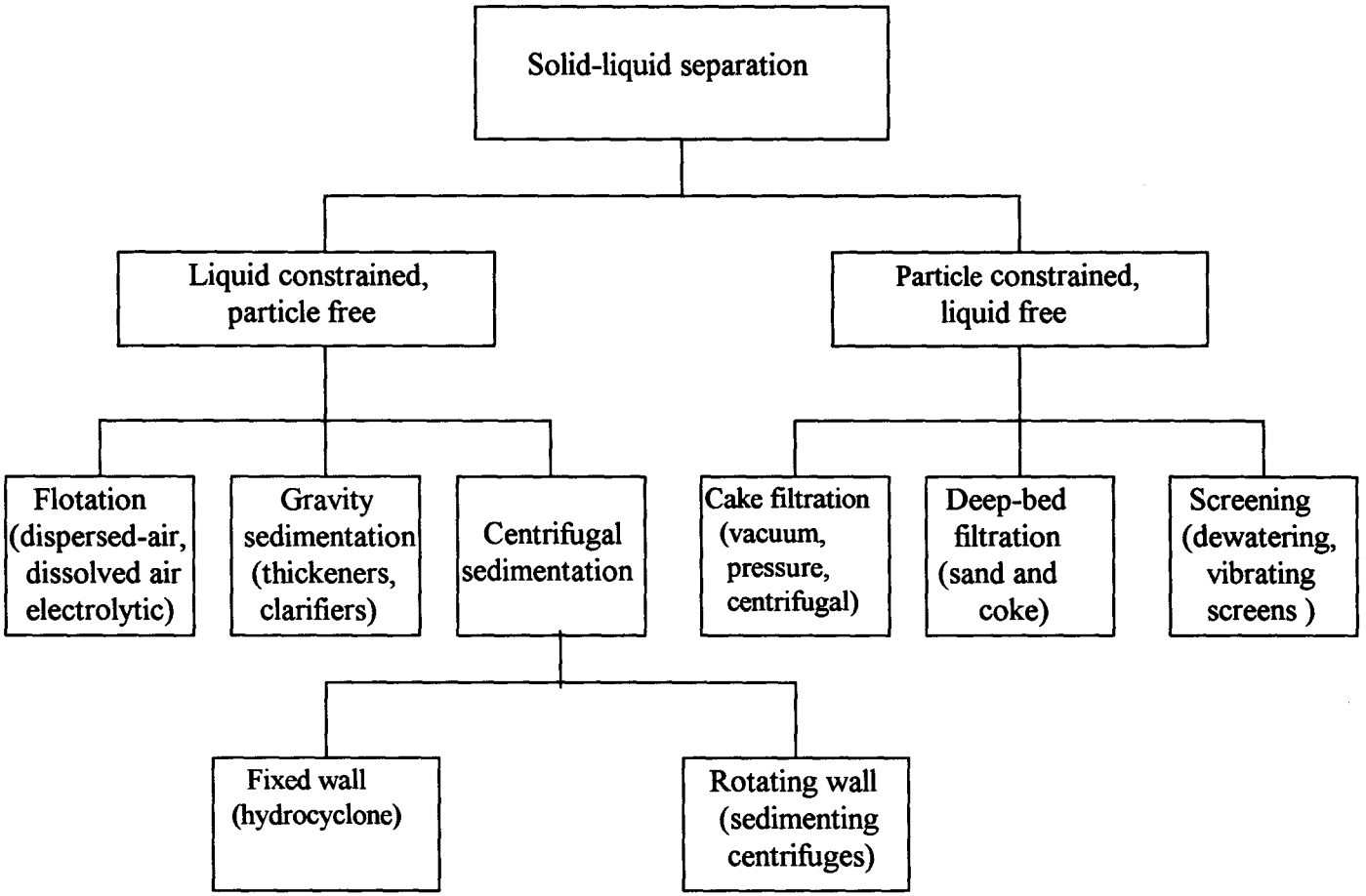


Figure 2: Classification of common industrial solid-liquid separation techniques

Filtration and Screening

Many types of filters have been used to harvest algae but all suffer the universal problem of rapid clogging (Borchard and Omelia, 1961) except for harvest of some filamentous microalgae such as *Spirulina* which are easily removed from their liquid media on vibrating, oscillating or cascade screens because their relative large size and filaments which bridge the screen openings. Oswald (1988) found that the fine screens (50 to 100 μm openings) are highly effective for *Spirulina*, but screens with openings greater than 100 μm capture only a fraction of the filaments.

Because of the clogging problem, moving filters such as the rotary microstrain and the continuous screen filters were developed. However, the microalgae sticking on the filters are still hard to collect and backwashing these microalgae will dilute the microalgae slurry again. One method to slow down the clogging problem is to cover the filter with a potato starch precoat (Mohn, 1988), but the potato starch precoat needs to be separated from the algae paste later.

Filtration and screening devices can be divided into the following groups (Shelef et al., 1984):

1. Pressure Filter. For the pressure filter, the driving force for filtration is the liquid pressure developed by pumping or by the force of gas pressure in the feed vessel.
2. Vacuum Filter. For the vacuum filter, the driving force for filtration results from the application of a suction on the filtrate side of the medium.
3. Microstrainer. Microstrainers consist of a rotary drum covered by a straining fabric, stainless steel or polyester.

4. Vibrating Screen Filters.
5. Cartridge Filters.
6. Deep-bed Filtration.
7. Cross-flow Ultra-filtration.
8. Magnetic Separation.

Sedimentation

Only a few reports on microalgae sedimentation without any flocculation process were published (Shelef et al., 1984). This is because microalgae, with its combination of small size and low specific gravity, are too slow to settle to permit the use of settling as a routine procedure for harvesting microalgae.

The technique of using lime to coagulate and settle suspended microalgae has been known for at least a hundred years. The most effective coagulants to flocculate algae are aluminum sulfate and ferric sulfate. Aluminum chloride and ferric chloride are also effective (Golueke and Oswald, 1965). In each case, the insoluble material created by the coagulant tends to clump together, entrapping and enmeshing the algae cells while they settle. However, a long detention time is needed, and the settled material is quite difficult to collect. The flocculated biomass is around 1.5% TSS (Mohn, 1988).

Centrifugation

Most species of microalgae can be removed from their media up to nearly one hundred percent by centrifugation in the range 500 to 3600 times the acceleration of

gravity (g), but high capital and power cost prevent its use in large scale algae harvesting. The precise functions have been described by Mohn (1978, 1988).

Two major types of centrifuges have been developed for the purpose of algae harvesting. One is the solid bowl centrifuge, another is the plate centrifuge. The solid bowl centrifuge requires 500 g and 10 minutes of residence time to form 20% TSS algae, whereas in a plate centrifuge residence time is only a few seconds at 5000 g, but concentrating factors may be only 10 to 20 and removals is only fractional. Golueke and Oswald (1965) concluded that to be economical for algae recovery, very large (213 cm diameter) continuous plate centrifuges would be required. Such large centrifuges are not commercially available at this time. Smaller 76.2 cm (30 inch) centrifuges require more than 3500 kW-h to separate a ton of dry algae from 200 mg L^{-1} suspensions at a flow of about 1000 L/min. The solid bowl centrifuges are very sturdy, require little maintenance and function trouble-free for many weeks without interruption. There is a continuous feed of suspension and discharge of solids. However, the suspension must be preconcentrated to at least 1.5% to 2% TSS by flocculation, so they are of little value over other concentration systems that cost less (Oswald, 1988).

Centrifugation devices can be divided into the following groups (Shelef et al., 1984):

1. Tubular centrifuge
2. Multichamber centrifuge
3. Imperforate basket centrifuge
4. Decanter

5. Solid retaining disk centrifuge
6. Nozzle type centrifuge
7. Solid ejecting type disc centrifuge
8. Hydrocyclon

Flotation

Flotation is a gravity separation process based on the attachment of air or gas bubbles to solid particles, which are then carried to the liquid surface and accumulate as float which can be skimmed off.

The floatation processes are classified according to the method of bubble production into following groups: dissolved air flotation, electrolytic flotation and dispersed air flotation (Svarovsky, 1979).

Compared with sedimentation, floatation is much more efficient for harvesting algae because algae particles can float upwards much more rapidly than settle downwards (Parker, 1975). It is also generally possible to remove the surface material more completely than the settled material, which is so light that it is difficult to remove from the bottom of a settling tank. Algae float will also generally have a much higher content of water-free solids than will algae sludge. The concentration of algae in the float can reach as high as 7% TSS (Mohn, 1988).

Dissolved Air Flotation

The dissolved air flotation (DAF) process is based on the higher solubility of air in water as pressure increases. Bare (1975) reported that 10 to 40% algae removal for exponential growth algae and 60% algae removal for endogenous growth algae were achieved with no coagulants added. When coagulants such as ferric sulfate and aluminum sulfate were added, the DAF system achieved over 90% algae removal.

Koopman and Lincoln (1983) described the application of autoflotation. Alum or C-31 polymer was mixed with algae culture in the afternoon before the cells entered the flotation tank. The O_2 developing in the tank the next day forced the resulting flocs upwards. With the aid of special withdrawal funnels, it was possible to pump off the O_2-rich suspension from the upper few centimeters of the tank. Eighty to ninety percent of algae removal was achieved with algae float concentrations averaging more than 6% TSS.

At present time the most reliable and economical method of harvesting algae is flocculation followed by DAF (Oswald, 1988). However, the product contains such coagulants as aluminum sulfate which is believed to interfere with phosphate metabolism in bone development in young chickens and young animals. Efforts have been made to remove alum from algae concentrates after they are harvested. The usual process is to add sufficient acid to bring the pH value to 1 or 2. At that point about two-thirds of the aluminum ion is rendered soluble and can be removed with the supernatant in centrifugation. According to Shelef et al. (1975) two-thirds of the alum that is removed by this technique is sufficient to avoid most nutritional problems associated with alum

algae. Also, the recovered soluble alum can be used to acidify and harvest additional algae.

Mohn (1988) pointed out that although flotation processes operate more efficiently and rapidly than sedimentation process, and also achieve a higher solid fraction (up to 7%) in the concentrate, the high investment costs for dissolved air flotation facilities and the additional energy requirements for pumps and generation of compressed air are disadvantages. If costs for flocculating agents are included, then the cost of separation by means of dissolved air flotation is similar to that of centrifugation.

Electroflotation

In this method fine gas bubbles are formed by electrolysis. Various microalgae species were harvested by this method and the collected algae float contained up to 5% solid (Sandbank et al., 1974). The energy requirement of the electroflotation is high but Svarovsky (1979) noted that for small units of $5m^2$ area or less, the electric-flotation operating cost is cheaper than that of dissolved air flotation.

Dispersed Air Flotation

Dispersed air flotation uses large bubbles of about one millimeter, which are produced by agitation combined with air injection (froth flotation) or by bubbling air through porous media (foam flotation or foam fractionation). Although dispersed air flotation is known to be relatively economical and can be applied to large volumes of liquid (Levin et al., 1962), it hasn't been well studied for microalgae harvesting.

A summary of the comparison of microalgae harvesting by flotation methods (Shelef et al., 1984) is given in Table 1.

Table 1. Comparison of microalgae harvesting by flotation methods

Device	Final slurry concentration % TSS	Relative energy required	Reliability	Recommendable for algae size group	Remarks
Dissolved air flotation (DAF)	1-6	high	very good	a+ b	Flocculants required
Electroflotation	3-5	very high	very good	a + b	Flocculants required
Dispersed air flotation	un.	Un.	Low	un.	Surfactants required

a: *Chlorella* type tiny algae
b: *Coelastrum, Micractinium* type grouped algae
un.: unknown

B. Foam fractionation

Foam fractionation is a water treatment technology that is often used to remove dissolved and suspended solids in the water. It is a gas/water interfacial phenomenon which is accomplished by bubbling air or another gas through water to produce a foam that can be removed from the system (Timmons, 1994).

The key component needed for fractionation to occur is the surfactant whose molecule possesses at least one polar and one nonpolar portion. Since a water molecule has a polar structure, the nonpolar end of a surfactant then becomes hydrophobic and tends to stick out of the water into the air bubble. As a result, surfactants are found

concentrated on gas/water interfaces whenever available. Once an air bubble provides the gas/water interface during fractionation, surfactants in the vicinity of the interface will concentrate on the bubble surface. Bubbles whose surfaces have attached surfactants will be collected and removed later as foam once they have emerged from the surface of a foam fractionator. The polar structure of a bubble and surfactant interface is depicted in Figure 3.

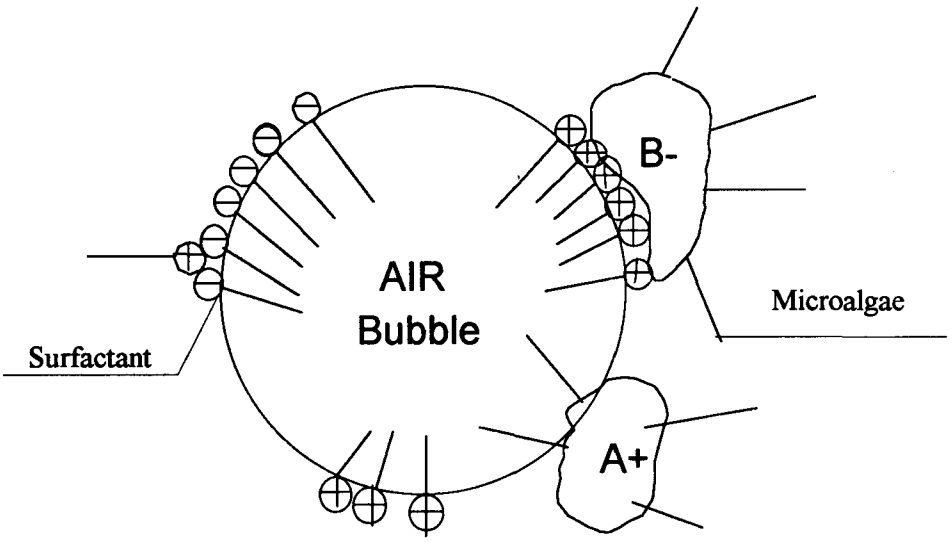


Figure 3. The polar structure of a bubble and surfactant interface

Spotte (1979) stated that the main factors affecting the efficiency of foam fractionation are (1) contact time between the air bubbles and the water, and (2) bubble size. Contact time is related to diffuser submergence depth, column diameter, air flow rate, and water flow rate. Collection of the foam produced is affected mostly by the geometry of the foam concentrating device. Therefore, operational and design parameters

of interest for foam fractionation are: bubble size, column height, column diameter, air flow rate, water flow rate, and foam overflow height.

Bubble size

Bubble size is the major factor determining the surface to volume ratio of the gas phase and the flow pattern around the air bubbles in a gas-liquid system. The smaller the bubble is, the larger the relative surface of the bubble would be. Chen et al. (1991) showed mathematically that reducing bubble size greatly increased the solids removal efficiency of foam fractionation. Chen et al. (1992) also showed that bubble size increased with air flow rate and air stone pore size, but decreased with protein concentration.

Column height

Increasing column height increases the bubble-water contact time which will increase the solids pickup. Kown (1971) studied the effects of column height on the effectiveness of a continuous bubble fractionation system and concluded that column heights above 30 cm have little effect on the performance of the columns, but column heights below 30 cm affect the performance of the columns significantly.

Diameter of column

Know (1971) studied the effects of column diameter on the effectiveness of a continuous foam fractionation system, and concluded that increasing the column diameter greatly reduced the effectiveness of the system.

Air flow rate

Higher air flow rates result in more bubbles, and since bubbles are solids carriers, more bubbles will increase the solids pickup. Weeks et al. (1992) demonstrated that as air flow rate increases, foam condensate production increases, but concentration decreases.

Foam collection height

Foam collection height is the height above the water surface in the column that the foam is collected. Weeks et al. (1992) investigated foam collection heights of 0, 4, and 8 centimeters and found that the higher the foam collection height the less foam was collected but the greater the concentration of solids in the foam. This is because with higher foam collection heights, the water in the foam had more chance to drain from the foam.

Foam fractionation is not yet well understood. Most of the past research effort has been devoted to the removal of dissolved solids. In the few papers which deal with the removal of suspended solids, the results varied greatly. There is no basic data that can be

used either to describe or to assess the performance of foam fractionation for algae concentrating.

Foam fractionation is theoretically a good method for algae concentration. Timmons (1994) reviewed the application of foam fractionation and concluded that foam fractionation is very efficient at removing fine particles whose diameters are less than 30 microns. The sizes of most microalgae are in that range.

Foam fractionation has been used with some success to remove fine suspended solids and excessive nutrient concentration from aquaculture systems. Dwivedy (1973) found that foam fractionation removed suspended and dissolved organics from oyster culture water. Lomax (1976) compared fish culture systems that used a biofilter in combination with either a sedimentation tank, foam fractionation units or mechanical filters. Lomax's opinion was that in terms of cost and effectiveness the biofilter with fractionation was the best design combination. Chen et al. (1993B) found that a fractionator reduced the number of particles per liter from $4.1*10^6$ to $2.1*10^6$ after fractionation with the condensate having a $55.2*10^6$ concentration of particles.

Foam fractionation has also been used in algae concentration. This relies on the charged nature of the algae cell surface. This charge appears to cause the cells to be attracted to bubbles that carry an opposite charge which is controlled by surfactants. When a constant stream of bubbles is passed upward through the algae culture, the bubbles raise algae cells to the surface and trap them in foam. Raymond (1978) used a foam fractionation device to concentrate algae and simultaneously recirculate the growing culture in shallow layers through an interconnecting series of hemicylindrical channels.

Honeycutt et al. (1983) demonstrated that colloidal gas aphrons (a kind of foam fractionation technique) is a very promising technique in concentrating unicellular algae from dilute suspensions.

Raymond (1978) reported that foam fractionation can be used for algae concentration with no additional surfactants added; but the reason for this performance was not included in that research. It is well known that the surfactant is the key component for the foam fractionation process. This indicates that the biosurfactant, which is produced by algae, must have played an important role in the foam fractionation for algae concentration.

C. Biosurfactant

The term biosurfactant has been used very loosely, and refers to any usable and isolatable compound obtained from microorganisms that has some influence on interfaces (Desai and Desai, 1993). Many microbes appear to produce a complex mixture of biosurfactants, particularly during their growth. Generally, biosurfactants are microbial metabolites with the typical amphiphilic structure of a surfactant, where the hydrophobic moiety is either a long-chain fatty acid, hydroxy fatty acid, or α-alkyl-β-hydroxy fatty acid and the hydrophilic moiety can be a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid, alcohol, etc.

Kosaric et al. (1987) divided biosurfactants into six major classes:

1. Hydroxylated and crosslinked fatty acid (mycolic acid)
2. Glycolipids

3. Polysaccharide-lipid complexes
4. Lipoproteins-lipopeptides
5. Phospholipids
6. Complete cell surface itself

The releasing of organic compounds by microalgae has been reported since the 1950's (Fogg, 1966, 1977, 1983). A multitude of compounds has been found: sugars, sugar-alcohols, organic acids, polysaccharides, lipids, vitamins, growth factors, toxins, amino acids, peptides and proteins (Hellebust, 1974). It is well known that algae release organic compounds during their stationary growth phase, and more and more evidence supports that algae also release organic compounds during their exponential growth phase (Fogg, 1977; Zutic et al., 1981; Myklestad et al., 1989; Mopper et al., 1995). Myklestad and Haug (1972) showed that algae *Chaetoceros affinis* produces a homogeneous, extracellular polysaccharide with a composition quite distinct from that of the intracellular reserve polysaccharide or the cell wall polysaccharide. It thus appeares that the excreted carbohydrate is not produced by leakage from cells in poor conditions. It is produced in all growth phases and reaches relatively high concentrations in the medium during nutrient-limited growth.

The extent, chemical composition and molecular size of these compounds released by algae are varied under different conditions and among different species (Hellebust, 1965, Ignatiades and Fogg, 1973). Ignatiades and Fogg (1973) identified that compounds released by algae were affected by light intensity, the combined effect of light intensity and

bicarbonate concentration, senescence, exposure to darkness, population density and nutrient supply.

Microalgae release 1- 70% of the total carbon fixed in photosynthesis (Fogg, 1966; Chrost and Faust, 1983). The rate of organic carbon release varied from 1.3 to 123.7 $\mu g\ C\ l^{-1}\ h^{-1}$ (Chrost and Faust, 1983). Chrost and Faust (1983) demonstrated that the dominant parts in released organic carbon (ROC) are a low molecular weight fraction less than 500 daltons (18.5% of ROC), a fraction of molecular weight 10,000-30,000 daltons (30% of ROC), and high molecular fraction of > 300,00 daltons (15.4% of ROC).

Fogg (1966) distinguished two types of extracellular products: Type *I.* metabolic intermediates, usually of low molecular weight substances for which a quasi-equilibrium exists between intra- and extra-cellular concentrations; Type *II.* metabolic end products, usually of higher molecular weight but quantitatively much less important, for which the rate of liberation is proportional to the growth.

The release of compounds has been demonstrated with individual algae species in culture, as well as natural populations in marine, freshwater and estuarine ecosystems. Hellebust (1965) studied 22 species of marine microalgae and found that glycolic acid forms 9-38% of the total carbon release in *Olisthodiscus sp., Chaetoceros pelagicus, Chlorococcum sp.* and *skeletonema costatum;* the amount of release of carbon as protein ranges from 0.2 to 5.9%, and the amount of release of carbon as chloroform-soluble material ranges from 2.8 to 10.3%.

There are two common experimental approaches to measure the release of compounds. One is using the radioactive carbon method and another is using various

separation procedures to identify and quantify individual compounds. The inherent limitation of using the radioactive carbon method has been discussed by Williams et al. (1976) and Storch and Saunders (1978). Mague et al. (1980) reported that at best ^{14}C values of extra-cellular release represent the minimum amount possible, since the release of unlabelled polymeric compounds is not accounted for.

A significant fraction of these organic compounds released by algae is surface-active (Zutic et al., 1981). Wilson and Collier (1972) showed that various marine microalgae are capable of producing biosurfactants, although not all species studied have equal ability in this respect. It has been reported that processes such as the flocculation of algae bloom (Mopper et al., 1995) and bioflocculation (Aaronson, 1973) are regulated by the biosurfactants.

The production of biosurfactants by microalgae is depended on the particular species (Wilson and Collier, 1972) and the age of the culture (Zutic et al., 1981). In the first phase, immediately after inoculation, there is no detectable increase in surfactant content. In the exponential growth phase, there is a net increase of surfactant activity which proves that the release from algae is an important source of surfactants in the algae culture medium and that it is not solely a result of an abnormal process of old culture. Generally, total surfactant content in culture media increases with cell density, while surfactant per cell shows an inverse relation to cell density (Zutic et al., 1981).

Several methods have been used to test the surfactant activity in the algae culture. Wilson and Collier (1972) measured the surface tension and viscosity with a Tensionmeter and a Viscosimeter. The precision and accuracy of such measurements were not

satisfactory, apparently because of the nature of the material produced by the organisms and because of the insensitivity of these methods to such materials. On the other hand, foam volumes obtained by shaking and the foam decay time, especially the latter, produced a more sensitive measure of the surfactants than the instruments. Zutic et al. (1981) used electrochemical methods based on the adsorption of organic molecules at a mercury electrode-solution. It provided a very efficient, simple and nondestructive way of rapidly determining surface-active constituents in natural sea water, as well as in microalgae culture media. The results showed that surfactant activity of an algae culture corresponds to approximately 5 - 100 mg/L of model surfactants such as polysaccarides, glycoproteins, peptides, lipids or humic acid.

Mopper et al. (1995) found that microalgae (3-51μm equivalent spherical diameter) were readily concentrated in the foam by bubbling. At the beginning of the bubbling (0-0.5 h), both microalgae and surface-active carbohydrates were extracted at a high rate; however, these rates dropped off steeply after about 0.5 h of bubbling. The result of this study indicates that there are two major kinds of surface-active materials in algae culture: highly surface-active, deoxysugar- and galactose-rich material that was preferentially extracted into foam at the beginning (0-0.5 h) of bubbling, and a glucose-rich 'background' material that was extracted from water into foam at a more uniform, low rate during bubbling. The latter may have been masked during periods when there was a high concentration of carbohydrates released by the algae.

Because of the variation of biosurfactant production in algae culture, different performances of foam fractionation for algae concentration is expected. Shelef et al.

(1984) showed that there was a great variation in results when foam fractionation is used for algae concentration. However, no research paper was found to deal with the relationship between biosurfactant production by algae and foam fractionation performance.

D. Summary of literature review

The lack of an economical method for harvesting microalgae from the relatively dilute suspensions is one of the major problems in the mass cultivation of microalgae. Because it is relatively economical and can apply to large volumes of liquid, foam fractionation has its potential for concentrating microalgae; but it hasn't been well studied. In the limited research, the results varied greatly. These variations may be related to biosurfactant production during microalgae growth. The relationship between biosurfactant production during microalgae growth and foam fractionation performance for concentrating microalgae is a new field for research, and it may result in a new method of using foam fractionation for concentrating microalgae with no additional artificial surfactant.

Chapter 3. METHODOLOGY

A. Algae culturing

1. Algae culturing system

Marine algae *Chaetoceros gracilis* was grown outdoor at sand island, Oahu, in an open culture system. The system consisted of sixteen clear fiberglass columns (Figure 4). The sixteen columns could be operated together or as a separate operating unit. Table 2 and Figure 5 show the components of the operating unit.

Table 2. Components of an operating unit

Device	Description	Brand
Clear fiberglass column	45.72 cm diameter and 1.83 m height	Sun Lite
pH controller	pH/ORP controller model 5656-00	Cole-parmer
pH probe	In-line electrode 05993-90	Cole-parmer
Air stone (dispersing air)	dimensions: 2.45 cm x 2.45 cm x 3.8 cm 140 microns pore size	Sweetwater
Air stone (dispersing CO_2)	dimensions: 15.2 cm x 2.86 cm producing 100-150 micron bubbles	Point Four Systems

Figure 4. Microalgae culturing system

The major part of the operating unit was an 45.72 ϛm diameter and 1.83 m height clear fiberglass column. There were two air stones on the bottom of the column, one for dispersing air and another for dispersing carbon dioxide. Air was supplied continuously to mix the algae culture. Carbon dioxide injection was controlled by a pH controller which was able to keep the pH within 0.2 pH units of the setpoint.

Well sea water was filtered by sand bed and delivered directly to the operating unit. There was a hole on the bottom of the column to connect the operating unit to a harvesting unit and to a freshwater line for cleaning purposes.

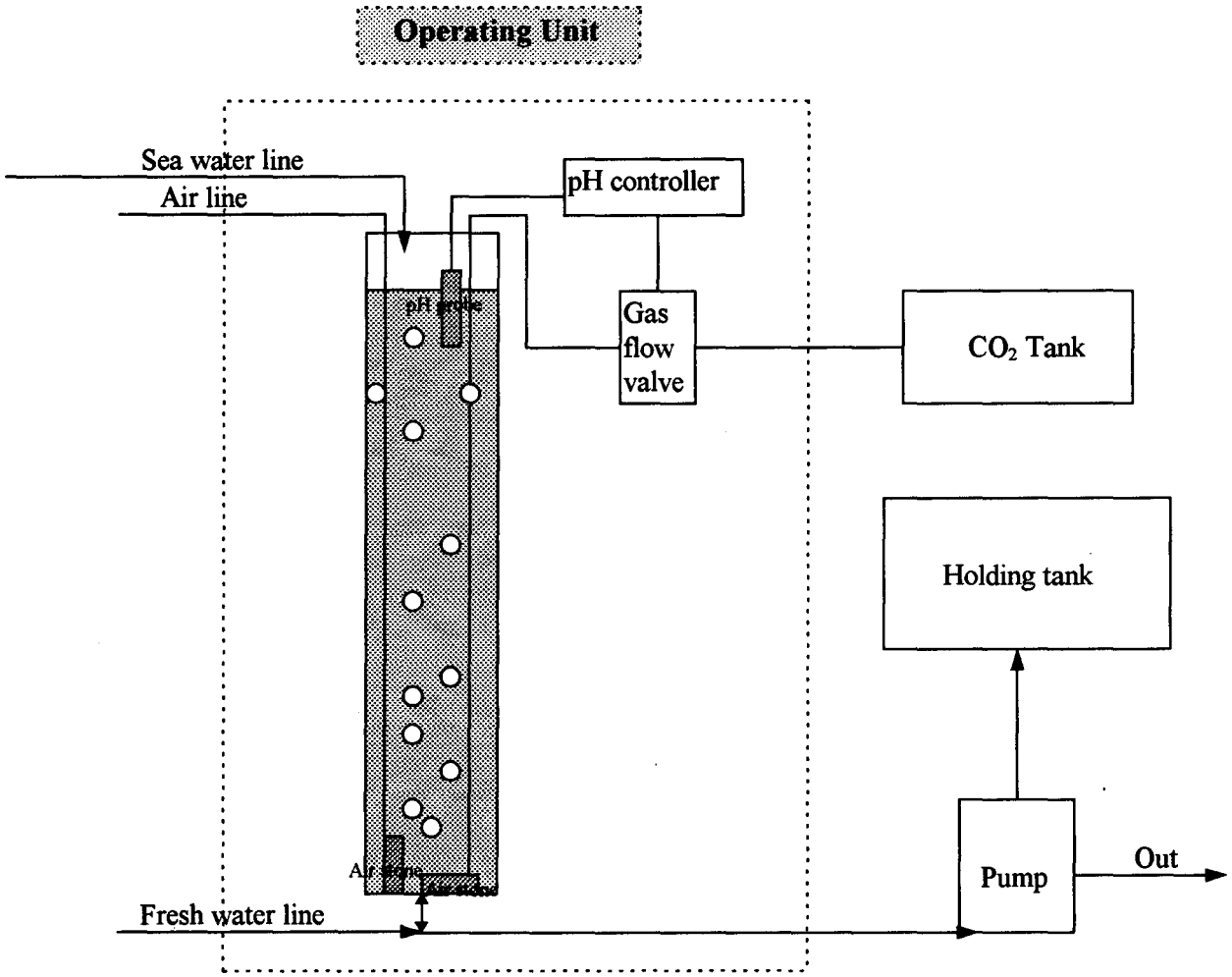


Figure 5. The operating unit in the open microalgae culturing system

2. Algae seed

Algae seed came from surface sea water at Keehi Lagoon, Oahu. After mixing the surface sea water (10%) with well sea water (90%) from Sand Island in the columns, Guillard's f/2 media (Guillard and Ryther, 1962) was added to enrich the culture. The

ingredients of the f/2 media are listed in Table 3. Aeration was provided continuously and pH was maintained around 8.2.

Table 3. Guillard's f/2 media

Item	Concentration per liter
$NaNO_3$	75 mg
$NaH_2PO_4 \cdot H_2O$	5 mg
$Na_2SiO_3 \cdot 9H_2O$	30 mg
Na_2EDTA	5 mg EDTA
Thiamin HCL	0.1 mg
Biotin	0.5 μg
Vitamin B12	0.5 μg
$CuSO_4 \cdot 5H_2O$	2.5 μg Cu
$ZnSO_4 \cdot 7H_2O$	5.0 μg Zn
$CoCl_2 \cdot 6H_2O$	2.5 μg Co
$MnCl_2 \cdot 4H_2O$	5.0 μg Mn
$Na_2MoO_4 \cdot 2H_2O$	2.5 μg Mo
$FeCl_3 \cdot 6H_2O$	0.65 mg Fe

A naturally mixed population of algae bloomed after culturing for three to five days depending on the weather and season. The mixed algae population was dominated by *Chaetoceros* all year around. In the warmer season, *Chaetoceros* made up over 90 percent of the population. Other species, like *Phardactylum tricornutum* and *Thalassiosira sp.*, were observed in the seed as well.

3. Batch algae culture

Batch algae culture was used to study the growth pattern of the algae and the performance of foam fractionation for concentrating the algae during their growth.

The batch algae culture started with the algae seed described above. After mixing the seed (10%) with well sea water (90%) in the columns, Guillard's f/2 media was added. Aeration was provided continuously and pure carbon dioxide was injected into the cultures to keep the pH around 8.2.

The batch algae culture usually lasted for four days until the algae began to die.

4. Semi-continuous algae culture

Semi-continuous algae culture was used to provide algae production for the research project "Naturally Occurring Antibacterial and Antifungal Substances from Marine Algae *Chaetoceros* sp.".

The semi-continuous algae culture started with the algae culture described above. Part of these cultures was then harvested on a daily basis. Hering (1996), working under Wang, has developed a nutrient mixture for the continuous production of *Chaetoceros spp.*. This nutrient mixture and well sea water were added after harvesting. Full ingredients of Hering's media are listed in Table 4. Aeration was provided continuously and pure carbon dioxide was injected into the cultures to keep the pH around 8.2.

Daily management of the semi-continuous algae culture included: taking algae growth data; cleaning columns, air stones and pH probes; reseeding algae; refilling columns with new media and well sea water; and adjusting pH meters.

Table 4. Hering's media

Compound	Name	Concentration
$(NH_2)_2CO$	Urea	3.0 mg N/L
Na_2HPO_4	Sodium Phosphate	2.75 mg P/L
$Na_2SiO_3 \cdot 5H_2O$	Sodium Silicate	10 mg SiO_2/L
Na_2EDTA	Chelating Agent	5.53 mg/L
Cyanocobalamine	Vitamin B12	2.75 μg/L
$CuSO_4 \cdot 5H_2O$	Copper Sulfate	0.0083 Cu mg/L
$FeCl_3 \cdot 6H_2O$	Iron Chloride	0.31 mg Fe/L

Because the semi-continuous algae culture was operated in an outdoor and open culture system, algae growth was greatly affected by the weather and season. The reseeding rate was 10-20% in summer, but 20-30% in winter. A new culture was brought to the system whenever *Chaetoceros* was no longer the dominant species.

It was critical to keep the semi-continuous algae culture system clean, because benthonic algae grow very fast on the surface of columns. Whenever benthonic algae bloomed, they used up nutrients in the system and reduced microalgae production.

B. Measurement of algae density

Both turbidity of algae culture and algae cell count were used to measure algae density. Turbidity was measured by a HACH turbidity meter model 2100P. Cell count was performed under a microscope using a haemocytometer slide.

The variation of turbidity and cell count was tested by measuring the same sample 15 times. The results are listed in Table 5. These results are also plotted in Figure 6 and Figure 7. Although both turbidity and cell counts had shown certain variations, the variation for cell count was much greater than that for turbidity.

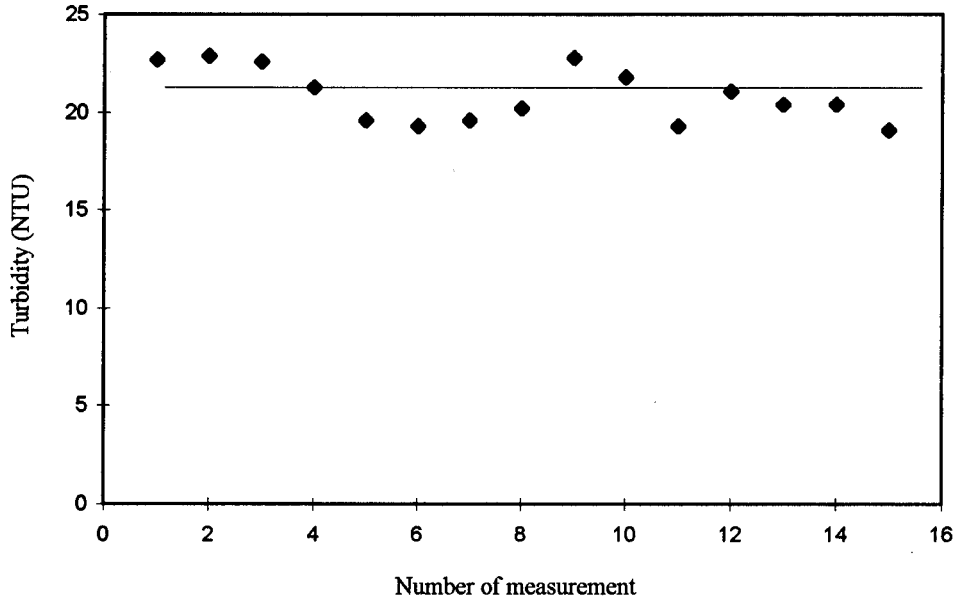


Figure 6. The variation of turbidity measurements

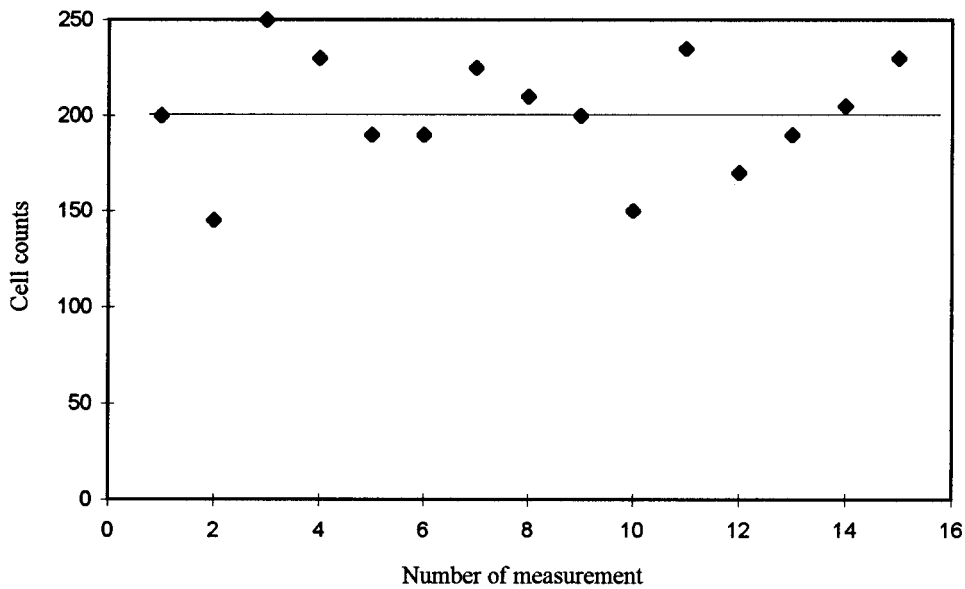


Figure 7. The variation of cell counts

Table 5. The variation of turbidity measurements and cell counts

No. of measurements	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Turbidity (NTU)	22.7	22.9	22.6	19.6	19.3	21.3	19.6	20.2	21.8	22.8	19.3	21.1	20.4	20.4	19.1
Cell counts (10^4)	200	145	230	190	190	225	210	200	150	235	170	190	205	230	250

Coefficients of variation for turbidity and cell count were calculated by:

Coefficient of variation =(Standard deviation)/(mean)*100 .....(1)

According to the measurements, the coefficient of variation for turbidity was 6.71, while the coefficient for cell count was 15.12, so turbidity was a more stable index than cell count. This difference may come from the different sample sizes which were used in the measurements. Turbidity tests used 10 ml samples per measurement, but cell counts used only 0.01 ml samples per measurement.

Because turbidity was more stable, it was used as a major index to measure the density of algae. The disadvantage of turbidity is that it only shows the total solids in the algae culture. Although algae was the major component of algae culture, some other particles existed in the culture as well. Furthermore, because a mixed algae culture was used in the experiments, turbidity was not able to distinguish between algae species. On the other hand, cell counts had the advantage of showing the details of the algae culture. So both of these indexes were used in the experiments.

A linear relationship between turbidity and cell count was found during the experiments. This relationship can be described as equation:

Cell counts (10^4)= 12.327 * Turbidity (NTU) +13.478 .....(2)

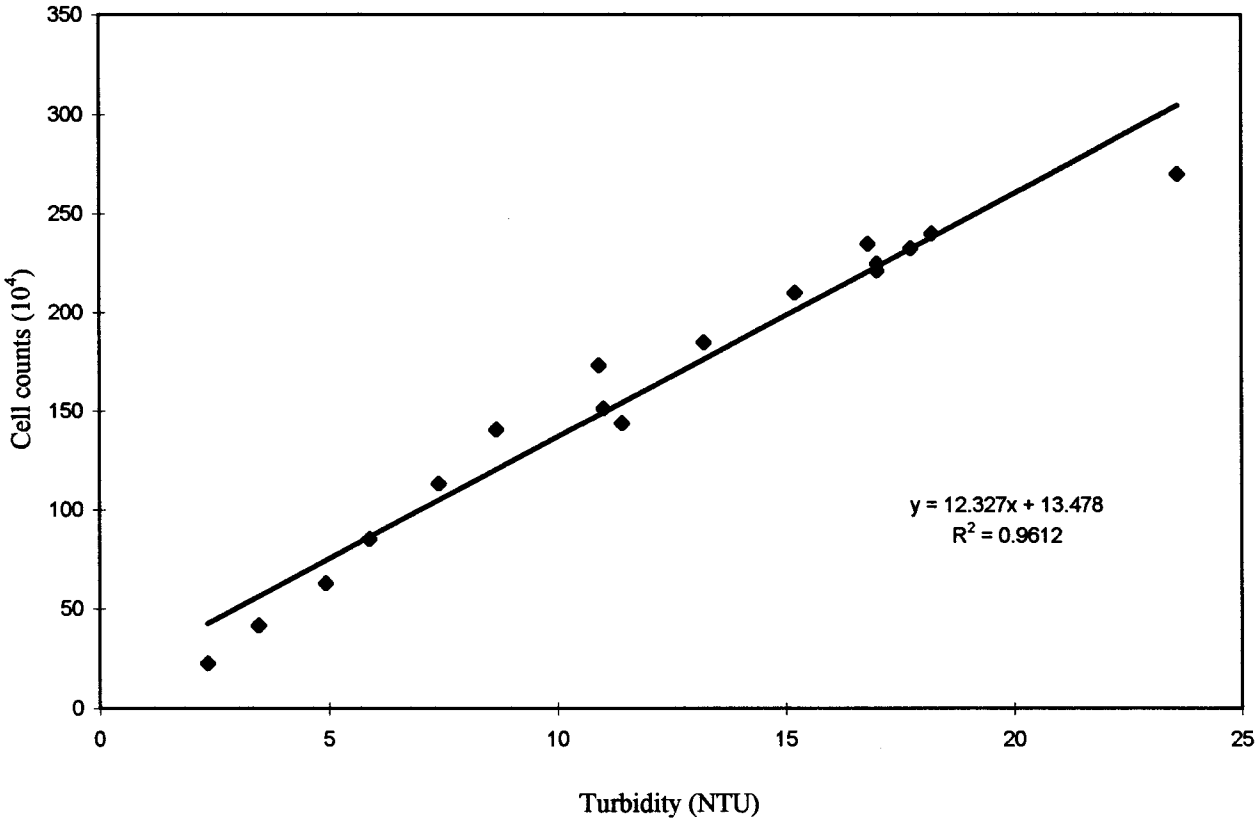


Figure 8. Turbidity vs. cell counts

According to the experiments, the R-square value for this equation was 0.96. The data and regression line are depicted in Figure 8.

In order to compare the results of the experiments with other references, turbidity measurements were calibrated against suspended solids (SS) analyses. Suspended solid analyses were performed according to the standard methods (APHA, 1992). The calibration data are depicted in Figure 9. Calibration function is:

Suspended solids (mg/L) = 10.886 * Turbidity (NTU) + 56.596 .....(3)

The R-square value for this function is 0.8461.

C. Measurement of surfactants

It is known that algae cultures contain a complex mixture of different surfactants, so the total activity of the surfactants was measured, rather than their mass. Wilson and Collier (1972) showed that foam volumes obtained by shaking and especially the foam decay time produced a more sensitive measure of the surfactants than did the instrument. In our experiments, the foam decay time was used to measure the activity of the surfactants.

The foam decay times were measured in the 15 ml test tubes which were filled with 10 ml samples by first shaking the samples by hand, with 30 strokes of the forearm within a 15 second period, then placing the tubes on the table and recording the period of time from the cessation of shaking to the disappearance of the bubbles. Five measurements were made for each sample and the average value was used as the foam decay time.

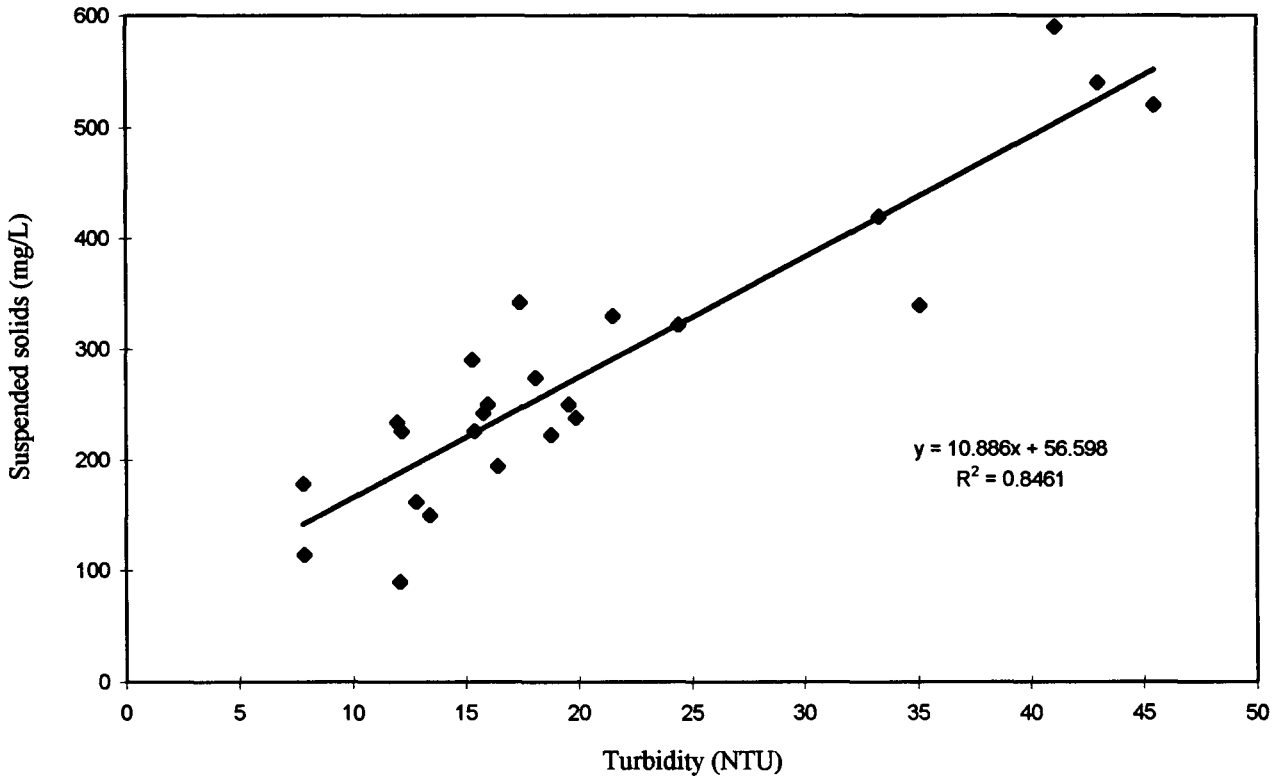


Figure 9. Turbidity vs. suspended solids

D. Algae concentration

1. Batch operation

Foam fractionation was used to concentrate algae in the experiments. The foam fractionator design used for batch operation was a slight alteration of a design previously presented by Chen et al., (1993b). A schematic diagram of this foam fractionator is presented in Figure 10. It consisted of a feed supply system, a bubble generation system, a foam creating column and a foam collection system.

The feed supply system consisted of a water pump and a control valve to introduce algae into the foam creating column. The bubble generation system included an air pump to generate compressed air, a flow meter and a control valve to control air flow rate, and a diffuser (Sweetwater fine-pore diffuser AS-4-0) to produce air bubbles. The air flow rate ranged from 9.3 to 11.8 L/min during the experiments. The foam creating column was made from a clear PVC pipe (Harrington 400CL-060). The diameter and height of the column were 2.0 m and 15.24 cm respectively. The foam created on top of the liquid was forced by gage pressure into the foam collector through a foam collection pipe.

Batch operation was used to test the maximum algae removal rate by foam factionation and the relationship between biosurfactant activity in the algae culture and the algae removal rate by foam fractionation. Configuration of the foam fractionator was not included in this research, so a typical configuration for aquaculture system was used. The operating variables (e.g., air flow rate and foam collection height) were adjusted according to the experimental purpose and equipment capacity.

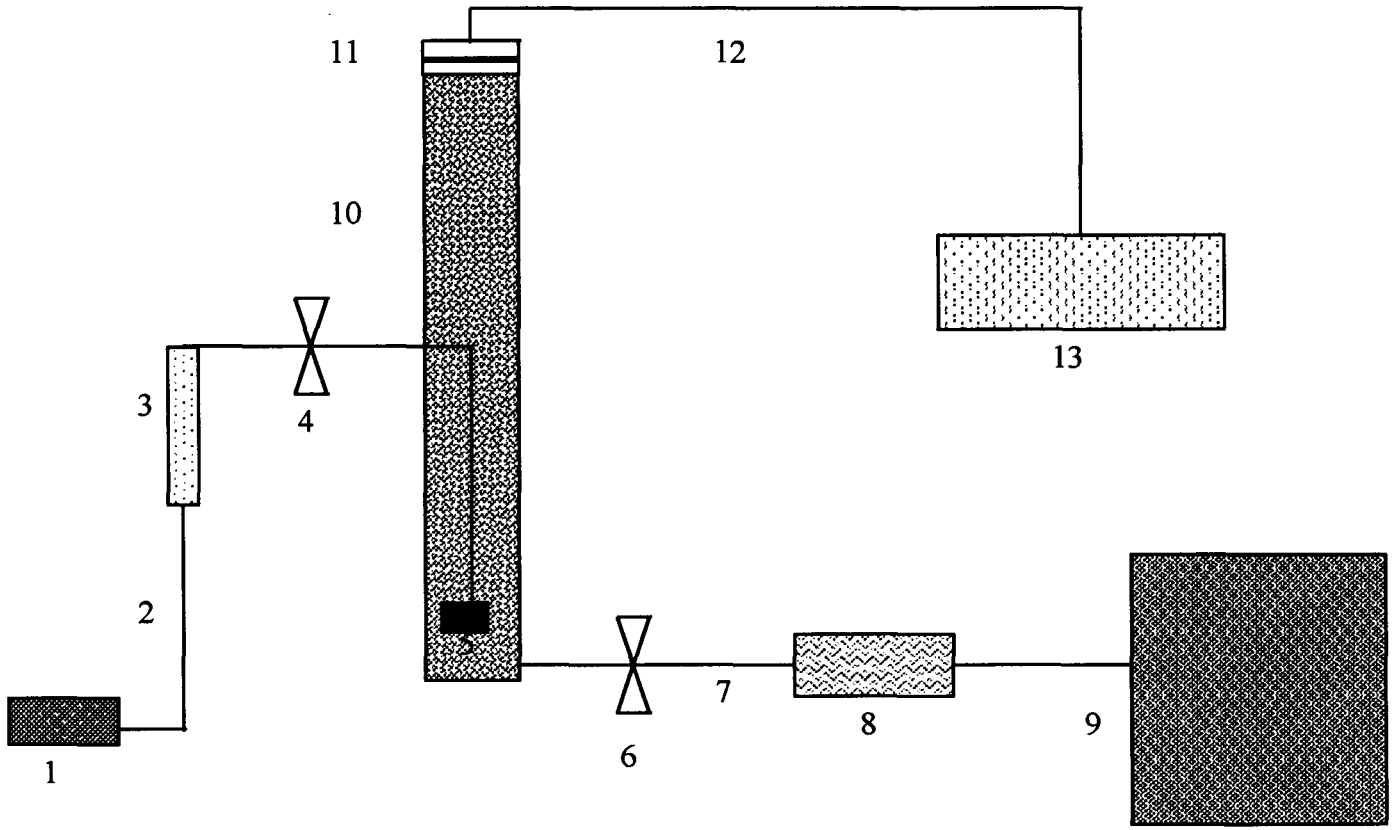


Figure 10. The experimental apparatus for batch operation

1. air pump, 2. air line, 3. air flow meter, 4. air control valve, 5. diffuser, 6. water control valve, 7. feeding and draining line, 8. water pump, 9. algae culture, 10. PVC column, 11. PVC cap, 12. foam collection tube, 13. foam collector

2. Continuous operation

The foam fractionation design used for continuous operation was an alteration of a design used by Weeks et al. (1992). A picture of this foam fractionator is presented in Figure 11 and a schematic diagram is depicted in Figure 12. It also consisted of an algae supply system, a bubble generation system, a foam creating column and a foam collection system.

The algae supply system consisted of a submersible water pump in the algae holding tank and delivered algae culture into a foam creating column through a water flow meter and a control valve. The water flow rate was 15 L/min during the experiments. Two diffusers (Sweetwater fine-pore diffuser AS-4-0) were connected to Anuenue Hatchery's (located in Sand Island, Oahu) compressed air line to produce air bubbles. The air flow rate was around 10 L/min during the experiments. The foam creating column was made from a clear PVC pipe. The diameter and height of the column were 2.0 m and 15.24 cm respectively. The foam created on top of the liquid was forced by gage pressure into a foam collector through a reverse funnel and foam collection pipe.

Continuous operation was used to continuously concentrate algae from the algae culture. It was used to test the possibility and efficiency of using a foam fractionator for algae concentration.

Figure 11. Microalgae harvesting system

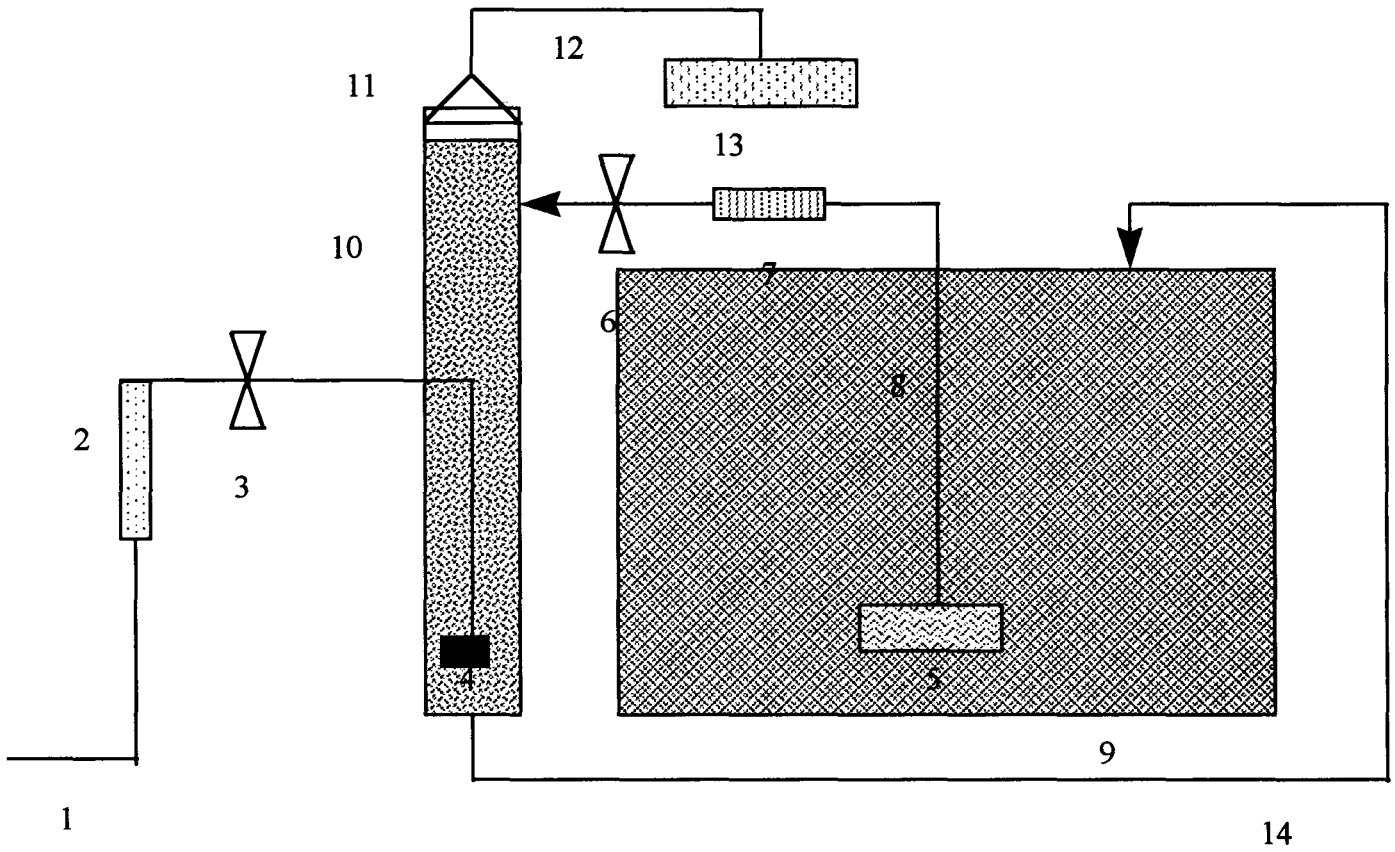


Figure 12. The experimental apparatus for continuous operation

1. air line, 2. air flow meter, 3. air control valve, 4. diffuser, 5. water pump, 6. water control valve, 7. water flow meter, 8. algae feeding line, 9. algae holding tank, 10. PVC column, 11. funnel, 12. foam collection tube, 13. foam collector, 14. algae return line.

Chapter 4. RESULTS

A. The microalgae concentrating factors by foam fractionation

The microalgae concentrating factor is defined as the ratio of the microalgae density in foam condensate to the microalgae density in algae culture. A series of experiments was performed to test the microalgae concentrating factors by foam fractionation. The results are summarized in Table 6.

Table 6. Concentrating factors by foam fractionation

Date	Turbidity of algae culture (NTU)	Turbidity of foam condensate (NTU)	Concentration factors
Jan. 29th, 1996	12	2708	225.7
Jan. 31st, 1996	5.5	275	47.6
Feb. 2nd, 1996	13.7	284	20.7
Jul. 27th, 1996	20.4	667	32.6
Jul. 29th, 1996	16.6	858	51.7
Jul. 30th, 1996	9.9	110	11.1
Jul. 31st, 1996	29.5	800	27.1

The concentrating factor reached as high as 225 in the experiments, and the average was 60. This indicates that foam fractionation is a good way to concentrate microalgae from fairly diluted algae culture, but in order to make this technique provide steady and efficient performance for concentrating microalgae, it is critical to find out the reasons for the variation of the performances. This variation can either come from the variation of algae cultures in the experiments or come from the unstable performances of the foam fractionator. So, a series of experiments was performed to test the repeatability of the foam fractionator.

B. Repeatability of foam fractionation

The repeatability of the foam fractionator was tested by repeatedly extracting microalgae from the same batch of algae culture five to six times in each of the four days. The results are listed in Table 7.

Table 7. Repeatability of the foam fractionator

Date	No. of experiment	Initial NTU	Final NTU	Foam NTU	Concentrating factor*	Removal efficiency**
27-Jul-96	Experiment 1	19.6	10.2	668	34.1	48
27-Jul-96	Experiment 2	20.3	9.09	661	32.6	55.2
27-Jul-96	Experiment 3	20.3	10.3	630	31	49.3
27-Jul-96	Experiment 4	21	9.51	672	32	54.7
27-Jul-96	Experiment 5	21	9.43	702	33.4	55.1
29-Jul-96	Experiment 1	14.7	7.14	751	51.1	51.4
29-Jul-96	Experiment 2	15.3	6.7	867	56.7	56.2
29-Jul-96	Experiment 3	15.6	7.14	751	48.1	54.2
29-Jul-96	Experiment 4	17.5	6.57	990	56.6	62.5
29-Jul-96	Experiment 5	18.2	7.35	881	48.4	59.6
29-Jul-96	Experiment 6	18.3	7.45	905	49.4	59.3
30-Jul-96	Experiment 1	8.34	6.29	104	12.5	24.6
30-Jul-96	Experiment 2	9.54	7.29	104	10.9	23.9
30-Jul-96	Experiment 3	9.61	6.91	94.1	9.79	28.1
30-Jul-96	Experiment 4	10.5	6.98	121	11.5	33.5
30-Jul-96	Experiment 5	10.6	7.2	125	11.8	32.1
30-Jul-96	Experiment 6	10.7	7.2	110	10.3	32.7
31-Jul-96	Experiment 1	28.5	20.2	719	25.2	29.1
31-Jul-96	Experiment 2	28.7	21.7	743	25.9	24.4
31-Jul-96	Experiment 3	29.3	20.1	830	28.3	31.4
31-Jul-96	Experiment 4	29.5	20.2	850	28.8	31.5
31-Jul-96	Experiment 5	30.1	20.1	800	26.6	33.2
31-Jul-96	Experiment 6	30.8	20	860	27.9	35.1

* Concentrating factor: The ratio of algae density in the foam condensate to algae density in the algae culture

** Removal efficiency: The ratio of algae density after concentrating to before concentrating

Two indexes, concentrating factor and algae removal efficiency, were used to measure the variation of the performances. The experimental results are depicted in

Figure 13 and Figure 14. Both the concentrating factor and algae removal efficiency were very stable for the same batch of algae, but there was a significant difference between different batches.

Using the one-way random effects model (Shoukri, 1996), Let Yij (concentrating factor and algae removal efficiency) denote the jth day on the ith experiment (i=1,2,3,4,5,6; j=1, 2,3,4). The intraclass correlation r_1 is calculated as an index of repeatability:

$$r_1 = (MSB-MSW)/(MSB+(n_0-1)MSW) \quad \text{.....(4)}$$

MSB: Mean squares between group;

MSW: Mean squares within group;

$$n_0 = \frac{1}{k-1}\left(N-\frac{\sum_{i=1}^{k} n_i^2}{N}\right) \quad \text{.....(5)}$$

$$N = \sum_{i=1}^{k} n_i \quad \text{.....(6)}$$

The intraclass correlation for the algae removal efficiency and concentrating factor in the experiments were 0.93 and 0.98 respectively. These are extremely good repeatability score based on the "standard values" of comparison which are: excellent (>.75), good (.40-.75) and poor (<.40).

Using ANOVA for the one-way random effects model to test the differences between different batches of algae, the results are listed in Table 8 and Table 9.

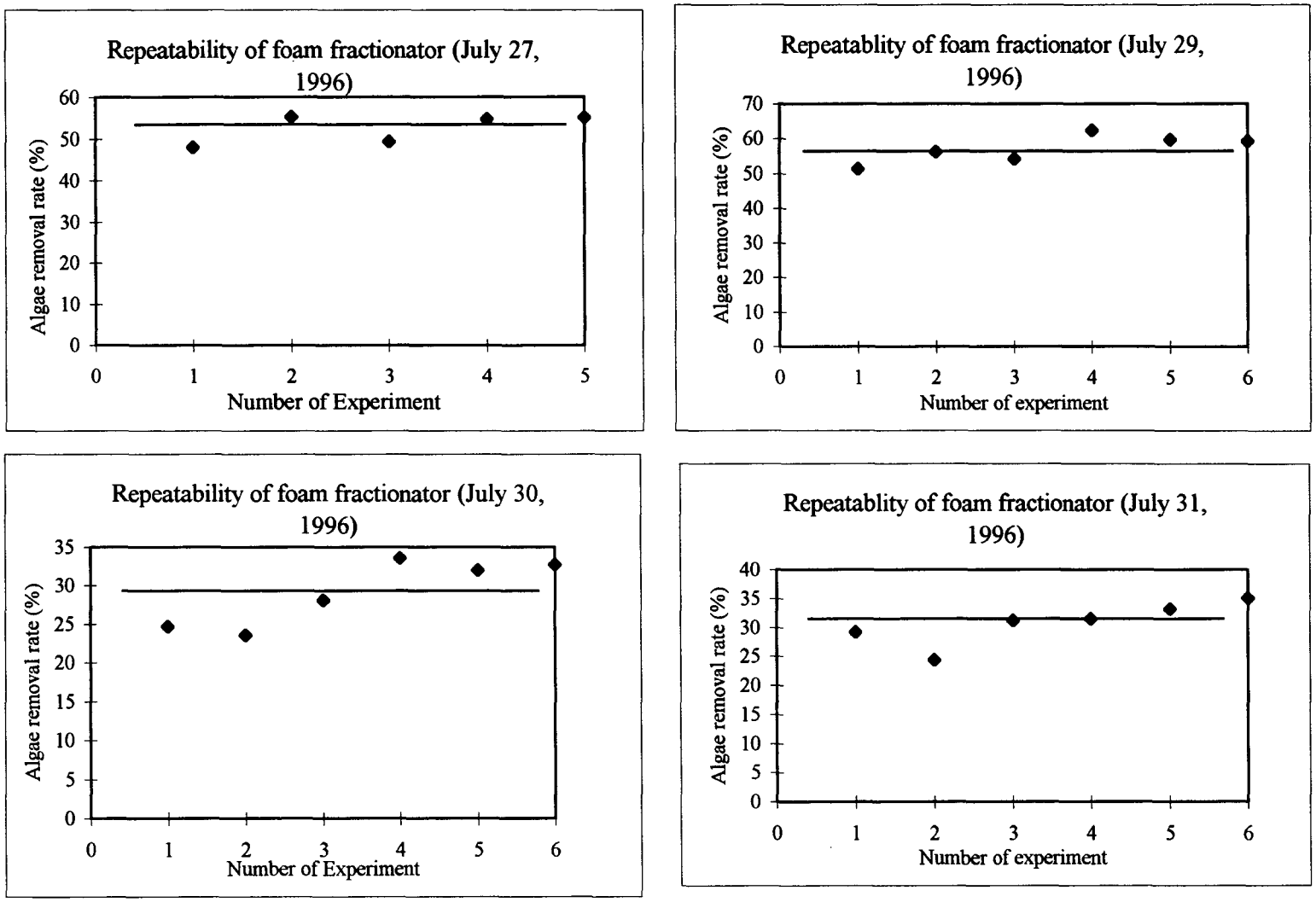


Figure 13. Repeatability of foam fractionation (algae removal efficiency)

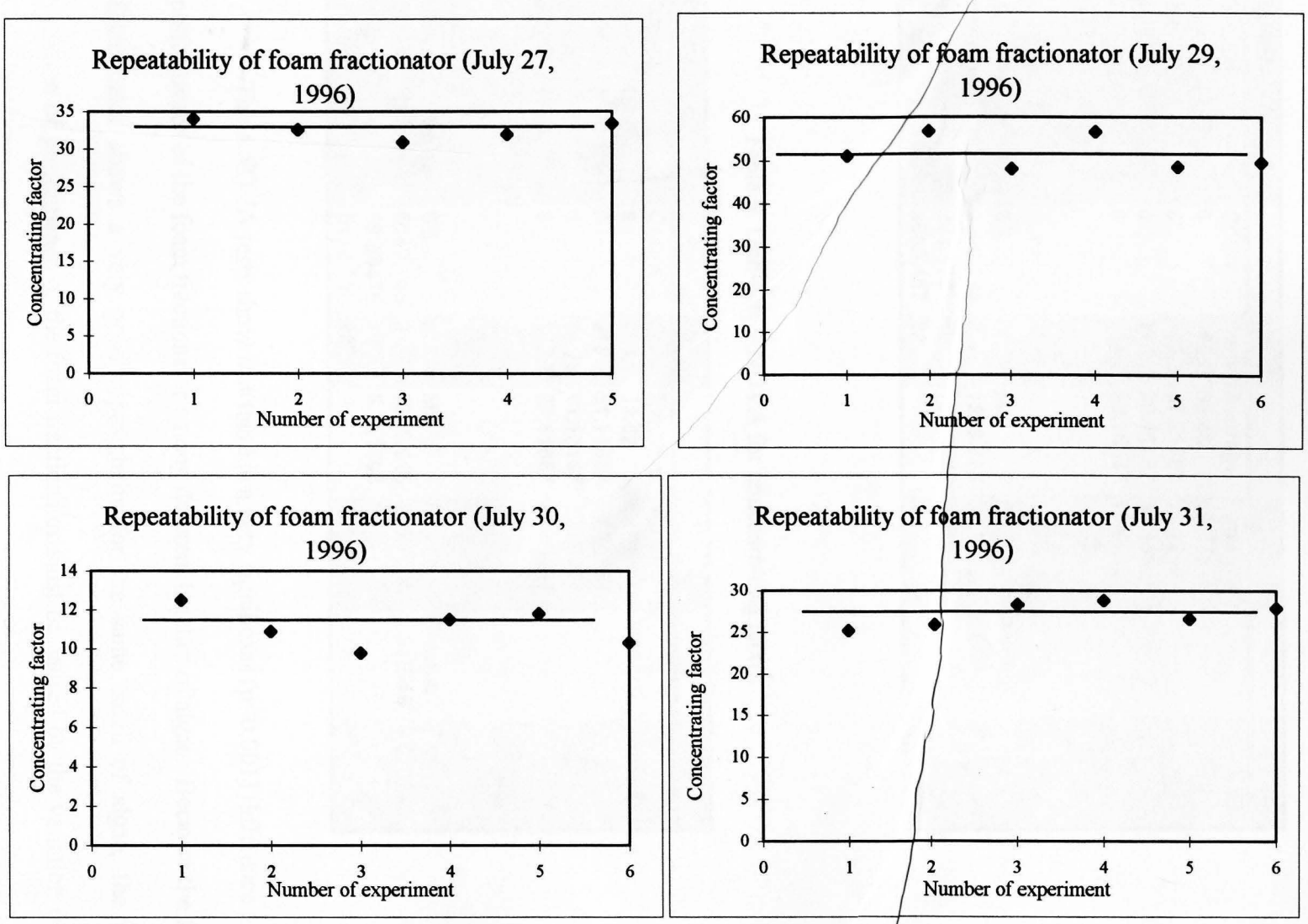


Figure 14. Repeatability of foam fractionation (concentrating factor)

Table 8. One-way ANOVA for algae removal efficiency

SUMMARY						
Groups	Count	Sum	Average	Variance		
July 27, 1996	5	262.3	52.46	12.343		
July 29, 1996	6	343.3	57.21667	16.22967		
July 30, 1996	6	174.7	29.11667	18.44567		
July 31, 1996	6	184.6	30.76667	13.68267		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3689.295	3	1229.765	80.24927	5.66E-11	5.010293
Within Groups	291.162	19	15.32432			
Total	3980.457	22				

Table 9. One-way ANOVA for concentrating factor

SUMMARY						
Groups	Count	Sum	Average	Variance		
July 27, 1996	5	163.1	32.62	1.452		
July 29, 1996	6	310.3	51.71667	15.70167		
July 30, 1996	6	66.79	11.13167	1.000017		
July 31, 1996	6	162.7	27.11667	2.053667		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5042.745	3	1680.915	320.7056	1.91E-16	5.010293
Within Groups	99.58475	19	5.241303			
Total	5142.33	22				

The ANOVA tests show that there is a very significant ($p<0.001$) difference in the performance of the foam fractionator among different batches of algae. Because the foam fractionator shows a very good repeatability for the same batch of algae, the daily variation of performance of the foam fractionator must be caused by the variation of the

algae cultures. It is very important for setting up a microalgae culturing and harvesting system to find the reasons for these variations.

C. Repeatability of foam decay time for measuring activity of surfactant

According to Wilson and Collier (1972), foam decay time produced a very sensitive measurement of the surfactant. It was more sensitive than instruments such as the DuNouy Tensionmeter and Oswald Viscosimeter. But, they did not check the repeatability of foam decay time for measuring activity of surfactant.

The repeatability of using foam decay time to measure activity of surfactant was tested by repeatedly measuring foam decay time for the same sample five times on each of thirteen samples. The results are listed in Table 10.

Table 10. Repeatability of foam decay time (seconds)

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
Sample 1	4	4	5	4	5
Sample 2	7	8	8	7	9
Sample 3	10	8	12	12	14
Sample 4	7	8	7	6	7
Sample 5	14	14	9	13	11
Sample 6	9	10	8	10	12
Sample 7	13	9	15	14	11
Sample 8	20	23	44	22	50
Sample 9	33	43	33	44	25
Sample 10	20	26	40	30	58
Sample 11	40	42	18	20	60
Sample 12	80	100	110	60	140
Sample 13	120	100	90	80	110

Using the one-way random effects model, let Yij (foam decay time) denote the jth sample on the ith experiment (i=1,2,...5; j=1, 2,...13). The intraclass corelation r_1 is calculated as an index of repeatability.

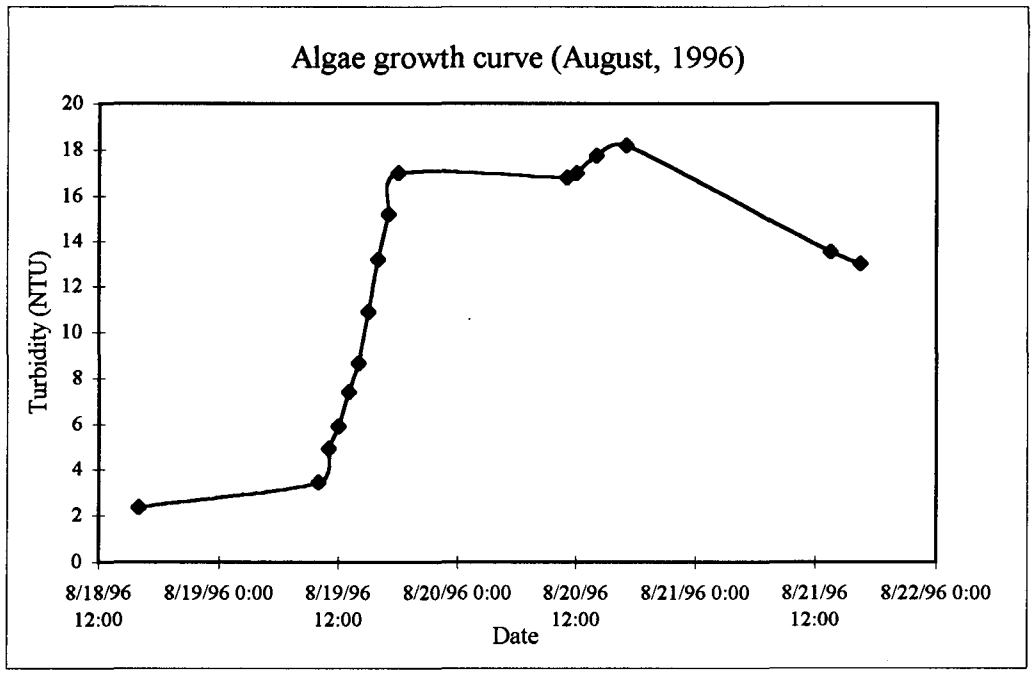
The intraclass corelations calculated from the experiment data was 0.87. This is a good repeatability score. So, foam decay time for measuring activity of surfactant is not only sensitive, but also repeatable.

D. Microalgae growth curve

A series of experiments was conducted to test the growth curve of microalgae. The results are presented in Figure 15. Although an open outdoor system was used to culture microalgae, i.e., culturing condition was changing daily, the microalgae growth followed a similar pattern. After seeding, the microalgae stayed in a lag growth phase during the first night. An exponential growth phase appeared the next morning and lasted to the next evening. Then the microalgae stopped growing and entered a stationary growth phase. From the afternoon of the third day, the microalgae began to die starting a decay phase.

A batch algae culture usually follows a four stage pattern, i.e., lag growth phase, exponential growth phase, stationary growth phase and decay phase, but the period in each stage depends on the culture condition and algae species. In the experiments, the mixed algae population was dominated by *Chaetoceros gracilis*.

Chaetoceros gracilis was one of the fastest growing algae species. It had two to four divisions per day during its exponential growth phase in summer. Because of its fast



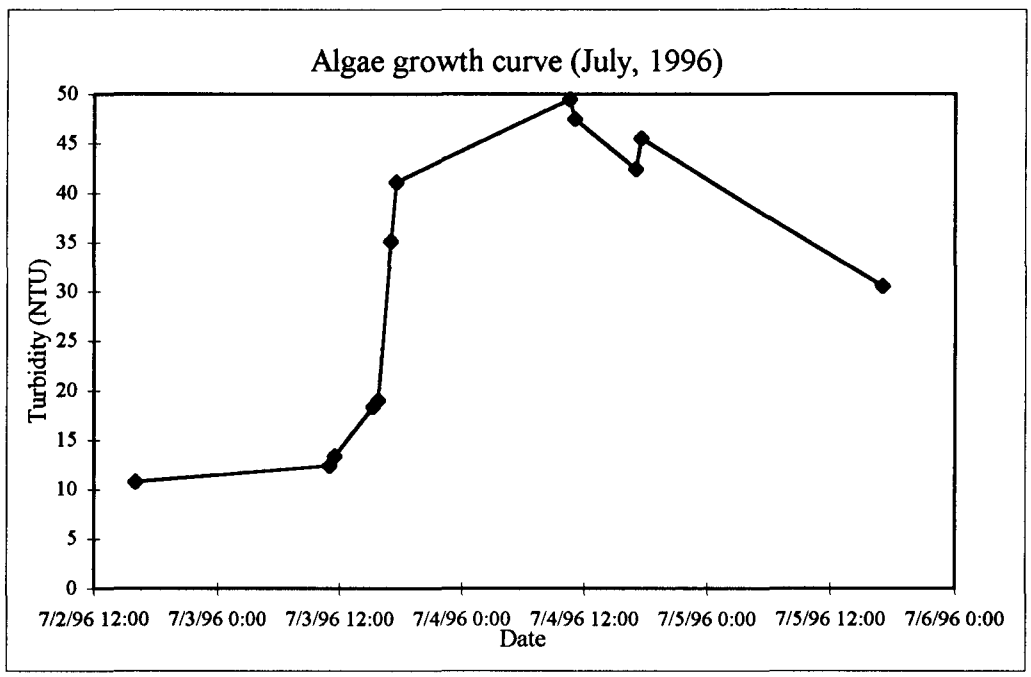


Figure 15. Algae Growth Curve

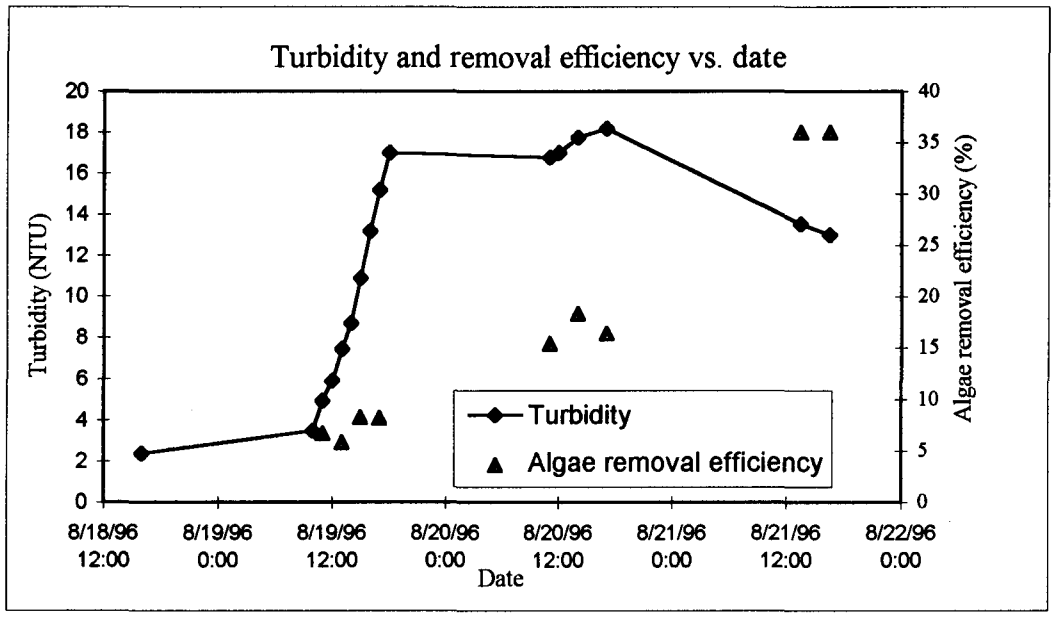
growth, *Chaetoceros gracilis* moves from one stage to another very quickly. The exponential growth phase in the experiments lasted for one day only when starting algae concentration was around $5*10^5$ cells per milliliter.

Similar results were found by Myklestad et al. (1989) when *Chaetoceros affinis* was investigated in a batch culture. The growth rate of *Chaetoceros affinis* was 3.8 divisions per day and the exponential growth phase lasted less than two days. A short transition time between different stages was found also.

Fast growth and short transition time between stages are the characteristics of the growth curve of *Chaetoceros*. It is very important to use these characteristics to manage the naturally mixed algae population and keep *Chaetoceros* dominant. Because *Chaetoceros* is a fast growing species, it dominants the mixed population quickly. It was the dominant species all year round when algae seed was started from surface sea water; but after *Chaetoceros* bloomed, new media had to be added in the same day, otherwise *Chaetoceros* would get into the stationary phase and the other species would catch up and take over. So, for the semi-continuos culture, seeding time and seeding rate are crucial for maintaining a *Chaetoceros* dominant culture.

E. Foam fractionation performance during microalgae growth

A series of experiments were performed to test foam fractionation performance during microalgae growth. The results are presented in Figure 16. Algae removal efficiency was very low in the lag growth phase and the exponential growth phase. When



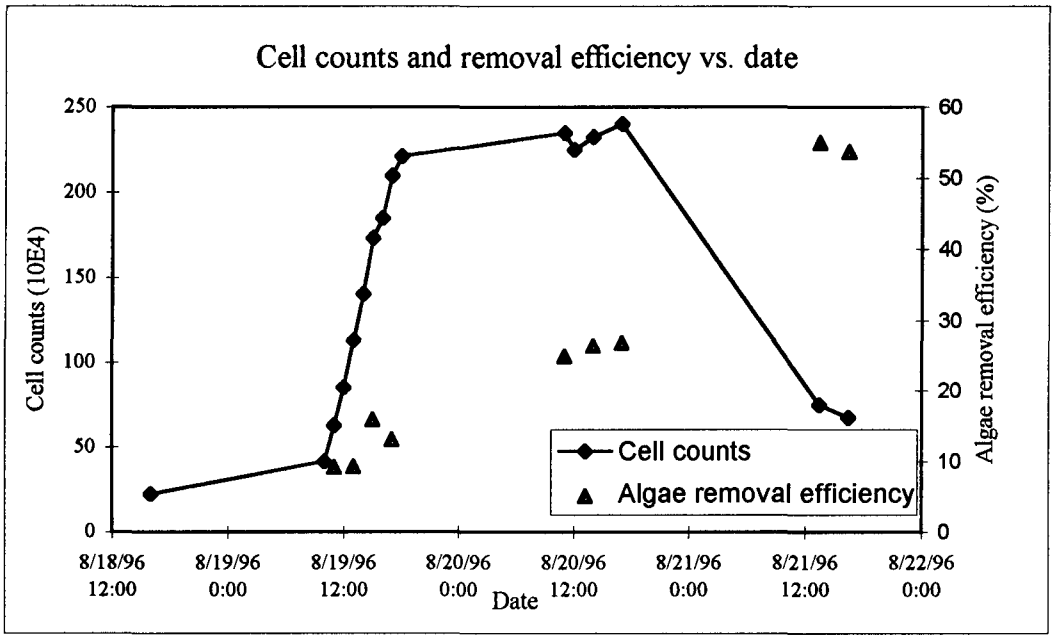


Figure 16. Foam fractionation performance during algae growth

the stationary growth phase was reached, algae removal efficiency was much higher. In the decay phase, algae removal efficiency was the highest, but the algae began to decompose during the decay phase. So the best time to harvest algae was in the stationary phase.

Although a sufficient increase in algae removal was noted when the stationary growth phase was reached, the results were still far below those desired. Even in the stationary phase, algae removal efficiency was only 20% according to NTU changes. The results from the cell count were a little more encouraging. The algae removal efficiency in the stationary phase was 30% and in the decay phase, the algae removal rate was higher than 50%.

The difference between NTU measurements and cell counts came from the nature of the mixed algae population in the experiments. Although *Chaetoceros* was the dominant species in the culture, other species like *Thalassiosira* was found also. Because *Thalasiossira* is much larger than *Chaetoceros*, it contributes more to NTU measurements than cell counts. On the other hand, foam fractionation is much more efficient in removing small particles than the larger ones.

Several experiments were conducted to test the concentration factor for *Thalassiosira* and the results are list in Table 11.

Table 11. Concentration factors by foam fractionation for *Thalassiosira*

Date	Turbidity of algae culture (NTU)	Turbidity of foam condensate (NTU)	Concentration factors
Jan. 22th, 1996	11.5	83	7.2
	10.3	79	7.7
	10.5	79	7.5
	28.4	232	8.2

The average concentration factor for *Thalassiosira* was only 7.65. Compared to the value of the concentration factor for *Chaetoceros*, 7.65 is much lower.

Although the algae removal efficiency was still less than expected, the results are comparable with other algae harvesting techniques. Bare et al. (1975) reported that only ten percent of the algae removal efficiency was achieved by using dissolved air flotation without combination with coagulants in the exponential growth phase of algae. The algae removal efficiency was increased to sixty percent when algae in the decay phase was used. But when dissolved air flotation was combined with coagulation, up to 90 percent of algae removal was achieved in Bare's experiments.

F. Biosurfactant activity during microalgae growth

Biosurfactant activity was measured by the foam decay time during microalgae growth. The results are presented in Figure 17. In the lag growth phase, the rapid decay of foam (less than 10 seconds) indicated insignificant production of biosurfactant. The foam decay time in the lag growth phase was only slightly longer than that of the uninoculated media (2-5 seconds). A slow increase of biosurfactant activity was observed during the exponential growth phase, while an abrupt increase of surfactant activity in the culture took place in the stationary growth phase. In the decay phase, surfactant activity reached the highest level.

Experiments showed that the release of surfactant from algae took place in all growth phases and had a marked increase in the stationary phase. This result strongly suggests that healthy cells excrete surfactant during growth and this should be interpreted

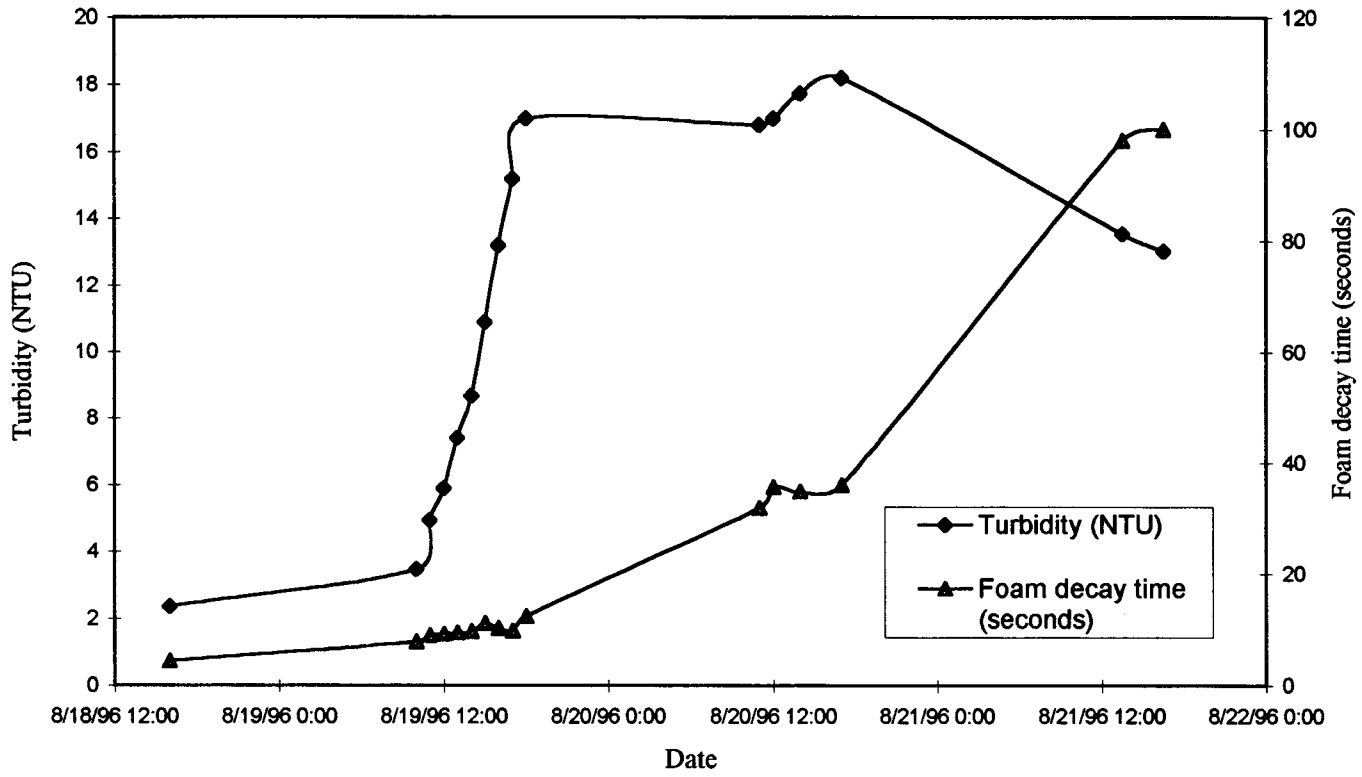


Figure 17. Biosurfactant activity during algae growth

as a natural physiological phenomenon in algae. The sharp increase of surfactant activity in the decay phase may be caused by leakage from cells in poor conditions.

The surfactant property during growth of *Chaetoceros gracilis* in the experiment was generally similar to that of the *Chaetoceros galvestonensis* which was reported by Wilson and Collier (1972). They found that the maximum foam decay time for *Chaetoceros galvestonensis* culture was about 120 seconds and occurred following the attainment of a maximum population level.

G. Relationship between biosurfactant activity and foam fractionation performance

From the previous experiments, there was a dramatic change of biosurfactant activity and foam fractionation performance during microalgae growth. The similar trend of these changes predicts that there is a close relationship between these changes. The biosurfactant activity and algae removal efficiency during algae growth is presented in Figure 18. When biosurfactant activity increased, the algae removal efficiency increased as well.

Foam fractionation concentrated not only microalgae but also biosurfactant in the foam condensate at the same time. After separating the solid part from the liquid part in the foam condensate by centrifuging, the solid part, algae paste, is the final product expected and the liquid part contains a very high activity of biosurfactant. The foam decay time for this liquid was longer than one hour and sometimes longer than one day. This liquid mixed with the algae culture and used to increase the activity of biosurfactant.

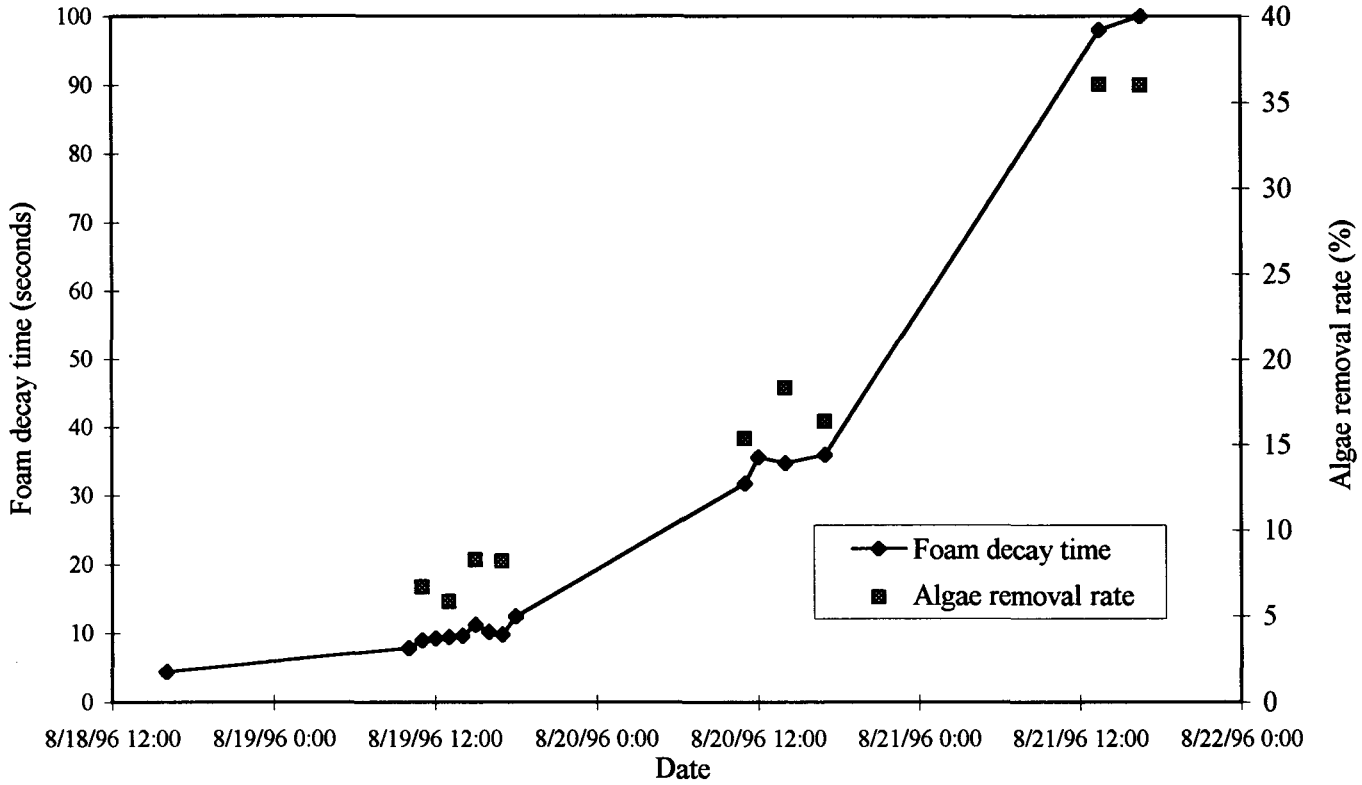


Figure 18. Biosurfactant activity and foam fractionation performance during algae growth

A series of experiments was conducted using the mixture of algae culture and liquid from foam condensate to test the relationship between the activity of biosurfactants in the algae culture and algae removal efficiency by the foam fractionator. The results are presented in Figure 19. A logarithmic relationship was found between the activity of biosurfactant and algae removal efficiency during the experiments. When using foam decay time as the index for the activity of biosurfactants and turbidity as the density of microalgae, the relationship between foam decay time and algae removal efficiency could be summarized as equation:

Algae removal efficiency = 14.374Ln(foam decay time) - 19.492 .....(7)

The R-square value for this equation was 0.84. Up to seventy percent of the algae was removed when the foam decay time was longer than 500 seconds in the algae culture.

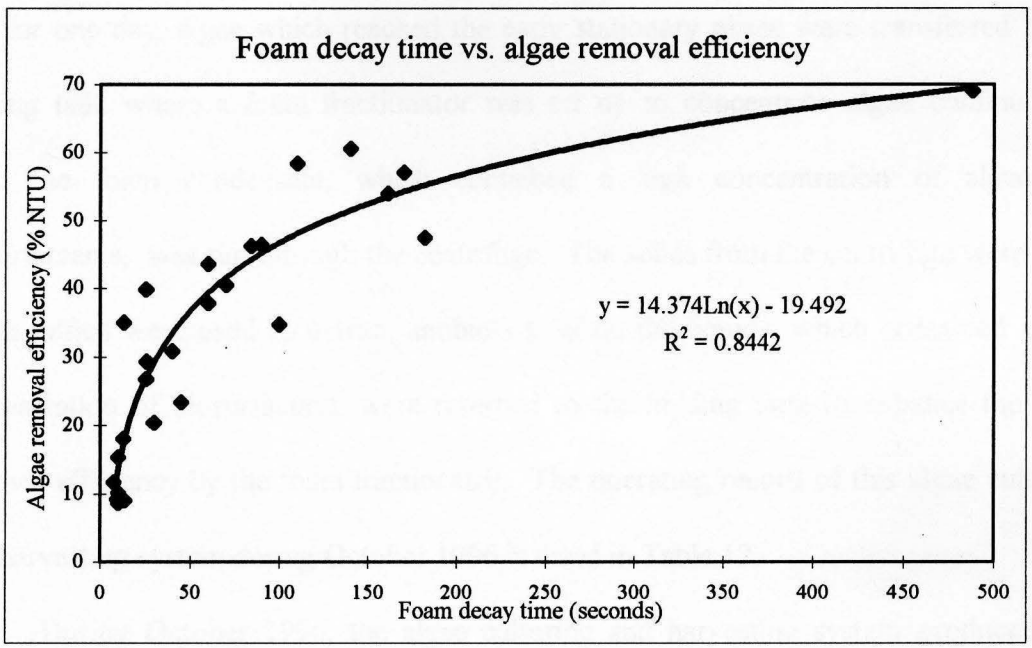
When using *Chaetoceros* cell counts as the index of algae density, the relationship between the foam decay time and algae removal efficiency could be expressed as:

Algae removal efficiency = 22.745Ln(foam decay time) - 40.564 .....(8)

The R-square for this equation was 0.95. More than ninety percent of the *Chaetoceros* was removed when the foam decay time was longer than 500 seconds in the algae culture.

H. Semi-continuous algae culturing and harvesting system

Based on batch operations, a semi-continuous algae culturing and harvesting system was set up. A schematic diagram of this system is presented in Figure 20. The algae culturing system consisted of sixteen operating units which is described previously in the method section. The algae harvesting system consisted of a holding tank (1500 liter),



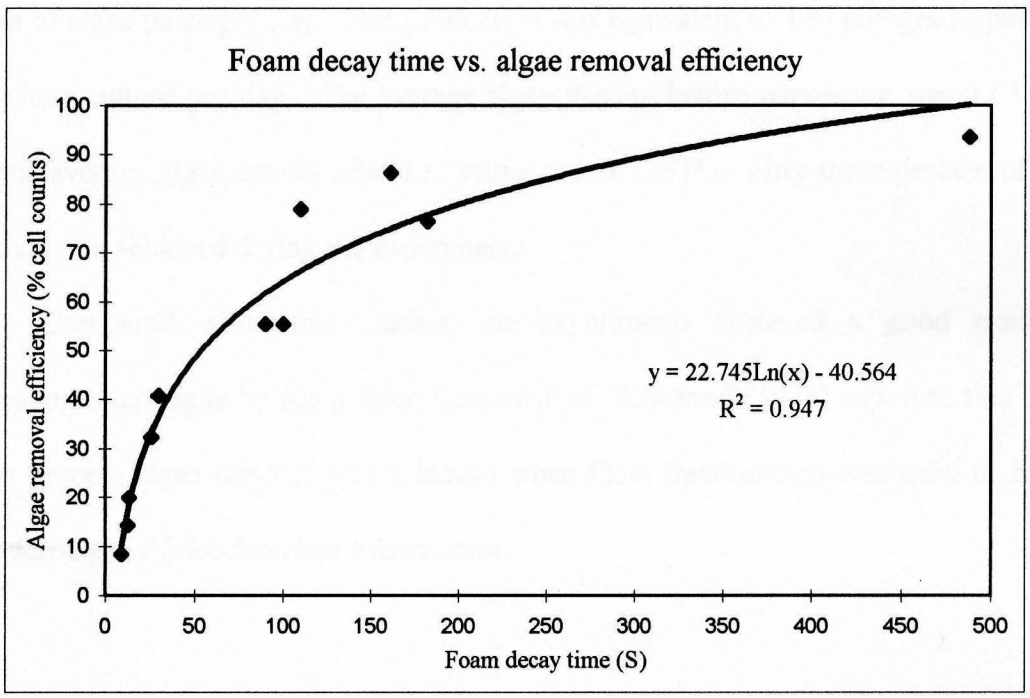


Figure 19. Relationship between biosurfactant activity and algae removal

a foam fractionator and a centrifuge (Beckman J2-HS). After growing in the operating unit for one day, algae which reached the early stationary phase were transferred to the holding tank where a foam fractionator was set up to concentrate algae continuously. Then the foam condensate, which contained a high concentration of algae and biosurfactants, was run through the centrifuge. The solids from the centrifuge were algae pastes which were used to extract antibiotics, while the liquids, which contained a high concentration of biosurfactant, were returned to the holding tank to enhance the algae removal efficiency by the foam fractionator. The operating record of this algae culturing and harvesting system during October 1996 is listed in Table 12.

During October 1996, the algae culturing and harvesting system produced 207 grams of algae paste per day. This production was equivalent to 140 milligrams paste per liter algae culture per day. The average algae density before harvesting was 12.1 NTU and the average algae density after harvesting was 5.3 NTU. Fifty-three percent of algae removal was achieved during the experiments.

Compared with other studies, the experiments achieved a good result for harvesting microalgae by using foam fractionation. Raymond (1978) reported that thirty-seven percent algae removal was achieved when foam fractionation was used to harvest the microalgae *Phaeodactylum tricornutum*.

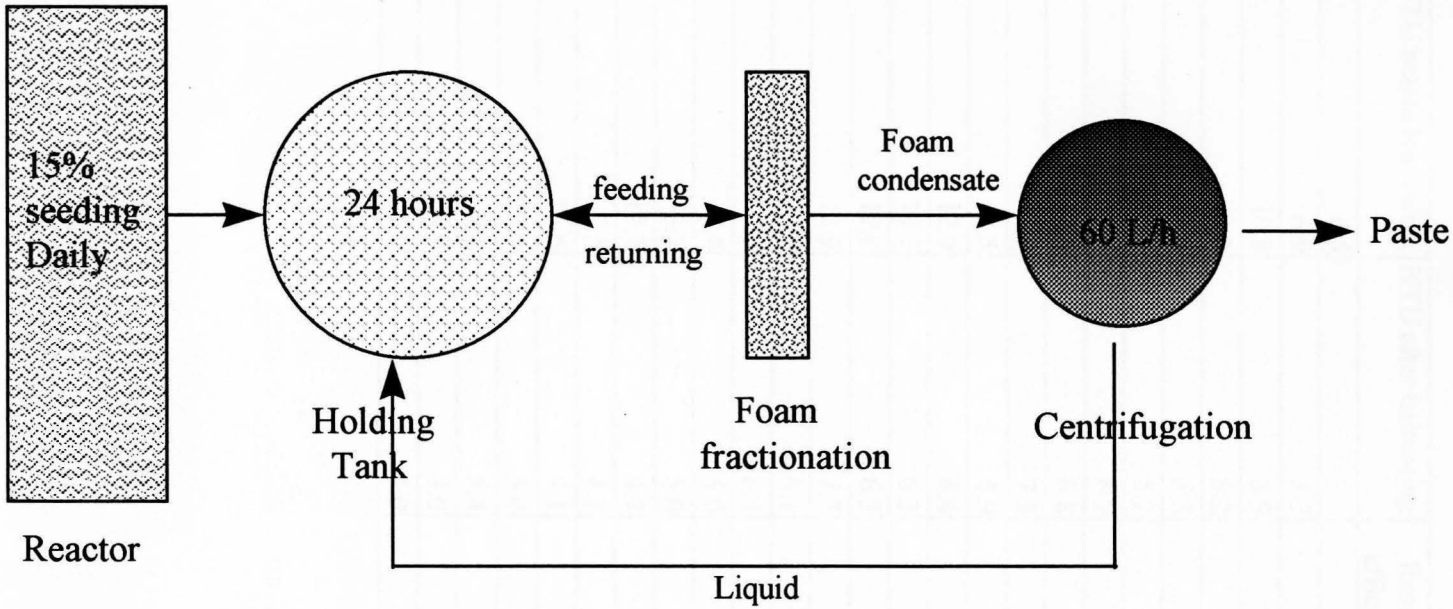


Figure 20.Microalgae culturing and harvesting system

Table 12. Operating record of algae culturing and harvesting system during October 1996

Date	NTU before harvesting	NTU after harvesting	Removal efficiency	Paste(g)
10/3/96	6.6			
10/4/96	6.9	3.7	0.44	88
10/5/96	10.8	3.9	0.43	124
10/6/96	14.2	5.2	0.52	176
10/7/96	13.5	5.3	0.63	198
10/8/96	16.6	5.2	0.61	212
10/9/96	17.9	6.2	0.63	280
10/10/96	17.5	6.8	0.62	298
10/11/96	9.2	7.3	0.58	278
10/12/96	8.5	4.9	0.47	162
10/13/96	12.9	4.4	0.48	160
10/14/96	14.5	6.3	0.51	215
10/15/96	17.0	6.3	0.57	257
10/16/96	12.9	7.3	0.57	289
10/17/96	14.7	6.1	0.53	225
10/18/96	9.5	6.3	0.57	270
10/19/96	7.4	5.0	0.47	165
10/20/96	8.3	5.0	0.32	107
10/21/96	12.7	4.7	0.43	110
10/22/96	7.5	4.1	0.68	298
10/23/96	15.6	4.1	0.45	140
10/24/96	19.6	5.4	0.65	305
10/25/96	8.5	5.4	0.72	350
10/26/96	6.9	4.0	0.53	175
10/27/96	8.0	5.0	0.28	96

Chapter 5. DISCUSSION

Foam fractionation has shown a very impressive potential for microalgae concentration in the experiments. Unlike the findings of Shelef et al. (1984) that the reliability of using foam fractionation for algae concentration was low, the experiments showed a very stable performance when same batches of algae were used. The variation of using foam fractionation for algae concentration was caused mainly by the variation of algae cultures. So this research concentrated on two questions: what changes in algae culture cause the variation of using foam fractionation for algae concentration and how these changes can be used to improve the performance of foam fractionation.

Although performances of foam fractionation are also dependent upon the geometry of the foam fractionators and operating variables (Chen et al., 1993b and Weeks et al., 1992), the operational and design parameters were not included in this research. A typical design of foam fractionation for aquaculture systems was used and the foam fractionators were operated in normal conditions.

The presence of biosurfactants in the algae culture was found to be the major factor which affected the algae removal by foam fractionation in the experiments. Although no attempt was made to identify the biosurfactants, total activity of these biosurfactants was measured by foam decay time in the experiments. Since it is known that algae cultures contain a complex mixture of different classes of biosurfactants, biosurfactant activity provides a quick and simple index to predict the total amount of

biosurfactants present in the algae culture and has been used by many researchers (Wilson and Collier, 1972 and Zutic et al., 1981).

It was reported that biosurfactant activity in algae cultures was related to the age of cultures (Zutic et al., 1981). Experiments showed a similar result that release of surfactants from algae occurred in all growth phases and an accumulation led to a marked increase in concentration during the stationary growth phase. This result suggests that foam fractionation is more efficient when algae are harvested during their stationary growth phase.

Surfactants have been recognized as a key component needed for foam fractionation (Rubin, 1981) for a long time and the release of biosurfactants by microalgae has been reported since the 1950's (Fogg, 1966, 1977, 1983), but no report was found to study the relationship between biosurfactant production in the algae culture and foam fractionation performance for algae concentration.

A logarithmic relationship between biosurfactant activity in the algae cultures and algae removal efficiency by the foam fractionator was found during the experiments. This relationship could be summarized as equation:

Algae removal efficiency = 14.374Ln(foam decay time) - 19.492 .....(7)

when using turbidity as the density of microalgae, or:

Algae removal efficiency = 22.745Ln(foam decay time) - 40.564 .....(8)

when using *Chaetoceros* cell counts as the density of microalgae. The R-square values for these two equations were 0.84 and 0.95 respectively, according to the experiments. This

further confirmed the key role of biosurfactant activity in the performance of foam fractionation.

Up to seventy percent of algae and ninety percent of *Chaetoceros* were removed during the experiments when foam decay time was longer than 500 seconds in the algae culture. It is not practical to keep foam decay time in the algae culture as long as 500 seconds, but the logarithm relationship between the activity of the biosurfactants and algae removal efficiency indicates that a small change of surfactant activity in the low range will dramatically affect the algae removal efficiency. For example, when foam decay time increased from four seconds to one hundred seconds, algae removal efficiency increased from zero to fifty percent. But when foam decay time increased from one hundred seconds to five hundred seconds, algae removal efficiency increased only from fifty percent to seventy percent. Foam decay time is usually far below one hundred seconds in the algae culture, so if additional biosurfactants are added to the algae culture, the algae removal efficiency will increase dramatically.

Foam condensate is a good source of biosurfactants, because foam fractionation concentrates not only microalgae but also biosurfactants in the foam condensate at the same time. After separating the microalgae from the foam condensate through centrifuging, the liquid part which contains a very high activity of biosurfactants could be reused to increase the biosurfactant activity in the algae culture and therefore to enhance algae removal by foam fractionators.

Based on these experiments, a two step algae harvesting technique was developed in our pilot scale algae culturing and harvesting system. Foam fractionation was used as

the first step to preconcentrate algae from fairly diluted algae cultures into foam condensates. The concentrating factor for this step was about fifty. Centrifugation was used as the second step to concentrate foam condensates into algae pastes and the liquid coming out from the centrifuge which contained a high concentration of biosurfactant was returned to the first step to increase the algae removal efficiency of foam fractionation.

Although it is still too early to determine accurately the cost of this two step algae harvesting system, the advantages of this system are obvious. It fully uses the advantages of foam fractionation which is regarded as a relatively economical method to concentrate small particles and can be applied to large volumes of liquid. Because biosurfactants are reused in this system, the final products do not contain any artificial surfactant. It has been proved that this system is a very efficient system with a low maintenance and operating cost.

The two step algae harvesting system achieved certain success in the experiments, but much remains to be improved including the optimal foam fractionation configuration, the optimal operating condition for algae harvesting and an accurate cost estimation.

Chapter 6. CONCLUSIONS

Foam fractionation was proven to be able to concentrate marine microalgae *Cheatoceros gracilis,* which may help reduce microalgae harvesting cost. However, foam fractionation performance varies and needs further study. The biosurfactant produced by microalgae is the major factor affecting foam fractionation performance. By reusing biosurfactant, it is possible to use two step microalgae harvesting technique to harvest microalgae without additional artificial surfactant.

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