Bio-production and bioenergetics: Bio-reactor production of microalgae and growth of filter-feeding bivalves

Background

Aquatic food chains depend on microalgae, microscopic primary producers living suspended in the water. Many aquatic animals graze and feed on microalgae and have developed the capacity to filtrate large water volumes for microalgae. For the establishment of artificial food chains in the laboratory, it is necessary to grow dense cultures of microalgae in photobioreactors, and subsequently dilute the algae into the containers holding the grazing animals. Microalgal cultures need light, and the light supply is the limiting factor that determines their productivity [1]. Light will penetrate only a few centimetres into dense cultures and deeper zones will be dark and unproductive, and this is a severe limitation to how much production can be increased by scale up of photobioreactors. Furthermore, the higher the light intensity at the culture surface is, the lower is the efficiency by which the microalgae converts light energy into chemical energy stored in the biomass. This effect along with technical limitations prevents production from being increased simply by supplying more light. The production of microalgae is therefore a major bottleneck for the productivity of artificial aquatic food chains whether these are established for experimental purposes or used in aquaculture.

Cultures of heterotrophic microalgae do not suffer from the limitations imposed by the need for external light supply and can therefore be grown to much higher densities and give rise to productivities that are orders of magnitude higher than can be obtained in phototrophic cultures. A limited number of heterotrophic species have been able to support growth of oysters and rotifers [2] but all microalgae are not equally valuable as feeds, which depend on their digestibility, biochemical composition, cell size and other characteristics. Identification of microalgal species that grows well under heterotrophic conditions and are eaten and digested by filter feeding animals, and design of cultivation processes those result in algae of high nutritional value are therefore of central steps in the process of replacing phototrophic microalgae by heterotrophic species.

Microalgal species that are able to grow heterotrophically have been identified from several algal divisions and some species, which for different purposes have been developed into highly productive, high-cell-density cultures, may also be promising candidates as 'feed-algae'. These include the chlorophyte *Chlorella* spp. [3, 4], the diatom *Nitschia laevis* [5], the dinoflagellate *Crypthecodinium cohnii* [6, 7], and the rhodophyte *Galdieria sulphuraria* [8, 9]. In particular the lipid content of phototrophic microalgae are important for their nutritional value as oyster feed [10] and *Chlorella* spp., *N. laevis*, and *C. cohnii* may all accumulate large quantities of lipids rich in poly-unsaturated fatty acids under the right conditions. *G. sulphuraria*, which accumulates starch rather than lipids, can be grown at extremely low pH, a property which may prove valuable in order to minimise contamination of cultures by other microorganisms.

Aims

The aim of this PhD project is to design and optimise cultures of ’feed-algae’ with specific, nutritional compositions, to develop heterotrophic microalgal cultures of high nutritional value in order to create sufficient productivity for quantitative feeding experiments with mussels at larger scale, and to study growth and bioenergetics in mussels living on a diet of heterotrophic ’feed-algae’.

Objectives

This PhD project is divided in two parts that will enable the PhD student to experience different scientific environments:

The objectives of the first part are to develop and characterise heterotrophic microalgal cultures with respect to growth and biochemical composition, and to develop batch and continuous processes resulting in algal cells of nutritionally high values and high productivities. This part of the project will mainly be conducted at Department of Biotechnology, Chemistry, and Environmental Engineering, Aalborg University, Aalborg.

The objectives of the second part of the project are to characterise growth and bioenergetics in blue mussels, *Mytilus edulis* of all sizes and compare mussels feeding on phototrophic microalgae, *Rhodomonas salina*, to mussels feeding on heterotrophic microalgae from cultures developed in the first part of the project, and to mussels living in natural water bodies and raised in mussel farms. This part of the project will mainly be carried out at the Marine Biological Research Centre, University of Southern Denmark, Kerteminde.

Perspectives
This project will result in novel knowledge on the production of heterotrophic microalgal cultures of optimised biochemical composition and their use as feed in blue mussels. It is expected that this knowledge can be used also for the future use of heterotrophic microalgae of optimised biochemical composition as feed for other aquatic organisms in the aquaculture sector. The growth and bioenergetics experiments on mussels will result in new knowledge on the nutritional demands of these animals that can be used to predict their growth in natural waters, and the production potential of mussel farms at different locations.

Methods
Phototrophic microalgae will be grown in continuous, light limited photobioreactors, in which CO_2 is added as acidic titrant in order to control pH and at optimised light intensities [11, 12].

Heterotrophic microalgae will be grown in bioreactors in batch, fed-batch and continuous flow cultures under well-controlled culture conditions and in media resulting in lipid-rich or lipid-poor/protein-rich cells. The process designs will be characterised and optimised for the specific biochemical composition of the cells, and for the production of high cell density continuous flow and batch cultures.

Pigment composition of those algae that produce pigments will be measured spectrophotometrically and by reverse phase HPLC since specific pigment contents often can be correlated the nutritional status and biochemical composition of the algae [13].

Lipid contents in microalgal cultures will be measured by staining with the fluorescent dyes and by gravimetry, while the composition of fatty acids in microalgae and mussel tissue will be measured by gas chromatography after forming ester bonds to methanol (FAME analysis). Protein content in microalgae will be measured spectrophotometrically after staining with Coomassie Brilliant Blue, while starch contents will be measured by HPLC after amylolytic degradation into glucose.

Organic substrates in heterotrophic algal cultures will be followed by HPLC, and inorganic substrates by colorimetric methods.

Feeding experiments will be conducted on mussels placed in aquaria with continuous supply of sea water, and where various microalgae will be fed at various concentrations.

Feeding rates will be based on balances of the algae, which will be counted in inlet and in the aquaria using Coulter Counters. By measuring specific molecular markers of the various algal species (pigments or fatty acids) in faeces from the mussels and in their tissues, it will be determined if the algae are efficiently digested.

Growth and respiration rates of the mussels will be used to evaluate the nutritional quality of various microalgae, and from these measurements, bioenergetic budgets of the mussels will be established under various well-controlled feeding conditions.

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