CULTURE AND UTILIZATION OF FRESHWATER ALGAE AS PROTEIN SOURCE

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INTRODUCTION

Mankind is faced with great challenges in the years ahead. The future prospects would include an even higher incidence of hunger, starvation, and malnutrition. The production of food from unconventional sources may alleviate some of these problems. It is predicted that the earth's population will increase by at least 50% to a total of 6 billion by the end of the century. It is expected to double to 8 billion in the 21st century. Large cities such as Mexico City, Sao Paulo, and Calcutta will have population of 30,26, and 16 million, respectively, by the year 2000 (Blume 1979).

With the world's increasing population, large amounts of protein will be required for human and animal feed (Meadows et al. 1972). The protein deficit is markedly seen in many Third World countries and has led to massive investments in research on single-cell (microbial) protein (SCP). However, it is desirable that SCP be produced in a very large scale and with the cheapest available substrates (Meyer 1980).

UTILIZATION OF ALGAE FOR INDUSTRIAL PHOTOSYNTHESIS

The scientific and economic evaluation for the commercial production of algae in mass culture on inorganic media has been thoroughly studied by different groups. A comprehensive survey of the relevant work published was compiled by the Carnegie Institution of Washington (Burlew 1953). Tamiya (1957) gave a detailed review of the technical aspects of mass culture of algae and found reason to be moderately optimistic about the commercial future of the process.

With the advances in algal culture, three lines of development are distinguished: (1) culture of algae for the production of useful organic materials as food, feed, and some special organic materials; (2) culture of nitrogen-fixing algae for increasing soil fertility; and (3) culture of sewage algae in symbiosis with bacteria with a two-fold purpose - to accelerate stabilization of organic sewage material, and to utilize the harvested algae as in (1).

The culture techniques and species of algae are more or less different. In (1), fast-growing green algae such as *Chlorella* and *Scenedesmus;* in (2), blue-green algae which are strong nitrogen fixers like *Nostoc, Anabaena, Tolypothrix,* etc.; and (3), mixed cultures of various algal strains in harmony with sewage bacteria.

Although different in aims, these projects have many common problems related to the physiology and biochemistry of algae under carefully controlled laboratory conditions. The main physiological characteristics of the algae involves the variety and flexibility of their nutrient requirements and the chemical composition of their cells.

Pioneering investigations on large-scale culture of algae under controlled conditions in inorganic media were conducted in the US, Germany, and Japan in the 1950s. In the 60s, cultivation of algae was investigated as a means of bioregeneration of wastes in chemocycle systems for extended space exploration missions or in treatment of wastes in seawage oxidation ponds (Oswald & Golueke 1968). More recently, algae were again considered for producing protein as food or feed in developing countries (Durand-Chastel & Clement 1975).

Considered algal pilot plant operations were those conducted at the University of California; Czechoslovakia; the Indian/West German System in Mysore, India; the Sosa Texcoco Process in Mexico City; that in Technion, Haifa, Israel; and in Kyoto University in Japan.

SOCIOECONOMIC IMPLICATION OF ALGAL MASS CULTURE

Out of about 17,000 algal species that have been described since the turn of the last century, only a few have been investigated and described as excellent for possible sources of protein. Among the important ones are species of *Chlorella* (*C. pyrenoidosa,* C. *vulgaris, C. ellipsoidea), Scenedesmus,* and the nitrogen-fixing blue-green algae *(Anabaena variabilis, A. cylindrica, Nostoc commune, N. muscorum, N. punctiforme, Phormidium molle, Tolypothrix, Stigonema, Nodularia*).

The fertility of rice fields in Southeast Asia and the Malay Archipelago is said to depend in some measure on the nitrogen-fixing species of *Tolypothrix.* In India, mixed cultures of *Aulosira* and *Cylindrospermum* are used.

Chlorella has been studied in great detail due to its high rate of photosynthesis, carbon dioxide consumption and release of equimolar quantity of oxygen, and high content of protein (40-60%, dry weight). That *Chlorella* could be used in a bioregenerative life support system of a space craft implies that it could serve also as an important source of food.

Detailed data on the chemical composition in terms of assimilation and biological value of *Chlorella* and *Scenedesmus* have been reported and discussed (Table 1) (Cook 1962). Assimiliation of *Chlorella* and *Scenedesmus* mixture (1:10) was insignificantly bettered by boiling and autoclaving.

In recent years, people in certain countries (e.g., Taiwan) have incorporated relatively small amounts of extracted algal cells in their diet. Unextracted algae produced a very disagreeable flavor when used as food ingredient. However, Hayami et al. (1969) found that Japanese women would accept 30 grams metnanol-extracted algae per day in their diet A drawback here is that the green color of algae is difficult to mask in most food mixtures.

The cell walls of algae are fibrous and can irritate the gastrointestinal tract. Algae used in Taiwan are steam-treated to rupture the cell walls and release the cell content. As much as 150 g of methanol-extracted algae per day could be consumed without complaints. The estimate of the nutritive value of *Chlorella* and *Scenedesmus* abated the original proposal on their utilization for human consumption and directed further studies along two lines: (1) examination of the practicability of using *Chlorella* and *Scenedesmus* as animal feed, and (2) preparation from the algae biomass of protein concentrates for human consumption.

However, difficulties involved in protein extraction, poor food properties and costly technological processes suggest that the preparation of protein products from *Chlorella and Scenedesmus* is not yet a primary and beneficial solution to the protein problem.

A study of the protoplast of various algae may give the key to a greater use of algal protein. The single-celled *Dunaliella* and *Cosmarium* whose protoplasts may become a potential protein source have been described. Interesting reports on the preparation of *Chlorella* protoplasts by means of

Table 1. Biological value of algae *(After* Cook 1962)

Protein Source	Biological Value
Dry algae	54.2 ±3.2
Autoclaved algae	55.5 ±2.6
Algae boiled for 30 min.	56.0 ±2.7
Algae boiled for 180 min.	48.7 ±2.6

hydrolytic enzymes had been published (Gibbs & Dorffres 1976; Berlines & Wenc 1976; Bruan & Aach 1975).

Of particular interest is the unicellular blue-green alga *Spirulina.* This alga is relatively large (about 100 times longer than *Chlorella).* There are good grounds for believing that it may be will suited for human consumption because the Africans on the shores of Lake Chad consume it as part of their diet (Table 2). The alga was also eaten by ancient Aztecs in Mexico.

Table 2. Chemical analysis of algal cakes on sale in the Republic of Chad (Azoulay & Senez 1960; Champynot 1965)

Protein	45-46%
Fats	5-6%
Carbohydrates	16-20%

Experiments to assess *Spirulina* assimilation have been conducted in France and Mexico (Table 3) (Clement et al. 1968). The results were so convincing that the Mexican government permitted the sale of *Spirulina* in 1973 (Clement 1975).

Table 3. Chemical analysis of *Spirulina* (Clement et al. 1968)

Protein	62-65%
Pats	2-3%
Carbohydrates	18-20%

The value of traditional recycling methods used in rural areas of Asia is also being increasingly widely recognized. An example is the Indonesian village pond in which wastes are converted to algae which are then eaten by fish. Lately, with the energy crisis in the 70s, the Asian Pacific countries got involved in a technique which started out as a means of waste utilization and opened the way to bioenergy through aquaculture on any scale desired. In principle, one and the same stock of mineral nutrients can maintain a constant supply of algae and methane. At present, capacities of 80 tons dry weight per hectare per year have been obtained but a further increase is still expected. These agricultural practices of recycling minerals by waste conversion to useful production opened up a new outlook toward aquafarming by rendering land more productive and making these minute plants accessible for human use.

FOOD SITUATION IN THE PHILIPPINES

The Filipinos are suffering from a deficiency of protein since our average daily protein intake of 42.5 g is far below our daily requirement of 68 g. Our total production of high protein foods seems barely sufficient to supply 50%

of our protein needs. Even with all our importation of high protein foods such as eggs, milk, fish and meat products and our eating of low-protein foods, our deficiency in protein is still enormous. If we add to this picture the fact that our population is increasing very fast and that to cope with this situation we have to constantly extend land area under cultivation, the thought naturally occur that a time will come when our fertile land will be limited by crowded population and could no longer supply our need for food. As a means of alleviating the threat of famine, particularly protein famine in this country, we should also explore the culturing of high-protein food algae not only for human but also for fish and animal nutrition.

The Philippines, being a tropical country, is naturally rich in algal flora. In raising high-protein algal food it is advantageous to use our local species as they are already accustomed to living under the existing local conditions, and therefore, their culture may not necessitate drastic modifications and adjustments of these conditions in order to favor their development. Strains which can tolerate high temperature or which may not be affected by diversified environment can be used. With such algae, the use of a cooling device similar to that used in culturing algae in temperature countries may be obviated.

NIST'S RESEARCHES ON ALGAL CULTURE

Early investigation was conducted in 1954 at the Biological Research Center, Institute of Science and Technology to explore the algal flora of Manila and nearby provinces for high-protein strains of unicellular green algae. The exploration yielded several *Chlorella* isolates in pure state. A detailed study of a select isolate, *C. pyremoidosa* Chick was made (Palo et al., 1965). This alga was found to contain more than 60% protein (dry weight basis), with all the essential amino acids and vitamins present; very prolific in growth (rate of more than 33 times the initial cell number in 4 days); and tolerate high temperatures (up to 46°C), fully exposed to sunlight during summer.

Later, further work was continued on four local *Chlorella* strains. The effects of media with low and high available nitrogen on the protein and lipid contents of the algae were determined (Rodulfo et al. 1972). The results were in agreement with those of Milner (1948, 1951) who showed that the chemical composition of *Chlorella* "can change in response to change in environment." Harvests with as high as 36.4% lipid and 22.7% fat were obtained by this method: The methanol extracts *of Chlorella* were active against *Micrococcus aureaus, Bacillus cereus* and *B. subtilis.* This confirmed the presence of some antibiotic substances in their cells. While the culture of algae did not reach pilot plant scale level at NIST, stock cultures of *Chlorella* were made available to other research institutions and to institutions of higher learning, fish culturists, etc. who would like to venture in algal culture.

Some other researches done at NIST were on *Scenedesmus obliguus,* another fast growing green alga which is equally rich in protein (50%, dry weight). A comparative study on bench-scale production of *Chlorella* and *Scenedesmus* was made for a year. Environmental conditions such as those of temperature and light were not controlled. Results showed growth of these algae to be favorable at summer time although proliferation of rotifers and other protozoa could not be avoided.

In the 70s, the NIST pursued further research on cultivation of the nitrogen-fixing blue-green algae with emphasis on edible forms commonly utilized by natives in Northern Luzon. One filamentous edible species, *Nostoc linckia,* locally called *tabtaba* was studied (Rodulfo 1980). This alga grew best in a nitrogen-free inorganic medium, had a protein content of 40-45% (dry weight), and grew well in an alkaline pH (7.5-8.0). Another very interesting species, *Nostoc commune,* was isolated from Albay in the Bicol region. The richness of the soil in the region could be due to the presence of this alga which was very prolific in the area. Growth was better in a nitrogen-free solution at pH 6.8-7.2 Maximum increase in colony size was 2.5 cm after two weeks (from initial pin size). Blue-green algae promise to be a very good source of protein fitted for human consumption.

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