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From Firenze to Sede-Boker and Back...

I. INTRODUCTION

Cooperation between the group of the Dipartimento di Biotecnologie Agrarie in Firenze working on microalgae and the Microalgal Biotechnology Laboratory has been initiated in 1986, when the Israeli group attended the workshop in Lamezia Terme (CA), followed by a visit of the Italian group at Sede-Boker. Later on, a few exchange visits have taken place under the framework of different programs, among them a few months stay of a student from CNR at Sede-Boker.

Also, researchers of the Sede-Boker group have been invited to serve as members in the International Advisory Board of conferences organized by the Italian group and held in Italy.

The Israeli and Italian laboratories have cooperated in a number of projects of common interests to both groups, such as photobioreactors, N₂-biofertilizers, aquaculture and carotenoids, funded by the CNR, European Community - BRITE-EuRAM, Alginet and Aquagris.

This cooperation has yielded quite a number of papers published in international scientific journals as well as chapters in books (the list can be found at the MBL web site: <http://bidr.bgu.ac.il/BIDR/research/algal/About.htm>)

2. THE «HAEMATOCOCCUS» CASE STUDY

The potential of microalgal biotechnology to yield a vast array of products

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Fig. 1 An overview of tubular photobioreactors at Algatechnology's site for the production of astaxanthin rich *Haematococcus* biomass

including foodstuffs, industrial chemicals, compounds with therapeutic potency and bioremediation solutions, has long been recognized. In view of the extensive variety of valuable bioproducts (natural or heterologous) to be produced and the wide physiological and functional diversity of known microalgal species, one may naively expect that the realization of the potential of microalgae as 'biofactories' should be trivial. However, to date only three *genera* from this group of photosynthetic microorganisms are cultivated in large-scale photobioreactors by several commercial companies; these are the cyanobacterium *Spirulina* and the green algae *Chlorella* and *Dunaliella*. Furthermore, they are used for production of a rather limited range of products, most of them directed to the nutraceutical market. Why only few microalgae and their bioproducts "made their way" into biotechnology?

Spirulina and *Dunaliella* thrive in rather extreme conditions of alkalinity and salinity, respectively, while *Chlorella* is endowed with a remarkably high growth rate. Therefore, contamination by parasite or competing microorganisms (microalgae, fungi and others) is naturally prevented and their cultivation in open reactors is possible, insuring a low cost of production for the biomass. Furthermore, the major share of the market for these strains is for health food, involving crude biomass production. This has the benefits of (i) simple processing (harvest and rudimentary handling), keeping production costs favorably low, and (ii) elu-

ding the competition with chemical industry, which cannot match the wealth in nutritional bioactive components and the attractiveness of natural products.

This concept is illustrated by comparing the respective production of secondary carotenoids β -carotene and astaxanthin by the halophilic *Dunaliella* and the fresh water *Haematococcus* chlorophytes. The latter growing more slowly and more prone to contamination must be cultured in tightly controlled closed reactors to yield astaxanthin, its unique product, at a relatively high cost compared to that for *Dunaliella* culture in natural open lagoons. The major market for astaxanthin (~200 ton/y) is fish (salmon) feed additive, prominently dominated by chemical industry with a price tag of US\$ 2,000/kg. In view of the high cost for *Haematococcus* culture, the production of natural astaxanthin, in contrast with that of β -carotene from *Dunaliella*, cannot compete the chemical synthesis, at least in the animal feed market. Following is an outline of the biotechnology developed by us for the production of astaxanthin-rich *Haematococcus* biomass.

The production of natural astaxanthin by Haematococcus pluvialis

The microalga *Haematococcus pluvialis* synthesizes and accumulates astaxanthin to relatively high levels. The commercial production process is based on two distinct cultivation stages. The first is called the "Green Stage", which starts indoors with a single -cell colony of the microalga, and continues outdoors in solar-powered photobioreactors. The aim of this stage is to produce plenty of viable, unstressed "green" algal cells by normal cell-division process. The "Green Stage" provides optimal growth conditions in order to achieve maximal biomass production rate. The second cultivation stage is the "Red Stage", in which the algal cells synthesize and accumulate the pigment astaxanthin. This stage starts by subjecting the cells to severe stress conditions, mainly high radiation intensity and changes in growth media. As a result, the *Haematococcus* cells start to form cysts by producing thick cell walls, and to synthesize and accumulate astaxanthin, in its esterified form. Cultivating the algal culture in closed systems allows an environmentally controlled process with less biological and chemical contamination. Following the "Red process", the level of astaxanthin in the "red cells" may reach up to ~4% of their dry weight. The astaxanthin content of the "red cells" is correlated to the severity of the stress conditions, mainly to the light flux through the culture. In due time, the "red culture" is pumped to the down-processing area, where the cells are cracked (to render the pigment bioavailable), dried and vacuum-

packed. *Haematococcus* oleoresin is produced in an additional step, using the CO₂ Supercritical Fluid Extraction process.

3. A DISPOSABLE FLAT PANEL PHOTOBIOREACTOR (DFPP)

Existing commercial microalgal culture systems range in volume from about 10² to > 10¹⁰ l (used for the culture of *D. salina*). However, aside from the specialized small-scale (<1000 l) culture systems, four types of culture systems predominate: large open ponds, circular ponds with a rotating arm to mix the cultures, raceway ponds and large bags. Other commercial large-scale systems include tanks used in aquaculture, the cascade system developed in Trebon, Czech Republic and heterotrophic fermentor systems used for the culture of *Chlorella* in Japan and Taiwan.

There are several considerations as to which culture system to use. Factors to be considered include: the biology of the alga, the cost of land, labor, energy, water, nutrients, climate (if the culture is outdoors) and the type of final product. The various large-scale culture systems also need to be compared on their basic properties such as their light utilization efficiency, ability to control temperature, the hydrodynamic stress placed on the algae, the ability to maintain the culture unialgal and/or axenic and how easy they are to scale up from laboratory scale to large-scale.

A common feature of most of the algal species currently produced commercially (i.e. *Chlorella*, *Spirulina* and *Dunaliella*) is that they grow in highly selective environments which means that they can be grown in open air cultures and still remain relatively free of contamination by other algae and protozoa. Thus, *Chlorella* grows well in nutrient-rich media, *Spirulina* requires a high pH and bicarbonate concentration and *D. salina* grows at very high salinity.

Algal species which do not have this selective advantage must be grown in closed systems. This includes most of the marine algae grown as aquaculture feeds (e.g. *Skeletonema*, *Chaetoceros*, *Thalassiosira*, *Tetraselmis* and *Isochrysis*), and the dinoflagellate *C. cohnii* grown as a source of long-chain polyunsaturated fatty acids. Such a close photobioreactor developed by us is described below.

Description of the disposable flat panel photobioreactor (DFPP)

The DFPP consists of a structure component, which is a cage inside this structures, plastic bags may be inserted with the liquid culture.



Fig. 2 On the left, the DFPP design showing different elements; on the right, green stage of *Haematococcus* cultivated in the DFPP

Other components of the reactor are: plastic bags, ca. 0.2 mm thick, sealed at the bottom or from the sides; a tube of PVC inserted in the bottom of the bag for aeration; a heating exchange unit plunged into the bag for temperature control (for heating or cooling).

Advantages

Like other close structures (bags, sleeves, fiberglass cylinder) used today for growing aquaculture desired microalgae, the DFPP is also simple to operate and can provide a sterile environment preventing contamination. Also, for the first time the DFPP is a very high efficient photobioreactor unlike all the previous structures described above, where light is limiting (a big light path) mainly due to the physical properties of the reactor (big bags or cylinders). Another big advantage is the low-cost construction and operation and its up scaling capability, for 100 L up to 500 L in volume.

The DFPP is the ideal solution for the cultivation of all microalgae hampered to contamination such as the fresh water; *Haematococcus*, *Chlamydomonas* and the marine one, *Chaetoceros*, *Tetraselmis* and *Nanochloropsis*.

