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Harvesting of Algae by Froth Flotation

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ABSTRACT

LEVIN, GILBERT V. (Resources Research, Inc., Washington, D. C.), JOHN R. CLENDENNING, AHRON GIBOR, AND FREDERICK D. BOGAR. Harvesting of algae by froth flotation. Appl. Microbiol. **10:**169–175. 1962.—A highly efficient froth flotation procedure has been developed for harvesting algae from dilute suspensions. The method does not depend upon the addition of flotants. Harvesting is carried out in a long column containing the feed solution which is aerated from below. A stable column of foam is produced and harvested from a side arm near the top of the column.

The cell concentration of the harvest is a function of pH, aeration rate, aerator porosity, feed concentration, and height of foam in the harvesting column. The economic aspects of this process seem favorable for mass harvesting of algae for food or other purposes.

One of the major problems in the mass cultivation of unicellular algae for food or other purposes is the lack of an economical method for harvesting the relatively dilute suspensions (Burlew, 1953).

Mention of flotation was made by Gotaas and Golueke (1957*a*) in the course of harvesting investigations carried out at the University of California. As applied there, the procedure required the addition of commercial flotants to the algal suspensions. Details of the process were not given, but the conclusions stated were that flotants were required in quantities that made the method too costly.

Continuously aerating cultures of unicellular algae grown in this laboratory were observed to froth and, on occasions, to produce rings of algae in the culture tubes just above the water level. In some instances, the culture medium was found to be almost devoid of cells with dense deposits of cells on the tube wall above the liquid level. Since no flotant had been added, the cultures themselves must have produced the frothing agent.

MATERIALS AND METHODS

Organisms. The following algal species obtained from the Indiana Culture Collection were tested for froth flotation harvesting: *Chlamydomonas simplex*, *Chlamydomonas inflexa*, *Chlamydomonas* sp. (marine), *Stichococcus bacillaris*, and *Chlamydomonas moewusii*. A high temperature *Chlorella* sp. (optimal temperature 39 C), obtained from Dean Burke of the National Institutes of Health, Bethesda, Md., was also tested.

All were amenable to froth flotation on the basis of preliminary studies. The high temperature strain of *Chlorella* was selected for intensive study since its high reproductive rate makes it of considerable practical interest in mass culturing for food production or for gas exchange in closed environments.

Culturing medium. The composition of the high temperature *Chlorella* medium is given in Table 1. The medium was adjusted to pH 6.8 with 5 N NaOH.

Culturing procedure. High temperature *Chlorella* cultures were grown to the desired densities in 1-gal bottles under cool white fluorescent illumination of approximately 600 ft-c intensity, and continuously aerated with a 5% CO_2-air mixture.

Harvesting procedures. The first approach was an attempt to harvest the algal cells by catching the droplets formed by the bursting of bubbles rising to the surface of aerating cultures. For this purpose, a trough-collar was fitted around the upper rim of a cylindrical culture vessel. Air was introduced into the bottom of the vessel.[2] The rising bubbles burst at the liquid surface and the fragments which were ejected from the culture were collected in the trough. This process was quite inefficient in that large volumes of air were required for small harvests. It did, however, demonstrate that a significant concentration of cells could be achieved, even when due allowance was made for evaporation of the harvest. The process was then altered to collect all froth produced in a manner somewhat similar to froth flotation as practiced in mineralogy.

The form of apparatus employed in subsequent in-

[2] Aeration was conducted at 15 psi in all experiments reported.

TABLE 1. *Composition of medium*

	g/liter
KNO_3	1.0000
$MgSO_4 \cdot 7H_2O$	0.2500
KH_2PO_4	0.2500
Sequestrene $NaFe$*	0.0052 (Fe)
Sequestrene Na_2Mn*	0.0030 (Mn)
Sequestrene Na_2Cu*	0.0010 (Cu)
Sequestrene Na_2Zn*	0.0010 (Zn)
Sequestrene Na_2Co*	0.0010 (Co)

* Geigy Chemical Corporation, Ardsley, N. Y.

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vestigations is shown in Fig. 1. Air is introduced into the bottom of the cylinder through a porous diffuser plate. The rate of aeration is controlled with float type rate meters. The culture to be harvested is introduced into the bottom of the device by gravity flow from an elevated reservoir. Aeration is applied and the froth formed rises up the tube and is delivered through the side arm. Operation may be batch or continuous. In the batch process, the desired amount of culture is introduced into the column, aeration is started and harvesting continues until the froth no longer rises to the elevation of the side arm. The continuous process permits the maintenance of any desired feed concentration in the column. This is achieved by balancing feed against withdrawal to maintain concentration and volume equilibria in the column. This simple apparatus has permitted the investigation of a number of important parameters affecting the quantities and concentrations of algae which may be harvested from suspensions.

Determination of algal concentration. An aliquot of algal suspension is centrifuged to constant volume in a capillary tube graduated to 0.001 ml. Algal concentrations are expressed in terms of packed cell volume (milliliters of cells per ml of suspension).

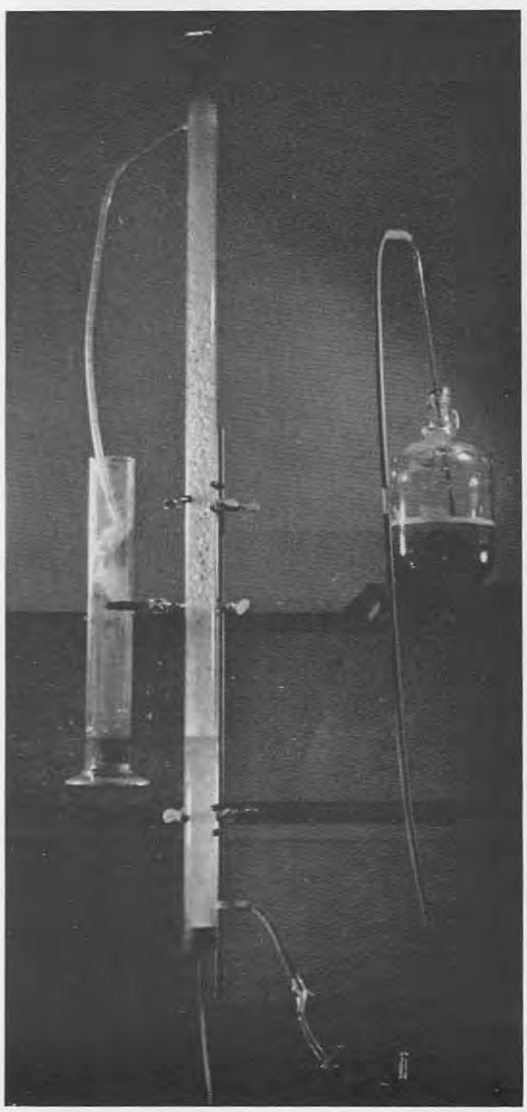

FIG. 1. *Froth flotation harvesting apparatus, batch operation*

RESULTS

Effect of pH on foam stability and harvest concentration. The high temperature strain of *Chlorella* was found to froth without the aid of flotant at the normal culturing pH. After several days of growth, the cultures exhibit a pH of 7.5 to 8.0 although the initial pH of the medium is 6.8. The effect of pH adjustment on the quantity and quality of froth is dramatic. As the pH of the culture is reduced, the foam becomes very dark green, rigid, and quite stable as seen in Fig. 2. The foam produced at a pH of 4.0 or lower will break only after prolonged standing or with the aid of an anti-foaming agent.

The apparatus illustrated in Fig. 1 was used in batchwise operation. A culture was grown from a light inoculum to an age of 7 days. Portions (100 ml) were adjusted to the desired pH values with concentrated HCl. The range selected was pH 2.1 to 4.8 in increments of 0.3. Each sample was transferred to a harvesting column, 2.4 cm diameter by 120 cm high, where air was applied at the rate of 65 standard cm^3 per min. The maximal height to which the foam would rise as a function of the pH was determined, all other variables being held constant. Harvests were made on columns of foam which had been aerated long enough to extend them to a height of approximately 5 cm below the maximal height attainable at the given pH. At this point, aeration was stopped and the top 15 cm of the foam were immediately removed by suction tube. The results, presented in Fig. 3, show the pronounced effect of pH on harvest concentrations. A straight line of least mean squares was fitted to the data. The packed cell volume of the harvest increased as the pH was lowered. However, as the pH decreased below 2, the organisms began to decompose. The effect of pH on foam height is seen in Fig. 4. Until the pH was reduced to approximately 4.5, relatively little frothing took place. At this point, the structure of the foam began to change and the foam height produced became inversely proportional to the pH. This relationship prevailed to a pH of approximately 2 and further pH reduction had little effect on foam height.

Presence of the frothing agent in culture supernatant. A *Chlorella* culture was centrifuged in a refrigerated centrifuge. The supernatant was diluted in fresh culture medium to 0.75, 0.50, 0.25, and 0.125 of the original supernatant concentration. These portions, together with undiluted supernatant control consisting of fresh culture medium, were aerated in the frothing apparatus. The pH was adjusted to 3.0 in all cases. The curve in Fig. 5 shows the frothing height to be directly proportional to the percentage of supernatant present. An attempt is underway to isolate and identify the frothing agent.

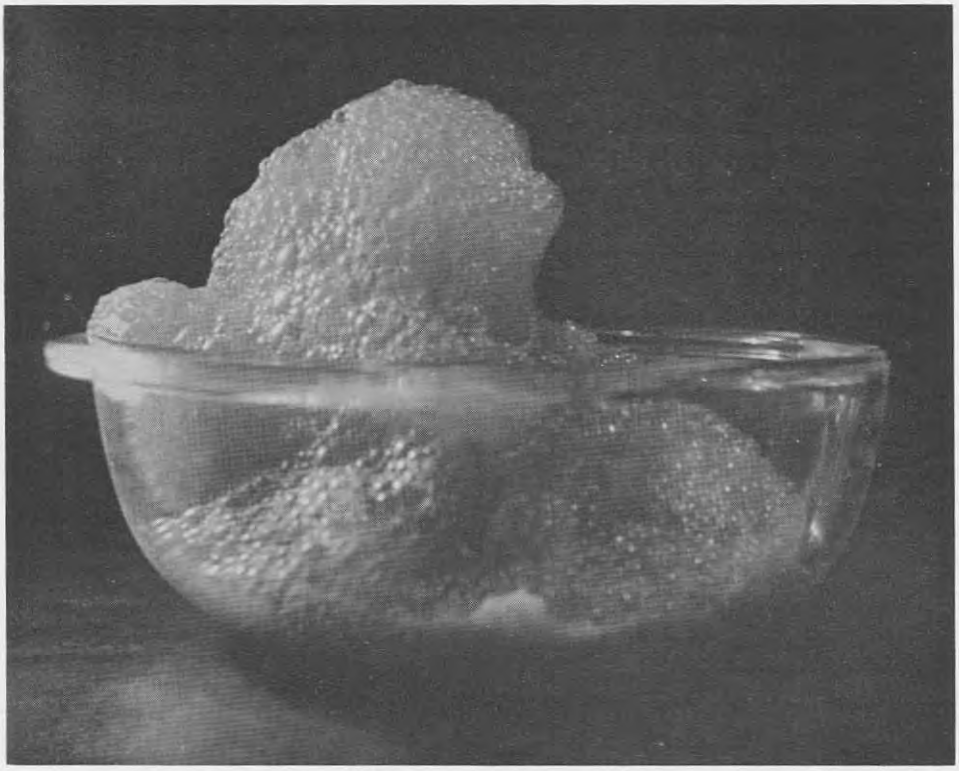

FIG. 2a. *Algal foam produced by froth flotation harvesting*

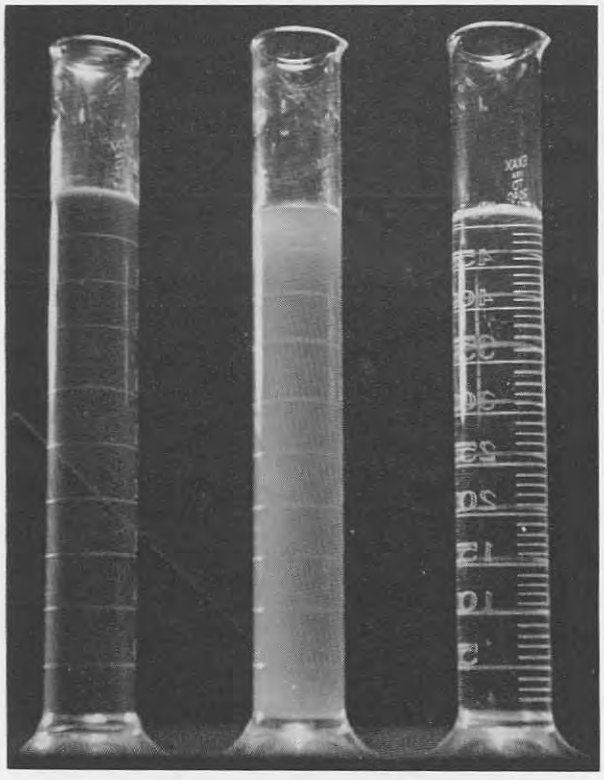

FIG. 2b. *Comparison of harvest, feed, and waste of froth flotation process. From left to right cylinders contain harvest, feed, and waste.*

Tolerance of Chlorella to low pH. In a scaled-up froth flotation harvesting process, it is likely that the algae will have to endure low pH for approximately ½ hr. The culture could be acidified as it enters the harvesting chambers and adjusted to an easily tolerated pH immediately after harvest has been effected. To determine whether the cells would suffer damage, the algae were subjected to low pH conditions, the pH readjusted to approximate neutrality, and the cultures observed for evidence of continued growth. Six 100-ml aliquots of high temperature *Chlorella* culture were adjusted to pH 2.05 with concentrated HCl. At 10-min intervals, aliquots were neutralized with 5 N NaOH. Of each neutralized sample, 20 ml were used to inoculate six 200-ml aliquots of fresh medium. Packed cell volumes were taken immediately after inoculation and after 16 hr of incubation under standard conditions of illumination and aeration. In each case, comparison of initial and final packed cell volumes revealed substantial growth. Moreover, there was no significant difference in amount of growth between the sample exposed to pH 2.05 for 10 min and the one exposed for 60 min. Thus, this organism can tolerate a low pH for at least 1 hr without significant loss of viability. Protein and vitamin analyses should be made on the harvested algae to complete the investigation of possible damage to the product.

Effect of foam height on harvest concentration. Figures 3 and 4 show that as a function of pH both the packed cell volume of the harvest and the maximal foam height increased. However, the packed cell volume of the harvest was obtained from the top 15 cm of each foam column. An experiment was run to determine whether the density of the algal material in a given foam column varied with height. A culture of high temperature *Chlorella* was grown

to a packed cell volume of 0.008 and the pH adjusted to 3.0. Harvesting was performed in a glass column of 52 mm diameter, fitted with a swaged steel diffuser with a porosity of 5 μ. Aeration was maintained at 90 standard cm^3 per min. The column was marked in 10-cm graduations. The algal culture was added to the column until the liquid level was 40 cm above the diffusing plate. Aeration was then started and maintained constant until the foam height attained a level of 120 cm above the liquid surface. At this point aeration was halted and the foam was immediately collected. This was accomplished by means of glass siphon tubes which were used to remove 10-cm segments of the foam column in descending fashion. Packed cell volumes of the collected fractions were determined. In this manner, the structure of the foam was determined under almost static condition. The results are plotted in Fig. 6. From the curve, the packed cell volume of the foam is seen to increase rapidly with height for the first 15 cm above the liquid level. Until a height of approximately 110 cm above the liquid level is reached, the constitution of the foam is surprisingly constant. At 115 cm, the foam density again rises sharply to the top of the column. This maximal value is 1⅓ times greater than the constant level value.

Effect of aerator porosity on harvest concentration. Aliquots of a culture adjusted to pH 3.07 were harvested at a constant aeration rate with aerators with a porosity of 5, 20, 35, and 65 μ. For this and subsequent experiments,

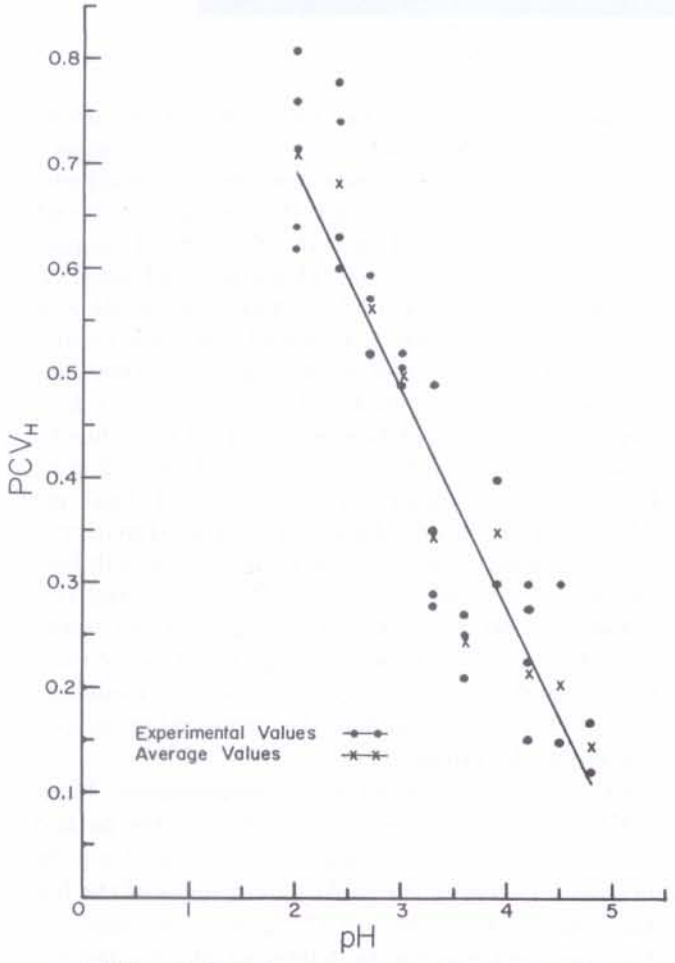

FIG. 3. *Effect of pH on harvest concentration. Packed cell volume of harvest (PCV_H). Concentration of harvest (PCV_H) vs. pH. PCV feed = 0.0065, aeration rate = 65 cm^3 air/min at 15 psi.*

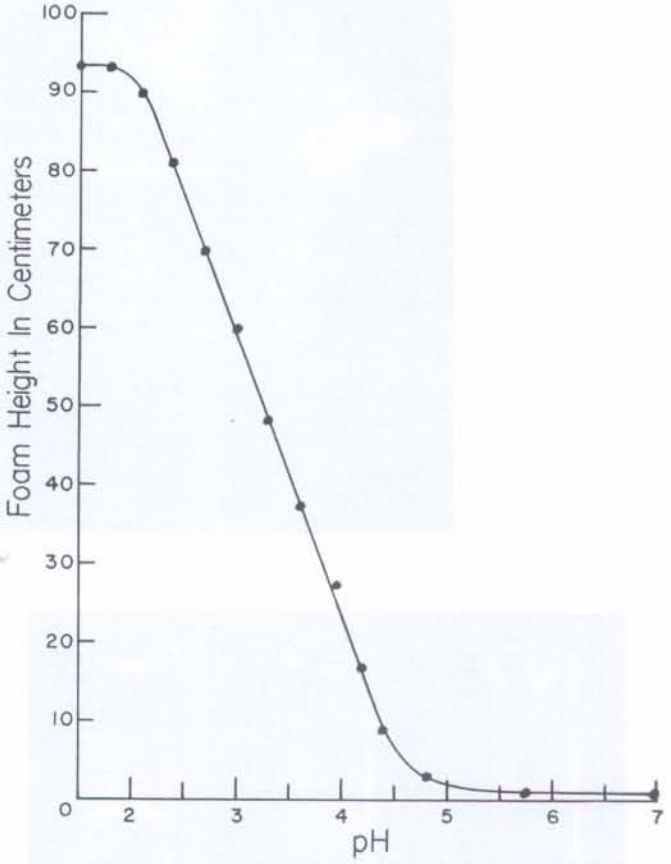

FIG. 4. *Effect of pH on foam height. Foam height vs. pH. PCV feed = 0.0065, aeration rate = 65 cm^3 air/min at 15 psi.*

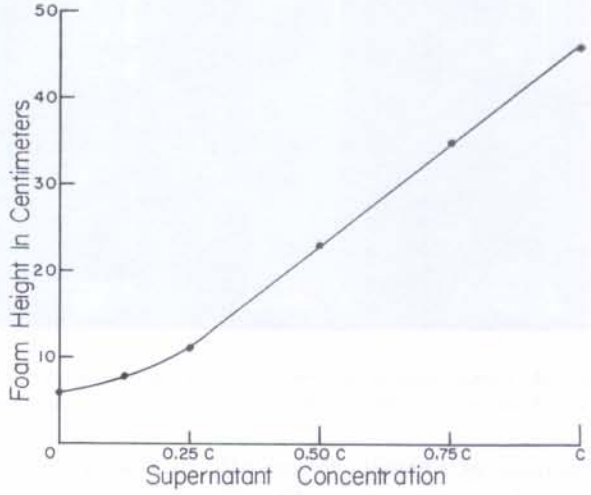

FIG. 5. *Relationship of foam height to supernatant concentration of the feed. Initial concentration of feed supernatant (C). Foam height vs. supernatant concentration of the feed. Aeration rate = 65 cm^3 air/min at 15 psi, pH = 3.0.*

harvest samples were taken, unless otherwise specified, several minutes after harvesting began, thus avoiding the heavy initial densities. An inverse, linear relationship between PCV_H/PCV_F[3] and the aerator porosity was demonstrated. This concentration factor was 34 with the 65-μ diffuser and 43 with the 5-μ diffuser.

[3] Packed cell volume of harvest/packed cell volume of feed.

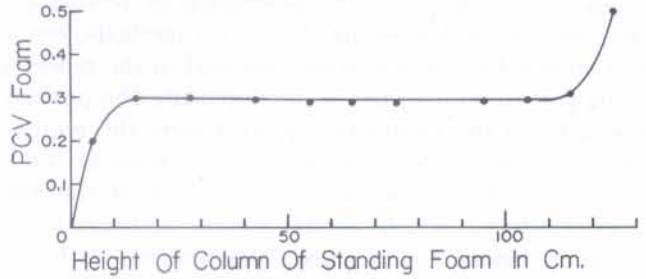

FIG. 6. *Algal concentrations in a column of standing foam. Packed cell volume (PCV). Concentration (PCV) of algae vs. height of foam. Aeration rate = 90 cm³ air/min at 15 psi, feed height = 40 cm, PCV feed = 0.008.*

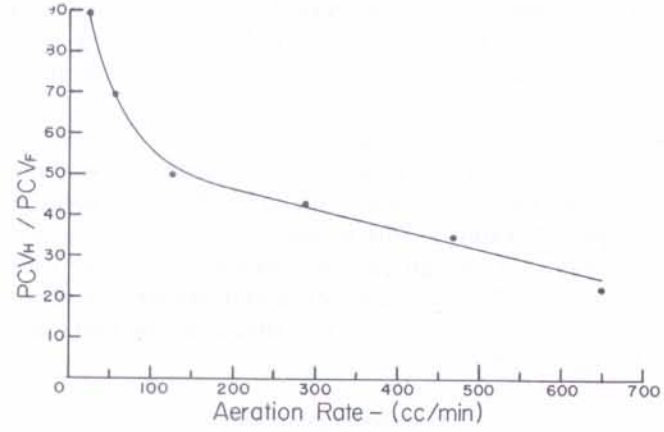

FIG. 7. *Effect of aeration rate on the concentration factor of the harvest. Concentration factor of harvest (PCV_H/PCV_F). Packed cell volume of the harvest (PCV_H) Packed cell volume of the feed (PCV_F). PCV_H/PCV_F vs. aeration rate. Diffuser porosity = 5μ.*

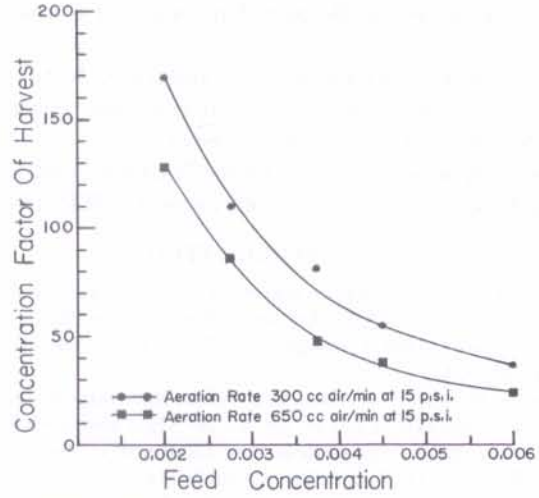

FIG. 8. *Effect of feed concentration on the concentration factor of the harvest at two aeration rates. For definition of concentration factor, see Fig. 7. Concentration factor of harvest vs. feed concentration. pH = 3.0.*

Effect of aeration rate on harvest concentration. Ten liters of high temperature *Chlorella* culture were adjusted to a pH of 3.0. Aliquots of the culture were harvested at different aeration rates using an aerator of 5-μ porosity. The aeration rate was varied between 25 and 650 standard cm³ per min. The plot in Fig. 7 depicts the interesting phenomenon which resulted. When the aeration was reduced, the concentration of the harvest and, therefore, the concentration factor achieved by the harvesting process, as expressed by PCV_H/PCV_F, rose sharply. This is a fortuitous circumstance in that it implies a practical harvesting process could be operated with great efficiency in the amount of air required.

Harvest concentration as a function of feed concentration. A 5-day-old *Chlorella* culture (packed cell volume 0.0060) was diluted with fresh medium to provide a packed cell volume range varying between 0.0020 and 0.0060. Air was applied at the rate of 650 standard cm³ per min through a 5-μ porosity aerator in one series of determinations and 300 standard cm³ per min in a second series. The results obtained from both series of runs are shown in Fig. 8. At a given aeration rate, within the range tested, reduced packed cell volume of the feed results in increased density of the harvest obtained. This figure demonstrates two economic virtues of froth harvesting of algae: (i) high harvest density at low rates of aeration; (ii) high harvest density from low feed density. The latter finding may have particular significance for mass cultures grown in sunlight or not highly intense artificial illumination. These circumstances might apply to the culture of algae for food or to sewage treatment processes utilizing algae.

Harvesting efficiency. High algal removal efficiencies are a characteristic of froth flotation harvesting. In typical results, 88% of the cells in 1,200 ml of feed culture were harvested in 18 min. Correspondingly, the culture medium from which the cells had been harvested was very low in algal content. Packed cell volume measurements of the medium after harvest were 0.000 for 5 ml of medium. Near the end of a batch run, the foam becomes exhausted and some cells are deposited on the wall of the apparatus above the liquid level. Thus, although removal efficiencies are essentially 100% with respect to the feed culture, in the tests made, part of the harvest was not recovered. In addition to the high removal efficiency, concentration factors were also high, frequently 50-fold or more and, on occasions, approaching 200-fold. The solids content of a harvest of packed cell volume 0.220 was 5.9% based on dry weight.

Discussion

Froth flotation harvesting has been shown to be accomplished most effectively at low pH. Standing for long periods or being stored at low pH values causes the harvested material to deteriorate. It will, therefore, be

necessary to readjust the pH of the harvest. This should be done before final drying and in a manner that will not dilute the harvest with water. A harvest with a packed cell volume of 0.120 and a pH of 3.0 resulting from feed adjustment with HCl would contain 0.073% NaCl after neutralization with NaOH as determined by neutralization experiments. Upon drying, the sodium chloride concentration would be somewhat over 1%. Various means for avoiding or reducing this addition of sodium chloride to the product are being explored. If none proves feasible, the harvest would be utilized by diluting it with other foodstuff.

It is still too early to determine accurately the cost of harvesting algae by froth flotation. However, estimates can be made for the cost of certain key processes in the method. The cost of adjusting a culture of *Chlorella* containing 0.5% algae may be calculated from the amount of HCl necessary to adjust the culture to pH 3.0 with HCl and the amount of NaOH required to neutralize it. Based on current prices of 20°Be′ HCl and solid NaOH and experimentally determined required quantities, the costs of the acid and base would be approximately 40 and 11 dollars, respectively, per ton of dry algae. If the clarified medium is not to be used over again, it may not be necessary to readjust the pH of the medium, but merely of the concentrated product. This would decrease the cost correspondingly. It would also be possible to acidify with nitric acid and neutralize with ammonium hydroxide, thus providing nitrogen for the medium which would then be reused. The cost of these latter reagents exceeds the cost of HCl and NaOH, but the total economics may warrant their use. This matter has not yet been given detailed study.

Based on 1960 experience at the District of Columbia Sewage Treatment Plant, aeration costs were estimated. The cost of producing air for the aeration of municipal sewage was 0.005 dollars per 1,000 ft^3 at 7.25 psi. Assuming a 0.5% algal suspension, and based on experiments in which 1,200 ml of such a suspension were essentially clarified in 20 min when frothed at an aeration rate of 625 standard cm^3 per min, the cost of air per ton of dry algae would be less than 4 dollars.

The froth flotation harvesting process concentrates the product to a point within the solids content, 5 to 8%, reported by Gotaas and Golueke, (1957*b*) as necessary for economical drying. The costs of such drying on a commercial basis have been estimated (Gotaas and Golueke, 1957*c*) at 20 dollars per dry ton of algae.

At this time it is not possible to estimate the installation costs of an algal harvesting plant based on froth flotation. However, the froth flotation process is known to be relatively economic and has been applied to large volumes of liquid. The principal cost is in initial installation. Operating costs, as indicated above by the cost of air, are relatively low.

The value of dried algae has been estimated at 80 dollars to something over 100 dollars per ton (Gotaas and Golueke, 1957*d*). It also has been reported (Gotaas and Golueke, 1957*d*) that as much as 35 to 40 dollars per ton may economically be spent in harvesting and processing the material. In the above cited studies at the University of California (Gotaas and Golueke, 1957*d*), extensive investigations were undertaken to develop economic means for harvesting. Only two methods, centrifugation and flocculation, were indicated in the report as having potential for economic development. The principal drawbacks to the flocculation process were the required addition of chemicals to the harvest which could not be easily removed and the low solids content of the algal sludge produced which would require additional concentration before final drying. The problem with centrifugation was primarily one of cost. Power for the industrial centrifuge used was estimated at costing from 65 to 200 dollars per dry ton of product (Gotaas and Golueke, 1957*e*). A striking comparison between the centrifugal method and the froth flotation process may be made in that the industrial centrifuge, operating on a continuous flow basis, produced a maximum of 0.7% solids in the harvest. It was stated that second and possibly third stages would be required to concentrate the harvest to 5 to 8% solids content required for economic drying. As has been shown, the froth flotation method, in its present form, readily produces a harvest containing 5.9% solids. Although, at the present time, the total costs of froth flotation harvesting and drying exceed the figure of 35 to 40 dollars cited as allowable, the costs may be brought within or near this range.

Acknowledgments

The deposition of algal cells above the liquid level in aerating test tube cultures was first observed by Donald Wetherell when he was engaged in algal investigations carried out at this laboratory. Dr. Wetherell is now Associate Professor of Botany, University of Connecticut, Storrs, Conn.

Dean Burke, National Cancer Institute, National Institutes of Health, kindly supplied the high temperature *Chlorella* culture from his algal collection.

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