Mass culture of microalgae in aquaculture systems: Progress and constraints

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Introduction

For several decades considerable efforts have been made to promote the production and use of microalgae as direct food for man. However, the high costs of production and harvesting, and the potential rejection of algal food by consumers for toxicological reasons or concerns, have so far been major obstacles to a real breakthrough (Soeder, 1980). As a result, human consumption of microalgae is limited at present to expensive 'health' foods. Hope is rising, however, that in the near future inexpensive *Spirulina* grown on wastes will be successfully used in human nutrition (Fox, 1980; Olguin & Vigueras, 1981; Becker & Venkataraman, 1982).

Aquacultural systems based jointly on microalgae and their animal consumers, which can be considered as an indirect use of microalgae in human food, have so far been much more successful. The uptake of microalgal biomass by commercially important filter-feeders is, in this regard, very promising from the energetic standpoint. Microalgae are indeed the biological starting point for energy flow through most aquatic ecosystems, and as such are the basis of the food chain in many aquaculture operations (Bardach, *et al* 1972).

Management of algal populations may thus be considered to be an integral part of aquaculture. A recent literature review (De Pauw & Pruder, 1984) clearly has shown that microalgae are used as (mostly live) food for rearing larvae and juveniles of many species of commercially important molluscs, crustaceans and fish (freshwater and marine); they are also indispensable for culturing several types of zooplankton (rotifers, cladocerans, copepods or brine shrimp) which are used as live food in crustacean and finfish farming.

So far, no completely satisfactory alternative has been found to the use of live microalgae in aquaculture. In spite of all efforts to replace live algae by inert feeds, aquaculturists are still dependent on the production and use of microalgae as live food for commercially important fish, molluscs and crustaceans, during at least part of their life cycle.

Modes of use of algae in aquaculture

Many aquaculture farms rely to a large extent on the use of phytoplankton from natural aquatic ecosystems. In this uncontrolled approach, the consumers are grown directly in the presence of their food (natural phytoplankton) produced in situ. Depending on consumer requirements (which often depend on life stages) the microalgae are consumed either directly (e.g., by herbivorous fish, bivalve molluscs, larval shrimp and prawns, zooplankton), or indirectly via the 'algae-zooplankton' food chain (e.g., most fish). Table 1 summarizes the ways in

Table 1. Modes of microalgae production and uses in aquaculture (for references of consumer types see De Pauw & Pruder, 1984).

Type of culture	Degree of control	Type of algae	Algae-consumer relation	State of algae	Inoculation	Enrichment	Water source	Type of enclosure	Volumes of cultures	Types of consumers
Extensive	Uncontrolled	Natural phyto-plankton	Grown together	Live	Natural	Natural	Natural Natural upwelling	Sea Lake Lagoon Pond	⩾1000 m^3	-Bivalve mollusc larvae, post-larvae, grow-out
Semi-intensive	Semi-controlled	Natural phyto-plankton	Grown separated (pumping)	Live	Natural	Natural	Natural	Sea Lake Lagoon Pond	⩾100 m^3	-Bivalve mollusc postlarvae
		Induced blooms of natural phyto-plankton	Grown together	Live	Natural	Artificial	Natural	Tank Pond		-Bivalve mollusc postlarvae -Zooplankton -Fish
		Induced blooms of natural phyto-plankton	Introduc-tion of consumer after bloom induction	Live	Natural	Artificial	Natural	Tank Pond		-Peneid shrimp larvae -Fish larvae -Abalone -*Brachionus*
Intensive	Controlled	Induced blooms of natural phyto-plankton	Separated	Live Preserved	Natural	Natural Artificial	Natural	Tank Raceway Pond	⩾liters ->100 m^3	-Bivalve mollusc larvae and post-larvae -Peneid shrimp larvae -Zooplankton
		Mono-specific algal cultures	Separated	Live Preserved	Artificial	Natural Artificial	Natural Artificial Artificial upwelling	Tube Plastic bag Bottle Tank		-Bivalve mollusc larvae -Peneid shrimp larvae -*Brachionus*

which microalgae are utilized in aquaculture.

The major problem encountered with the use of natural phytoplankton is the lack of control of the production and the composition of the algal populations; these two factors depend on many environmental variables, not the least of which is the availability of nutrients. Moreover, phytoplankton populations are subject to predation by undesirable consumers present in the natural environment. As a result, algal production in natural systems often fluctuates to a large extent and beyond any control. Last but not least, blooming of unwanted or toxic species such as blue-green algae or dinoflagellates (red tides) can result in mortality of the consumers and, in the worst case, even of man (Landner, 1976).

Many efforts have been made to optimize the availability of natural phytoplankton to the target species reared. Some of the systems developed for nursery rearing of bivalve molluscs bring the food to the consumers by taking advantage of tides or currents or by pumping the algae-rich water into offshore or land-based constructions (Claus, *et al* 1981).

In contrast to the extensive approach described above, the technical reliability of microalgal cultures is quite appealing. Aquaculture can now rely on industrial processes for the controlled production of algae for hatchery rearing of peneid shrimp larvae, bivalve mollusc larvae and production of live foods such as rotifers or *Artemia* (Ukeless, 1976; De Pauw & Pruder, 1984). Watson (1979) describes more than 50 patents which have been issued in the USA for intensive algal production; bivalve molluscs can now be reared from egg to market size in open and closed systems, on cultured phytoplankton (Roels, *et al* 1976; Pruder & Greenhaugh, 1978; Scura, *et al* 1979). In the most intensive forms of aquaculture, algae are always grown separately from their consumers.

Parallel to the controlled production of pure cultures of algae, procedures have been and are being developed for the induction of blooms of natural phytoplankton. This approach is widely adopted, not least for economic reasons, where large quantities of algae (hundreds of m^3 per day) are needed; this is the case, for example, in nursery rearing of bivalve molluscs and in cultue of phytoplanktophagous fish. In this regard, aquaculture systems involving microalgal production linked to wastewater treatment seem to be quite promising (De Pauw & Van Vaerenbergh, 1983). Usually, blooms of natural phytoplankton are induced in the enclosures where the consumers are grown. Pond fertilization for increase of primary production (mainly microalgae) has been practiced for centuries in Asian fish farming (Prowse, 1966; Huet, 1970; Bardach, *et al* 1972; Billard, 1980; Edwards, 1980; Noriega-Curtis, 1981).

A transition between intensive and semi-intensive culturing consists of inducing a bloom of natural phytoplankton in the enclosure before the introduction of the consumers. These techniques apply specifically to shrimp larvae, bivalve mollusc larvae and the rotifer *Brachionus* (De Pauw & Pruder, 1984).

A more controlled but at the same time more difficult approach consists of inducing and managing algal blooms completely separated from their consumers (Goldman & Ryther, 1976; Riva & Lelong, 1918; De Pauw, *et al* 1983).

Cultured microalgae may be used either directly while alive or indirectly in a concentrated form after harvesting and preserving; they are also utilized as additives in pelleted food. As examples of industrial production we can quote the culturing of the clean-water algae *Chlorella* in Asia (Kawaguchi, 1980; Soong, 1980) and *Spirulina* in Mexico (Durand-Chastel, 1980) as well as 'wastewater' algae, e.g., algae grown on domestic sewage (Sandbank & Hepher, 1980; Shelef, *et al* 1980) and algae grown on piggery waste (Soong, 1980). The production price of the algae determines their potential for small- or large-scale application in aquaculture.

Progress and constraints

Major areas of nutritional, biotechnological and economic constraints can be identified in the field of microalgal production for aquaculture (Table 2). These points apply not only to algal production for aquaculture purposes but are also valid for phycoculture in general.

In this paper the authors want to focus more particularly on progress made recently in the areas mentioned above.

Table 2. Major areas of progress and constraints in microalgae cultivation for aquaculture.

Area	Progress	Constraints
Nutritional value of algae	Discovery of importance of polyunsaturated fatty acids	Biochemical definition of nutritional value; variation in nutritional value due to varying culture conditions
Upscaling of pure algae cultures	Development of protocols for balanced growth conditions	Premature collapsing of cultures
Bloom induction in natural phytoplankton	Steering towards interesting species through operational management	Imperfect species control
Predation-contamination	Development of chemical and physical methods for control	Control of micropredators
Fertilization of large-scale cultures	Development of wastewater treatment systems in combination with algal production; new methods of application, use of inexpensive fertilizers	Application, dose effect
Harvesting and storage of microalgae	Discovery of non-toxic chemical flocculants	High cost price of harvested algae; presentation of preserved algae to the consumer
Economics of algae production	Reduction of production costs through: inexpensive culture devices and fertilizers, reducing mixing, automatization, replacement of pure algae cultures by induced blooms	Production costs still too high

Nutritional progress and constraints in mass production of microalgae

Over forty different algal species are currently used as live food for aquatic invertebrates and vertebrates (Ukeles, 1976; Walne, in COST, 1978; Imai, 1977; Persoone & Claus, 1980; De Pauw, 1981). The nutritional value of these species has been determined mainly through feeding trials on the commercial species of interest such as oysters, clams, shrimp and fish, or on zooplankters (De Pauw & Pruder, 1984).

So far, the exact reason why one algal species is a good food and another less so or not at all, has not yet been ascertained and many contradictory statements are found in the literature. A striking example is the case of the diatom *Phaeodactylum tricornutum,* which is usually considered a poor algal food for larval and juvenile bivalve molluscs (Epifanio, *et al* 1981). Wilson (1978) recently demonstrated that the ovate and the tricornate as well as the fusiform variants of this species can give the same growth results with larvae of the flat oyster *(Ostrea edulis)* and the Japanese oyster *(Crassostrea gigas)* as does the haptophyte *Isochrysis galbana,* generally considered the best food for larval bivalves. Another interesting example is the finding by Le Roux (1975) that the haptophyte *Prymnesium parvum,* generally considered a toxic species for fish and shellfish (Walne, 1970), can ensure a better growth of larvae of *Mytilus edulis* than does the chrysophyte *Pavlova (Monochrysis) lutheri,* one of the best algal food species known provided the densities of *P. parvum* do not exceed a certain threshold.

Although certain nutritional requirements for some consumer species have been defined, no general set of nutritional criteria for algal consumers can yet be advanced. Of course the algae must be nontoxic, be the proper size to be ingested, have a digestible cell wall and have sufficient essential biochemical constituents. Bivalve molluscs, for example, cannot digest thick cell walls, whereas rotifers are able to do so with the aid of their mastax. For this reason, *Chlorella* is well suited for *Brachionus* cultivation, but not for oysters or clams. However, breaking the cell wall (e.g., by thermal shock) can make chlorococcalean algae digestible; this has been demonstrated with drum-dried *Scenedesmus* as food for *Artemia* (Sorgeloos, 1974) and silver carp (Soeder, in Walne, 1976).

Another problem with regard to food quality is

that algae (even 'good' species) occasionally and unpredictably become toxic to oyster larvae (Loosanoff & Davis, 1963; Walne, 1974). The reasons for this phenomenon are not yet completely understood, although the age of the culture of algae when fed to the consumers, may be of importance. Stimulatory as well as inhibitory reactions have been observed with algal foods (Wilson, 1979) and there are indications that the bacterial flora associated with algal cultures may be inimical to the larvae as demonstrated by authors quoted in De Pauw (1981).

As a primary criterion for suitability, microalgae must fall within an acceptable size range to be ingested by the consumer. Once ingested, the nutritional value of the algae will depend on their biochemical composition and on the nutritional requirements of the consumer. Obviously, only when composition and requirements match, will growth of the consumer proceed, from hatchling eventually to fertile adults (Provasoli, *et al* 1970). A major drawback in the design of algal growth units is the lack of knowledge of the nutritional requirements of the consumer, as already pointed out by Taub (1970).

Although scientific literature has thus far been rather vague on the relationship between the nutritional value of an alga and its biochemical composition (De Pauw, 1981), it has been suggested recently that concentration of amino acids within the algae as well as the balance of amino acids play a role in growth and development of bivalve larvae (Webb & Chu, 1983). Growing evidence is also becoming available that the lipid quality rather than the quantity is of prime importance to the nutritional value of microalgae (Ackman, *et al* 1968; Chuecas & Riley, 1969; Epifanio, 1979a, b; Watanabe, *et al* 1983; Webb & Chu, 1982).

Fujita (1979, cited in Watanabe, *et al* 1983) demonstrated that different algal foods (e.g., marine *Chlorella,* freshwater *Chlorella,* dried *Chlorella*) fed to the rotifer *Brachionus plicatilis,* alone or in combination with yeast, resulted in different concentrations in the rotifers of certain polyunsaturated fatty acids (PUFA) that are essential for larvae of marine fish such as red sea bream. Depending on the amount of essential fatty acids (EFA), the fish larvae are either healthy or malformed. The same has been demonstrated with other crustacean species used as live feeds, such as *Artemia, Tigriopus, Daphnia* and *Moina* (Watanabe, *et al* 1983).

Langdon & Waldock (1981) demonstrated the importance of the PUFAs 20:5ω3 and 22:6ω3 for the growth and development of *Crassostrea gigas.* According to these authors, a deficiency in these fatty acids is the principal cause of the poor nutritive value of certain algae such as *Dunaliella tertiolecta,* for oyster larvae. The PUFAs 20:5ω3 and 22:6ω3 have also been cited as being essential for the growth and survival of marine fish (Yone, 1978; Howell, 1979; Scott & Middleton, 1979) and peneid shrimp (Kanazawa, *et al* 1977; Jones, *et al* 1979; Kayama, *et al* 1980; Aujero, *et al* 1983).

Webb & Chu (1983) established a classification of the nutritive value of microalgae for oyster larvae, based on the ratio of fatty acids of the ω6 group to the ω3 group. These authors found that algal species that are often cited as a good food for oyster larvae are those having a relatively high ω6 to ω3 ratio (1:2 to 1:3); species considered to have a moderate nutritive value are characterized by a lower ratio (1:5). These observations may eventually explain some of the synergistic interactions which have been observed with mixed algal diets (Epifanio, 1979b).

Also interesting is the finding by Walne (1970) that juvenile bivalve molluscs grow much better in unfiltered seawater to which 'good' algae such as *Isochrysis galbana* or *Tetraselmis suecica* have been added, than in filtered seawater containing the same algae. On the same line one can quote the observations of Sayed (1981) and Pruder & Ewart (in prep.), who found a positive effect of silt added to microalgae, on the growth rate of the eastern oyster *Crassostrea virginica.* From all this it should be concluded that in nature bacteria and other microorganisms and even extracellular compounds most probably supplement the shortcomings in the nutritional value of the algal food.

Variations in gross chemical composition of the algae and more specifically the lipid content and the degree of unsaturation in fatty acids are dependent on numerous factors which are linked and even inherent to the culture conditions, as shown in Table 3.

Flaak & Epifanio (1978) demonstrated that algal cultures in the exponential phase contain more protein, while cultures in the stationary phase have more carbohydrates; as a result, oysters fed algae of the former type not only grow less well than those

Table 3. Factors influencing lipid content and degree of fatty acid unsaturation in microalgae (modified after Sansregret & de la Noüe, 1983, 9.v. for bibliographic citation of the references listed).

I. Variation in lipid content
- temperature (Aaronson, 1973; Smith & Morris, 1980)
- light intensity (Tipnis & Pratt, 1960)
- nutrient limitation:
  a) nitrogen (Fogg & Collyer, 1953; Shifrin, 1980)
  b) silicon (Werner, 1977; Shifrin, 1980)
- nutrient source (Spoehr & Miller, 1949; Lee, 1980)
- osmotic pressure (Ben-Amotz & Avron, 1980; Dubinsky, *et al* 1978)
- growth phase (Collyer & Fogg, 1955; Materassi, *et al* 1980)

II. Variation in degree of fatty acid unsaturation
- temperature (Olson & Ingram, 1975; Materassi, *et al* 1980)
- light (Harris, *et al* 1967; Materassi, *et al* 1980; Shifrin, 1980)
- autotrophic and heterotrophic nutrition (Harris, *et al* 1967; Materassi, *et al* 1980)
- nutrient source (Lee, 1980)
- growth phase (quantity of nitrogen available) (Olson & Ingram, 1975).

fed on the latter but also contain less glycogen.

The influence of various N-fertilizers on growth and composition of algal cells has been shown by Witt, *et al* (1981).

Because some of the parameters mentioned in Table 3, such as light intensity in outdoor cultures, are beyond control, the nutritive value of outdoor algal cultures often cannot be predicted. Unwanted changes in nutritive value sometimes occur when one attempts to counteract or prevent environmentally induced unbalanced growth conditions (De Pauw & Pruder, 1984).

Research should be carried out not only on the nutritional value of pure cultures of algae but also on that of blooms of natural phytoplankton induced in large-scale units after enrichment of the water mass as demonstrated by Vos, *et al* (1984). The latter culturing technology will indeed be used more and more in the future for the rearing of phytoplankton-eating fish (Edwards, 1980), *Artemia* (Milligan, *et al* 1980), bivalve molluscs (De Pauw, *et al* 1983) and shrimp larvae and post-larvae (Shigueno, 1975; Hanson & Goodwin, 1977).

In this context, there seems to be much controversy whether or not phytoplanktophagous fish eat and digest blue-green algae (Edwards, *et al* 1981). Although it is well known that blue-green algae can be toxic to predators (Prowse, 1966), fish kills should, in general, be attributed more to oxygen depletion associated with the algal bloom than to poisoning (Swingle, 1968). An interesting finding was made by Moriarty (1973) on the existence of a diurnal cycle of digestion of blue-green algae by *Tilapia nilotica,* correlated with a cycle of acid production in the fish stomach. Observations of algal cells passing intact and alive through the gut (when stomach acid was at a minimum) led to the erroneous conclusion that the algae are indigestible.

Biotechnological progress of constraints in mass culturing of microalgae

1. Upscaling and collapsing of pure cultures of algae. A major handicap in the cultivation of algae is our inability to grow selected species with known food value in substantial volumes (hundreds of m^3). As mentioned earlier, there are a few exceptions to this statement, namely, the industrially produced freshwater alga *Chlorella* (Kawaguchi, 1980; Soong, 1980), and *Spirulina* (Durand-Chastel, 1980) which are, however, of restricted use in aquaculture.

For hatchery purposes, where only small quantities of algae are needed for a restricted period of time, numerous culture methods and devices have been developed. Well-defined (often axenic) indoor culturing conditions provide dense monospecific algal populations with predictable yields. Recent reviews on the achievements in this field have been published by Ukeles (1976), Watson (1979) and De Pauw & Pruder (1984).

Upscaling the cultures, however, to larger volumes (mostly in outdoor enclosures) and thus leaving the artificially protected environment of (semi)sterility, rapidly leads to collapse of the culture or take-over by other species better adapted to the prevailing outdoor conditions. As a result, outdoor cultures of several m^3 usually last but for short periods of time which rarely exceed a few weeks.

Premature collapse in fact means that the given species is developing under unbalanced growth conditions. Consequently, if more reliability is to be expected in the future, balanced growth conditions must be defined for high-density cultures in the

exponential phase of growth. Important contributions in this field have recently been made by Pruder (1981) who put forth a protocol for such defined conditions. This author points out not only that nutrients (e.g., nitrogen and phosphorus) and vitamins are vital for algal growth, but also that the equilibria between oxygen, carbon dioxide, pH, temperature and light intensity are of equal importance.

2. Species control in induced blooms of natural phytoplankton. As upscaling of pure algal cultures is difficult to achieve in the majority of cases, the alternative may be the induction of blooms in natural phytoplankton without inoculation of any particular species. A major problem in outdoor cultures of natural phytoplankton is obtaining control over the species composition of the bloom induced (Ryther & Goldman, 1975); this is, however, an absolute necessity if the nutritional requirements of the consumers are to be met. For example, in freshwater, the massive development of green chlorococcalean algae such as *Synechocystis* may be toxic for the consumers (Lincoln & Hill, 1980), and in seawater, the development of *Phaeodactylum* has been described as undesirable for bivalve molluscs (Ryther & Goldman, 1975).

It appears that by manipulation of operational parameters such as nutrient loading (BOD, N, P, Si), nutrient ratios (N:Si:P), retention time, pH, temperature, mixing, etc. one may succeed in obtaining a certain control over the composition of algal populations even on a large scale (several authors quoted in De Pauw, 1981, and De Pauw, *et al* 1983). In freshwater this has been achieved by Azov, *et al* (1980). In seawater De Pauw, *et al* (1983) could, in large-scale cultures, experimentally induce a replacement of algae like *Chlorella* and *Phaeodactylum,* unsuited for oysters and clams, by better genera such as *Skeletonema, Nitzschia* and *Chaetoceros*. Positive results were also obtained with properly enriched large-scale cultures of *Skeletonema* and *Chaetoceros* in France, Ireland and Japan, respectively, by Shigueno (1975), Riva & Lelong (1981), and Rodhouse, *et al* (1981) and with the mass production of *Chlorella* for rearing of *Brachionus* (Hirata, 1979) in Japan.

A desk study for an industrial oyster nursery has recently been worked out in the Netherlands, based on the technique of controlled bloom induction in natural phytoplankton (Claus & De Pauw, 1983). A major task for the future will be to work out practical guidelines to induce blooms of natural phytoplankton in different aquatic environments and under different climatic conditions.

3. Predation and contamination of algal cultures. The major difficulty with large-scale cultures, from the biological point of view, is that microalgae, like all monocultures, are susceptible to infection by viruses, bacteria, fungi or protozoans and are exposed to predators such as protozoans, rotifers, crustaceans and even microplantonic larvae of benthic organisms (De Pauw, 1981; Becker & Venkataraman, 1982).

Predators of a relatively large size can be washed out by reducing the retention time of the algal culture to a level less than the generation time of the predators. Alternatives include keeping the pH high during the day and the oxygen concentration low during the night (Groeneweg & Schlüter, 1981), chemical eradication (Loosanoff, *et al* 1957; Tamas, 1979; Becker & Venkataraman, 1982) or centrifugation (Hidu, in Persoone & Claus, 1980). From a modern standpoint of environmental protection, treatments with pesticides should be avoided as much as possible.

Although infection by unicellular fungi may be successfully treated by application of biocides (Becker & Venkataraman, 1982), treatment of bacterial and viral infections of algae is still a problem. Personal experience, however, has repeatedly shown that properly managed algal cultures are quite resistant and that infections are often an indication of poor culture conditions.

Most problems with marine microalgal cultures are related to predation by various types of protozoans: zooflagellates, ciliates, and rhizopods. It was observed that actively growing cells of *Phaeodactylum* are not attacked by *Monas* predators; only after the exponential growth phase does predation start (Raymont & Adams, 1958).

Presently no efficient treatment method exists to counteract predation by unicellular organisms. In small-scale cultures of *Chlorella* and *Dunaliella,* reasonable success was obtained with formalin, methylene blue and malachite green in low concentrations (Rothbard, 1975; De Pauw, *et al* 1979). Large-scale treatment with chemicals, however, remains to be evaluated, especially from the toxico-

logical but also from the economic point of view. More information is needed on the biology as well as on the ecology of the unicellular predators (Haas, 1979; Post, *et al* 1983).

As collapse of mass cultures of microalgae owing to predation is very unpredictable in practice, the maintenance of cultures in duplicate seems to be an absolute necessity in aquaculture operations (Scura, *et al* 1979; Rodhouse, *et al* 1981; De Pauw, *et al* 1983).

4. Enrichment of large-scale cultures. For small-scale production of monospecific cultures of microalgae (often axenic), numerous complex (and mostly expensive) media have been developed (Ukeles, 1976; Stein, 1977; Rohde, 1978). Contrariwise, in large-scale cultures, one is forced for reasons of costs to use cheaper media such as commercial agricultural fertilizers (Boyd, *et al* 1981; De Pauw, 1981; De Pauw, *et al* 1983) or organic fertilizers such as animal or human manures (Bardach, *et al* 1972; Wurtz-Arlet, 1980). Freshwater as well as marine algae are also grown outdoors on a large scale on domestic, agricultural and industrial wastewaters (De Pauw & Van Vaerenbergh, 1983; De Pauw & Pruder, 1984). The use of wastes, however, entails certain risks such as accumulation of heavy metals or pesticides or the presence of pathogenic bacteria or viruses (Shelef & Soeder, 1980); information on this important aspect of waste-algae culture is, however, scarce.

The use of nutrient-rich water from wells, or artificially upwelled water from the deep sea, for mass cultivation of microalgae has been investigated and may be promising to a certain extent, although it should be underlined that these waters are often not sufficiently 'nutrient-rich' (Scura, *et al* 1979; Roels, *et al* 1976). Problems related to the enrichment of water masses with nutrients to stimulate algal production, include the methodology for distributing the nutrients into the whole water mass, the dosage and the frequency of enrichment, the nature of the fertilizers, their price, their local availability, etc.

With regard to species control in induced blooms of natural phytoplankton, a better knowledge is needed of the nutrient requirements of the algal species desired. To this end Bonin & Maestrini (1981), Bonin, *et al* (1981) and Maestrini & Bonin (1981) have reviewed the influences of inorganic and organic macronutrients, hormones and vitamins on the competition among and succession of species in natural phytoplankton populations.

There is also a definite need for sound scientific evaluation of current pond-fertilization techniques, which are too often based on pure rule of thumb. An example of the problems encountered in trial-and-error cultivation is the production of lab-lab in Southeast Asia as food for milkfish (Buri, 1978), for which are too often based on pure rule of thumb.

Most studies on pond enrichment have been done in freshwater and brackish water in relation to fish and shrimp production (Huet, 1970; Bardach, *et al* 1972; Wurtz-Arlet, 1980). Studies on enrichment of seawater and hypersaline waters (for mollusc and shrimp production) are scarce, and much more research should be carried out in this area of growing interest (Davis, 1978; Robert, *et al* 1979; Rubright, *et al* 1981; De Pauw, *et al* 1983; Borowitzka, *et al* 1983).

A problem with the enrichment of very large natural areas such as bays or lagoons is that fertilizers may be taken up by seaweeds or higher plants that are not useful to man and are difficult to control (Korringa, 1976). Moreover, part of the fertilizer may be captured and trapped in the sediments as demonstrated by Boyd & Musig (1981).

In large-scale enclosures, part of the nutrients is also taken up by bacteria and periphyton developing on the walls and the bottom of the culture system. A temporary excess of fertilizer may also lead to a progressive build-up of nutrients that can contribute to the growth of macroalgae or higher plants, which in turn cause a reduction of microalgal production by shading (Trevaillon, *et al* 1973).

With regard to the nature of the fertilizers, different chemical types of nitrogen, phosphorus and silicon salts should be tested in relation to the chemical nature of the water body and the algal species desired, and simple guidelines worked out (De Pauw, 1981). The search for commercially available, inexpensive fertilizers should be encouraged; it has already been shown that expensive metasilicates can be replaced by inexpensive liquid silicates used in industry (De Pauw, *et al* 1983). The economic implications of CO_2 addition to large-scale cultures remains to date unsolved (Oswald, *et al* 1977). A partial solution could be to work with less concentrated cultures in deeper culture vessels, since this would automatically put a lower burden

on the bicarbonate buffer; this technique seems quite promising, especially for seawater culture (De Pauw, 1981).

Last but not least, the suggestion of Pruder (1981) that organic wastes may serve as a cheap carbon source is definitely worth further consideration.

5. Harvesting and preserving of microalgae. If economically feasible, the harvest and eventually the storage of algae grown at latitudes where there is no light limitation would represent a major breakthrough in aquaculture hatchery and nursery operations. The three constraints, however, are 1) the technology of harvesting particles smaller than 50 μm; 2) the processing and the storage of the algal harvest; 3) the (re)treatment of stored algae to make them acceptable again to the consumers.

Harvesting of microalgae can be achieved in several physical, chemical or biological ways: centrifugation, filtration, ultrafiltration, flocculation, air-flotation, autoflotation, etc. However, reliability and costs of the techniques are inversely related; the techniques such as centrifugation which are most reliable are unfortunately not cost-effective (Benemann, *et al* 1980; Mohn, 1980; De Pauw & Van Vaerenbergh, 1983). Inexpensive sand-filtration, on the other hand, has been shown to damage the algal product.

To date the most promising first-step technique to concentrate microalgae seems to be chemical flocculation. Most research on microalgal flocculation has been performed with the objective of wastewater treatment (Moraine, *et al* 1980; De Pauw & Van Vaerenbergh, 1983). Only a few studies relate to microalgal recovery for aquaculture (Nigam, *et al* 1980; Becker & Venkataraman, 1982; J. Morales & J. de la Noüe, unpubl.).

Among the flocculants tested, alum, cationic polyelectrolytes and chitosan seem to be the most promising ones. However, technical optimization of the flocculation process has not yet been achieved and needs to consider factors such as temperature, pH value, intensity and duration of agitation, composition of the culture medium, and the growth phase of the culture (Tenney & Stumm, 1965). Further problems relate to the concentration of flocculants in the harvested product which may reach toxic levels, as is the case for aluminum. The synthetic cationic polyelectrolytes have been reported to be carcinogenic. Presently, chitosan (a natural product derived from chitin) is most promising: it is as effective as the others mentioned above without being toxic. The only economic drawback is its high price (13.5 US $\$ \cdot kg^{-1}$) (Lavoie & de la Noüe, 1983). Research efforts should be directed towards process optimization and towards the identification of cheap chitosan sources to overcome this constraint.

Preservation of microalgae has progressed. Different methods such as drum-drying, spray-drying, freeze-drying, and deep-freezing with the addition of cryoprotectants have been successfully tested (e.g., Hidu & Ukeles, 1962; Walne, 1970; Ben-Amotz & Rosenthal, 1981).

Controversial opinions exist on the nutritive value of concentrated and preserved algae (De Pauw, 1981). Live-preservation aims not only at the provision of live food but also at instantaneous inoculation of new cultures.

A final problem to be solved concerns the presentation of the harvested and preserved algae to the consumer in an acceptable form. More specifically, microalgae entangled in the matrix of a flocculant are of too large particle size to be ingested by most filterfeeders, and techniques for the 'declustering' of such algal masses need to be developed (COST, 1983).

Finally, it should be mentioned that concentrated algae, live or dead, disintegrate rapidly in the culture medium. This can cause fouling problems, mass development of bacteria and deterioration of the culture medium (Walne, 1974).

Economic progress and constraints in the production of microalgae

Information on production costs of microalgae is very scarce. According to Soeder (1978), this is due to the small number of projects devoted to the technologies of microalgal production systems and to the lack of support for experts to explore this field in greater depth.

Table 4 summarizes the few data available. It is striking that, depending on the technology used, microalgae may cost up to 200 US $ per kg dry weight for monospecific algal cultures used in hatcheries (unharvested) and down to 0.2 US $ for harvested sewage-grown algae. In the latter case the

Table 4. Cost of microalgae.

Type of algal culture	Cost (approximate) US $ · kg^{-1} dry weight	References
Monospecific algal cultures produced indoors or in a greenhouse	120 –200	Walne, 1976 Loring, 1981 (pers. commun.) (in De Pauw, 1981)
Induced blooms of marine phytoplankton species	4 – 23	De Pauw, 1981; De Pauw, *et al* 1983
Chlorella (dried)	9 – 11	Kawaguchi, 1980; Soong, 1980
Spirulina (dried)	5	Sosa Texcoco, 1980 (sales price, pers. commun.)
Spirulina (dried)	1 – 1.5	Berend, *et al* 1980
Scenedesmus (dried)	1.5 – 1.9	Soeder, 1978; Becker & Venkataraman, 1982
Wastewater-grown microalgae (harvested-dried)	0.17– 0.29	Berend, *et al* 1980; Shelef, *et al* 1978; Lincoln & Hill, 1980

algae are a by-product of a wastewater treatment process (Shelef, *et al* 1978) and can eventually be used for animal feeding and fish rearing. As said earlier, a major constraint for the large-scale application of controlled microalgae culture for aquaculture is the prohibitive production price. Although the high production costs should always be considered in relation to the (high) commercial value of the end product (postlarvae of peneid shrimp, bivalve mollusc spat) it is clear that pure cultures of algae for nursery rearing and grow-out of bivalve mollusc and for farming herbivorous fish is financially excluded in temperate climates (De Pauw, 1981). Under tropical conditions and in sites where very pure well water or artificial upwelling water is available, it has been suggested that oyster rearing up to market size with cultured pure microalgae as the sole sources of food may be economically feasible (Burzell, 1978; Pryor, 1978). The positive cost-benefit of such enterprises has, however, not yet been proven at the commercial level.

As shown earlier, a serious alternative could be the use of reliable induced blooms of natural phytoplankton, which are much cheaper to produce (De Pauw, *et al* 1983).

Hatchery rearing of bivalve molluscs and *Brachionus* production on pure algae cultures seems at present to be economically feasible, although the real cost-benefit is often camouflaged by a large input of public funding and industrial support (Bougis, 1976).

Considering the difficulties and the constraints outlined above involved in culturing microalgae, scientists are continuously endeavoring to find alternative inexpensive feeds for rearing the different developmental stages of fish, molluscs, crustaceans and zooplankton (e.g., Hirata, *et al* 1975; Sorgeloos, *et al* 1980; De Pauw, 1981; De Pauw, *et al* 1981). It has already been noted by several authors (in De Pauw & Pruder, 1984) that dried algae such as *Spirulina* and *Chlorella* can be used for rearing *Brachionus, Artemia, Daphnia* and larvae of *Mercenaria.* However, the price of these harvested and preserved algae is very high in comparison to 'classic' inert feeds such as yeast, rice bran, starch, egg yolk, etc., which cost less than 0.5 US $ per kg, and some of which are presently used for rearing *Artemia* and *Daphnia* (Sorgeloos, *et al* 1980; De Pauw, *et al* 1981).

Success in replacing live algae by inert feeds is, however, still only partial because of the poor nutritional value of several types of inert feeds (e.g., Watanabe, *et al* 1983). Research is in progress at various places in the world to supplement 'poor' inert feeds with adequate nutritional components such as polyunsaturated fatty acids (e.g., Léger, *et al* 1984).

In conclusion, and keeping all the above-mentioned facts in mind, efforts in the production of microalgae should concentrate in the future on reducing the costs in small-scale as well as large-scale systems through

- cheaper design of culture systems;
- reduction of manual labor through automation;
- use of inexpensive fertilizers, nutrient-rich waters or waste nutrients;
- cheap sources of CO_2 for culture enrichment;
- reduction of energy needed for mixing and pumping cultures by use of alternative energy sources;
- use of greenhouses and heated effluents to increase algal yields;
- induction of blooms of natural phytoplankton as an alternative to monospecific algal cultures.

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