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OUTDOOR MASS PRODUCTION OF MARINE MICROALGAE FOR NURSERY CULTURING OF BIVALVE MOLLUSCS

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ABSTRACT

Nursery rearing of bivalve molluscs, as the intermediate step between the controlled production of larvae in hatcheries and the non controlled growing-out in the wild, is a practice in modern shellfishery management which receives more and more attention.

A major bottle-neck in the culturing process of the post-larval bivalves is, however, the production of large quantities of marine micro-algae suitable as food for the growing spat.

First, experience was gained by the authors in small 1 m^2 outdoor units with induced blooms of natural phytoplankton. The cultures were run either as a turbidostat or as a chemostat with discontinuous or continuous harvesting. Natural seawater (25 - 32 °/₀₀) was enriched with inorganic commercial, or organic fertilizers (manure), sole or in combination. The influence of environmental factors such as light, temperature, detention time, nutrients and turbulence on yield and species composition are reviewed briefly.

As a next step, a semi-industrial pilot plant for the controlled nursery growing of edible shellfish was designed and has been built at the belgian coast.

This pilot-scale should provide bio-technical guidelines and aims at assessing the cost-benefit of an industrial bivalve nursery.

INTRODUCTION

The controlled rearing of spat of edible bivalves such as oysters and clams, as an intermediate step between the controlled production and culturing of larvae in hatcheries, and the non-controlled further grow-out in the wild, is a problem in mollusc farming which receives more and more attention (Cost, 1978).

Nursery rearing is the culturing of postlarvae from a few millimeters in length up to a few centimeter (Bayes, 1979). Nursery rearing of bivalves can be done indoor as well as outdoor and is usually performed in specific devices containing spat in high densities in running seawater preferably enriched with live

unicellular algae (Claus et al., 1980, Persoone and Claus, 1980). Since today, no serious alternatives have been found for living algae as food source for juvenile bivalves (Claus et al., 1980), nursery-rearing of these bivalves thus implies as a prerequisite the presence of very large quantities of microalgae suitable as food for the spat (Persoone and Claus, 1980).

The classic, but very expensive indoor techniques used in hatcheries for producing monospecific algæ cultures cannot, for economic reasons, be scaled up to the very large volumes necessary for the nursery step.

Outdoor or greenhouse production of large quantities of monospecific algae is only possible under very specific conditions at a few places of the world (Persoone and Claus, 1980).

As a result, to date nursery culturing of bivalves is mostly performed with the natural phytoplankton present in the seawater pumped or flowing through the culturing devices, as sole food source for the juvenile bivalves.

Considering that the richness in algae present in the natural environment and/or the fluctuations in the primary productivity at a particular site, may both be limiting the growth of the molluscs at least during certain periods of the year, it is clear that mollusc farmers are endavouring to develop technologies for a more or less controlled production of algae.

In order to contribute to the solution of this problem, which is, to date, considered as one of the major bottle-necks in nursery culturing, experience has been gained for several years at the belgian coast in small outdoor units with continous culturing of natural marine phytoplankton.

The major goal set forward was to get control, as much as possible, of the species-composition of marine phytoplankton blooms induced artificially. This paper reviews the results obtained sofar with outdoor algal cultures of 1 m^2 surface area in seawater of 25 to 32 ‰S during more than 3 years. Factors which appeared to be important for the qualitative as well as the quantitative production of marine micro-algae are commented.

EXPERIENCE

Algal yield

In nutrient saturated and light limited algal cultures, yield is mainly determined by irradiation (Shelef et al., 1973 ; Stengel and Soeder, 1975 ; Goldman, 1979b ; De Pauw et al., 1980a).

To maximize the light energy conversion efficiency it is necessary to adapt the concentration of the algae to the changing light irradiation ; this implies that for a given depth the detention time of the culture is the variable factor (Shelef et al., 1973). A comparison of different detention times (or dilution rates), revealed that at our latitude (51° N), algal yields are maximal when the culture volume harvested ranges from 4 % to 60 % per day from winter to summer. These values correspond with detention times of 25 and 1.7 days respectively.

Based on this experience, a curve representing the ideal harvesting regime, which roughly follows the sun irradiation curve during the course of a year cycle, could be drawn. This enables us to steer the algal cultures in order to maximize the algal yields (De Pauw et al., 1980b).

In theory the dilution rate of the cultures has to be adapted each time to changes in irradiation. In practice, the harvesting regime determined to be optimal for a given period of time was changed each month.

At our latitude, sustained yields ranging from 0-1 g to about 10 g $m^{-2}d^{-1}$ (dry weight) may be obtained depending on the season. The corresponding light conversion efficiency, as calculated from dry weight figures, total irradiation and a caloric content of 23 KJ per g dry weight material, varied in average between 0.5 and 1.5 %.

Below irradiation values of 200 J $cm^{-2}d^{-1}$, no algal growth was observed anymore, even with heating of the cultures (De Pauw et al., 1980a). At our latitude, 51°N this only happens during a short period of the year (December).
Depending on the goal to be achieved, the algae cultures were steered as a turbidostat based on culture density and harvested discontinuously, or as a chemostat based on dilution rate with continuous harvesting and continuous addition of nutrients. Continuous cultures proved to be most practical to run.

Nutrients and pH

Enrichment of the seawater with macro-nutrients such as nitrogen and phosphorous and eventually silicates is often imperative to achieve maximal yields. The fertilizers can be of inorganic as well as organic origin, sole or in combination.
Our experience includes the succesful use of commercial fertilizers such as ammoniumsulphate, calciumnitrate, triple-superphosphate phosphoric acid, sodium metasilicate and swine manure (De Pauw et al., 1979 ; De Pauw and De Leenheer, 1979 ; De Pauw et al., 1980b).
In the case of the use of inorganic fertilizers addition of small amounts of organic fertilizers to supplement for micro nutrients has been shown to be useful. Addition f.ex. of 0.1 % swine manure proved to be beneficial to the algae and also indirectly to the reared bivalves (Claus, pers. commun.).
Algal yields were in average 20 % higher than without addition of manure. Analogous effects have been observed in freshwater algae cultures (Soeder et al., 1970) as well as in seawater cultures (Lee-Watson, pers. commun.).

It is important to saturate the cultures for nutrients if maximal yields are to be obtained ; the nutrients must be highly soluble and directly available to the algae. It should be underlined that the non availability of nutrients due to pH dependent physico-chemical reactions (stripping and/or precipitation) have to be taken into consideration in the calculation of the nutrient additions. Whenever possible, the pH should be stabilized at levels where precipitation and stripping of nutrients cannot occur (usually below 8.5). Unfortunately, in large-scale cultures, an efficient addition of CO_2 to lower the pH remains an unsolved

problem. A partial solution is to work with deeper cultures with a less concentrated algal biomass per volume which automatically put a lower burden on the bicarbonate buffer.

Depending on the algal species, group of species wanted, different proportions of nutrients will have to be added. For diatoms for example, many species of which are considered to be very good food source for bivalves, the seawater also has to be enriched with silicates. Diatoms on the other hand consume less phosporous than Chlorococcal algae.

The enrichment of course depends in the first place of the nutrient-content of the seawater used which is function of time and place.

In our cultures nitrogen consumption varied between 50 and 2200 mg N $m^{-2}d^{-1}$, phosphorous consumption between 10 and 270 mg P $m^{-2}d^{-1}$, and silicium consumption between 70 and 340 mg Si $m^{-2}d^{-1}$, depending on the season and the species developing. These values correspond in average with weighed ratios of inorganic N:Si:P of 6.5:3.4:1.0. We suspect however, that silicates were limiting in our cultures. Recently we adapted the N:Si:P ratio of the added nutrients to 10:10:1

Species composition

Large scale monospecific cultures of marine algae are very difficult to maintain for longer periods. A second drawback of this type of culturing is that they are labour intensive and costly because they involve maintenance and scaling up from very small pure cultures which are grown axenically. The maintainance of "open" cultures of induced natural phytoplankton blooms is on the contrary much less demanding. Epifanio (1979) has shown that mixed diets of algae generally give a better growth of bivalves than monospecific algal species used solely.

A major problem however, in outdoor mass culturing of natural phytoplankton is to get control over the species composition of the induced blooms, since not all algal species are suitable foodorganisms for juvenile bivalves (Persoone and Claus, 1980). This is the case for example for many chlorococcal algae.

Literature (Dunstan and Tenore, 1974 ; Goldman and Ryther, 1976 ; Graneli, 1978 ; Harisson and Davis, 1979) and personal experience (De Pauw *et al*., 1979 ; De Pauw *et al*., 1980a) indicate that it is feasible in practice to exert a certain control over the species composition of natural phytoplankton populations by manipulating different ecological parameters.

A correlation analysis of the data, showed that the following factors all influenced the species composition in our cultures : temperature, salinity, detention time (which affect the concentration of algae and as a result the light conditions in the culture), nutrients and pH. Another factor which has not been analysed so far, but which might also be of importance is turbulence.

Over a period of more than 3 years, and within a temperature range of 0°C to 25°C, about 10 species were dominating at different periods of the year in our induced cultures : 5 species of Diatoms (*Skeletonema costatum*, *Phaeodactylum tricornutum*, *Nitzschia*

longissima, Thalassiosira pseudonana, Thalassiosira sp., 3 species of green flagellates (Eutrepsiella sp., Pyramimonas sp. and Tetraselmis sp. and 2 Chlorella spp.).
Depending on the species, concentrations from 100,000 cell ml^{-1} up to 2.5 10^6 cells ml^{-1} were reached for these algae.
Other species which we can expect to bloom in our outdoor cultures are Amphora sp., Amphiprora sp., Chaetoceros sp., and some other centric diatoms.

In indoor laboratory conditions, all these species can be monocultured without problems within a broad range of different ecological conditions. In mixed cultures, however, competition occurs and any of these species will only dominate at a certain combination of environmental conditions.

We have observed that higher temperatures, a pH below 8, long detention times, high phosphorous levels and low silicate levels stimulate the development of Chlorococcal algae. Such as Chlorella spp. diatoms on the contrary are usually stimulated by silicate addition, lower temperatures, low phosphorous levels and short detention times. Depending on temperature, detention time, and silica levels, different types of small diatoms species will dominate the spectrum. Absence of turbulence will probably favor growth of motile instead of non-motile algae.

Predation

In our outdoor cultures which were kept continuously for more than 3 years, little or no predation by larger predators such as copepods, and rotifers has been observed. Development of pelagic predators may be precluded by decreasing the detention times below the generation time of the predator.
Regular collapsing of the cultures on the contrary has been observed simultaneously with the blooming of unicellular protozoan predators such as ciliates, zooflagellates and rhizopods.
It is not clear yet, if the mass development of these small predators is the consequence or the cause of the collapsing of the algal population. If the detention time can be reduced below the generation time of the protozoans (in case of a fast turn-over of the algae) the algal population can be restored rapidly.
Chemical or physical treatment of the unicellular predators is presently investigated. With Dunaliella cultures good results were obtained with alternating addition of small amounts (1 ppm) of Malachite green and Methylene Blue.

Technological aspects

Major technicological parameters of importance in a system for mass production of algae are mixing, depth and eventually pH stabilization.

For mixing, different technologies have been developed : paddle-wheels, air-bubbling, air-lift pumps, jet pumps, a.o. (Stengel, 1970, Oswald et al., 1977, Goldman, 1979a). All these systems aim at increasing the yield per unit of surface by a more effective utilization of incident light. Mixing also prevents sedimentation of the algae, thermal stratification, anaerobic conditions, photoinhibition ; it also keeps the nutrients in active

contact with the algal cell surface. With air lift pumps in outdoor and indoor cultures, up to 30 % more yield was obtained in comparison with non-mixed cultures (Persoone et al., 1980). However, the advantage of this increased biomass production must be weighed against the increased energy input for the mixing, in the total cost-benefit of the process.

Another beneficial effect of air-mixing in algal cultures is the pH stabilization of the medium by the increased quantity of carbondioxide (present in the air) bubbled in the water.

Since algal yield is theoretically independent of depth and the algal concentration inversally proportional to depth, different culture depths may be used for a same biomass output. Experience has been gained by us with culture depths ranging from less than 25 cm, up to nearly 2 meter (De Pauw and De Leenheer, 1979, Persoone et al., 1980).

Deeper cultures (1-2 m) have the advantage that they provide more stable temperature conditions and may decrease as such fluctuations in species composition. Deep layers also provide a larger amount and a greater buffer capacity of CO_2 for the algae which are less concentrated ; as a result there is less increase than in more shallow cultures.

Shallow cultures on the contrary have the advantage that they can produce very high algal concentrations ; this can be important in cases where direct harvesting of the algal biomass is considered.

PLANNING AND FUTURE

On the basis of the results obtained with our small scale experimental algal unit coupled to an experimental small indoor nursery (Claus et al., 1980) a semi-industrial pilot plant for the nursery rearing of bivalve molluscs has been designed and was recently built along the belgian coast near Ostend. The production capactity of this plant will be several hundred thousand oysters and clams per year grown from a size of few millimeter up to 1.5 to 2 cm.

The nursery consists of an outdoor algal production unit and an indoor unit for the rearing of the spat.
Mass production of micro-algae will be carried out in 4 tanks of 100 m^2 surface area and a depth of 0.5 m to 1 m ; each tank is equipped with a different technology for circulation of the algal cultures (air-lift pumps, air-bubbling system, paddle wheel).

The purpose of the experiments is to compare yield and species-composition as a function of different parameters such as detention time, temperature, irradiation, nutrients and mixing, to make up the cost-benefit for each individual case and to assess the biological technological and economic suitability of the different systems for extrapolation to future industrial units.

To conclude it may be underlined that the subject of nursery culturing of bivalve molluscs including the problematics of mass culturing of algae has retained the attention of several European governments ; a joint research action on mariculture has been voted upon and was adopted recently (COST-action 46)* the first item of which is nursery culturing of bivalve molluscs.

The laboratory for mariculture at the State University of Ghent who is coordinatiing this part of the program will organize end February 1981 an international workshop on this subject.

RESUMEN : "Produccion en masa de microalgas marinas para el cultivo en "nusery" de molluscos bivalvos".

El cultivo en "nursery" de moluscos bivalvos, como un paso intermedio entre la produccion controlada de larvas y el crecimiento no controlado al exterior ("grow-out"), es un proceso que recibe mas y mas atencion cada dia en las empresas modernas, dedicadas a la cria de moluscos bivalvos.

La mayor dificultad para el cultivo post larval de bivalvos, es la produccion de grandes cantidades de microalgas marinas validas como alimento para el crecimiento de las semillas ("spat").

Las experiencias se realizaron primeramente en pequenas unidades al exterior, de 1 m^2 de superficie, en las que se inducian afloramientos ("blooms") de fitoplancton natural.
Los cultivos se realizaron como si de un turbidostato o un quemostato se tratara, con recogidas continuas o discontinuas.

El agua de mar natural (25-32 °/oo) fue enriquecida con fertilizantes inorganicos comerciales, o con fertilizantes organicos (estiercoles), anadidos aisladamente o en combinacion.
La influencia, sobre la produccion y composicion de especies, de factores ambientales tales como la luz, la temperatura, el tiempo de retencion, los nutrientes o la turbulencia son revisados brevemente.

Como in nuevo paso, una planta piloto semiindustrial para el crecimiento controlado de moluscos comestibles, ha sido disenada y esta siendo construida en la costa belga.

Esta escala piloto nos permitira conocer, las lineas biotechnicas a seguit en el futuro, y nos ayudara en la evaluacion del estudio costes-beneficios de una "nursery" industrial para bivalvos.

REFERENCES

Bayes, J.C., 1979. How to rear oysters. Proc. 10th annual Shellfish Conference, the Shellfish Association of Great Britain, London : 7-13

Claus, C., Van Holderbeke, L., Maeckelberghe, H., Persoone, G., 1980. Nursery culturing of bivalve spat in heated seawater. FAO, EIFAC/80/ Symp. E/35. May 1980, 20 p.

Cost, 1978. Report from the secretariat to the cost senior officials committee. European Cooperation in the field of Scientific and Technical Research. COST/58/78, 13 p.

De Pauw, N., De Leenheer, L. Jr., 1979. Mass culturing of marine and freshwater algae on aerated swine manure, 441-473, in Styczynska-Jurewicz, E., Backiel, T., Jaspers, E., Persoone, G., Edts., Cultivation of fish fry and its live food, European Mariculture Society, Special Publication 4, Bredene, Belgium.

De Pauw, N., De Leenheer, L., Verlet, H., Dochy, M., 1979. Culturing of marine microscopic algae. International Council for the Exploration of the Sea. ICES, Mariculture Committee, F:54, 185-195.

De Pauw, N., Verlet, H., De Leenheer, L. Jr., 1980a. Heated and unheated outdoor cultures of marine algae with animal manure. In : Shelef, G., C.J. Soeder and M. Balaban, Edts. Algae Biomass, Elsevier/North-Holland Biomedical Press, in press.

De Pauw, N., De Leenheer, L. Jr., Laureys, P., Morales, J., Renates, J., 1980. Cultures d'Algues et d'Invertébrés sur déchets agricoles. In : Billard, R., Ed., La Pisciculture en Etang, I.N.R.A., Publ., Paris, in press.

Dunstan, W.M. and Tenore, K.R., 1974. Control of species composition in enriched mass cultures of natural phytoplankton populations. J. Appl. Ecol., 11, 529-536

Epifanio, C.E., 1979. Growth in bivalve molluscs : nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* and the hard clam *Mercenaria mercenaria*. Aquaculture, 18 : 1-12

Goldman, J.C., 1971a. Outdoor algal mass cultures. I. Applications. Water Res., 13, 1-19

Goldman, J.C., 1979b. Outdoor algal mass cultures. II. Photosynthetic yield limitations. Water Res., 13, 119-136.

Graneli, E., 1978. Algal assay of limiting nutrients for phytoplankton production in the Öresund. Vatten 38 : 117-128

Harrison, P.J. and Davis, C.O., 1979. The use of outdoor phytoplankton continuous cultures to analyse factors influencing species selection. J. exp. mar. Biol. Ecol., 41 : 9-23

Oswald, W.J., Benemann, J.R., Koopman, B.L., 1977. Production of biomass from fresh water aquatic systems-concepts of large-scale bioconversion systems using microalgae. 59-81, in Proceedings of Fuels from Biomass Symposium, Univ. of Ill., Champaign, III.

Persoone, G. and Claus, C., 1980. Mass culture of algae : a bottle-neck in the nursery culturing of molluscs. In : G. Shelef, C.J. Soeder and M. Balaban, Edts., Algae Biomass, Elsevier/North Holland Biomedical Press, in press.

Persoone, G., Morales, J., Verlet, H., De Pauw, N., 1980. Air-lift pumps and the effect of mixing on algal growth. In : G. Shelef, C.J. Soeder and M. Balaban, Edts., Algae Biomass, Elsevier/ North Holland Biometrical Press, in press.

Shelef, G., Schwarz, M., Schechter, H., 1973. Prediction of photosynthetic biomass production, in accelerated algal-bacterial waste-water treatment systems, 181-190, in Proc. 6th Int. Conf. Wat. Pollut. Res., Jenkins, S.H., ed., Pergamon Press, New York.

Soeder, C.J., Hegewald, E., Pabst, N., Payer, H.D., Rolle, I., Stengel, E., 1970. Das Dortmunder Verfahren zur Produktion eßbrare Mikroalgen. Kohlenstoffbiol. Forschungsstation e.V., Dortmund, Lensingdruck, Dortmund, 26 p.

Stengel, E., 1970. Anlagentypen und Verfahren der technischen Algenmassenproduktion., Ber. Dtsch. Bot. Ges., 83, 589-606.

Stengel, E., Soeder, C.J., 1975. Control of phytosynthetic production in aquatic ecosystems., 645-660, in Photosynthesis and Productivity in different environments, Cooper, J.P., Ed., Cambridge Univ. Press, Cambridge.

[illegible], G. and Collins, [illegible], 1980. Algal cultures as a [illegible] in the recovery [illegible] of nutrients. In: G. Shelef, C.J. Soeder and M. Balaban, Eds., Algae Biomass. Elsevier/North Holland Biomedical Press, in press.

Persoone, G., Morales, J., Verlet, H., De Pauw, N., 1980. Air-lift pumps and the effect of mixing on algal growth. In: G. Shelef, C.J. Soeder and M. Balaban, Eds., Algae Biomass, Elsevier/North Holland Biomedical Press, in press.

Shelef, G., Schwarz, M., Schechter, H., 1973. Prediction of photosynthetic biomass production in accelerated algal-bacterial wastewater treatment systems. 181-190, In Proc. 6th Int. Conf. Wat. Pollut. Res., Jenkins, S.H., ed., Pergamon Press, New York.

Soeder, C.J., Hegewald, E., Pabst, W., Payer, H.D., Rolle, I., Stengel, E., 1970. Der Dortmunder Verfahren zur [illegible] [illegible]. Forschungsberichte [illegible], Dortmund, 76 p.

Stengel, E., 1970. Anlagentypen und Verfahren der technischen Algenmassenproduktion. Ber. Dtsch. Bot. Ges., 83, 589-606.

[illegible], J., [illegible], 1975. Control of phytoplankton production in aquatic ecosystems, 645-[illegible]. In Photosynthesis and Productivity in Different Environments, Cooper, J.P., Ed., Cambridge University Press, Cambridge.