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Commercial development of microalgal biotechnology: from the test tube to the marketplace

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Abstract

While humans have taken limited advantage of natural populations of microalgae for centuries (*Nostoc* in Asia and *Spirulina* in Africa and North America for sustenance), it is only recently that we have come to realize the potential of microalgal biotechnology. Microalgal biotechnology has the potential to produce a vast array of products including foodstuffs, industrial chemicals, compounds with therapeutic applications and bioremediation solutions from a virtually untapped source. From an industrial (i.e. commercial) perspective, the goal of microalgal biotechnology is to make money by developing marketable products. For such a business to succeed the following steps must be taken: identify a desirable metabolite and a microalga that produces and accumulates the desired metabolite, establish a large-scale production process for the desired metabolite, and market the desired metabolite. So far, the commercial achievements of microalgal biotechnology have been modest. Microalgae that produce dozens of desirable metabolites have been identified. Aided by high throughput screening technology even more leads will become available. However, the successes in large-scale production and product marketing have been few. We will discuss those achievements and difficulties from the industrial point of view by considering examples from industry, specially our own experience at Mera Pharmaceuticals.
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1. Introduction

Microalgae are an extremely heterogeneous group of organisms. To be called a microalga, the organism needs to be small (usually microscopic), unicellular (but can be colonial with little or no cell differentiation), colorful (due to photosynthetic and accessory pigments), occur mostly in water (but not necessarily) and most likely be photoautotrophic (but not necessarily all the time). Phylogenetically, microalgae can be prokaryotic or eukaryotic and, in evolutionary terms, recent or very ancient. This very diversity makes microalgae, as a group, a potentially rich source of a vast array of chemical products with applications in the feed, food, nutritional, cosmetic, pharmaceutical and even fuel industries.

The history of microalgal utilization from natural populations is centuries old (*Nostoc* in Asia and *Spirulina* in Africa and Mexico). However, the purposeful cultivation of microalgae is only a few decades old. During the 20th century, researchers and commercial producers developed several cultivation technologies that are in use today to produce microalgal biomass: open ponds [1], enclosed photobioreactors (PBRs) [2] and fermentation reactors [3].

The status of microalgal applications in aquaculture, food, specialty chemicals and environmental applications has been reviewed recently [3–6]. In this paper we will concentrate on aspects of algal biotechnology that are related to producing high value compounds such as nutraceuticals and pharmaceuticals.

Microalgae are not a well-studied group from a biotechnological point of view. Of the tens of thousands of microalgal species believed to exist, only a few thousand strains are kept in collections around the world, only a few hundred have been investigated for chemical content and only a handful have been cultivated in industrial quantities (tons per year quantities).

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Because they are largely unexplored, the microalgae represent a rich opportunity for discovery; the expected rate of rediscovery (finding metabolites already described) is expected to be far lower than for other groups of better-studied organisms [7] (Fig. 1).

2. Discovery

Natural products are a consistent source of new drugs [8]. As opposed to other techniques used to generate compounds (e.g. combinatorial chemistry), natural products offer much diversity and chemistries that are under-represented in synthetic compounds. One can also expect that natural compounds (i.e. those made by living organisms) inherently possess advantageous properties such as water solubility, cell membrane permeability and bioavailability that need to be engineered into synthetic chemicals by trial and error. Whether through classic extract and fraction screens or newer pure compound libraries, it is expected that natural products will continue to be an excellent source of new compounds. This, combined with combinatorial biosynthesis techniques, offers a rich future in new compound discovery [9]. Furthermore, new techniques are expected to produce compound leads from presently unculturable microorganisms, including microalgae [10,11].

Several groups are actively screening microalgal isolates for high value compounds such as secondary carotenoids [12–16], fatty acids [17,18], polysaccharides [19,20] and other active compounds [21–24]. Except for microalgal strains that may be found in sufficient quantities and purity in nature (e.g. cyanobacterial mats), a minimum of laboratory scale up is necessary for this phase of the discovery efforts [25].

At Mera Pharmaceuticals, we are conducting two discovery programs to develop new compound leads. First, through a licensing agreement, we are working on developing new pharmaceuticals from a collection of over 2000 Cyanobacterial strains kept at the University of Hawaii. The UH collection has already produced over 100 bioactive molecules. We intend to revisit these compounds and, utilizing enzymatic biocatalysis techniques, multiply the number of compound leads. By creating new compound leads we expect to not only increase the number of compounds but also produce new compounds that may offer desirable characteristics (more potency, less toxicity) and that are unknown (i.e. patentable). We believe that the generated compound libraries will be sought after by the pharmaceutical industry (Fig. 2).

Our second discovery program deals not only with the specific identification of new compounds but also with the scale up of culture volumes needed to produce enough material for structural elucidation and further testing, including clinical trials. This program is supported in part by a grant awarded by the United States Department of Energy (DOE) to a consortium formed by Physical Sciences Inc., the Hawaii Natural Energy Institute and Mera Pharmaceuticals to study the suitability of utilizing microalgae for carbon sequestration. The goal of the DOE is to find technologies that will lower the cost of CO_2 capture and sequestration. The costs of removing CO_2 from a conventional coal-fired power plant with flue gas desulfurization is estimated to be in the range of \$35–264 per ton of CO_2 [26]. DOE's goal is to reduce the cost of carbon sequestration to below \$10 per ton of avoided net cost.

Our vision of a viable strategy for carbon sequestration based on photosynthetic microalgae entails combining CO_2 from the fossil fuel combustion system and nutrients in a PBR where microalgae photosynthetically convert the CO_2 into either compounds of high commercial value or mineralized carbon for sequestration (Fig. 3). While the cost of producing microalgae is much

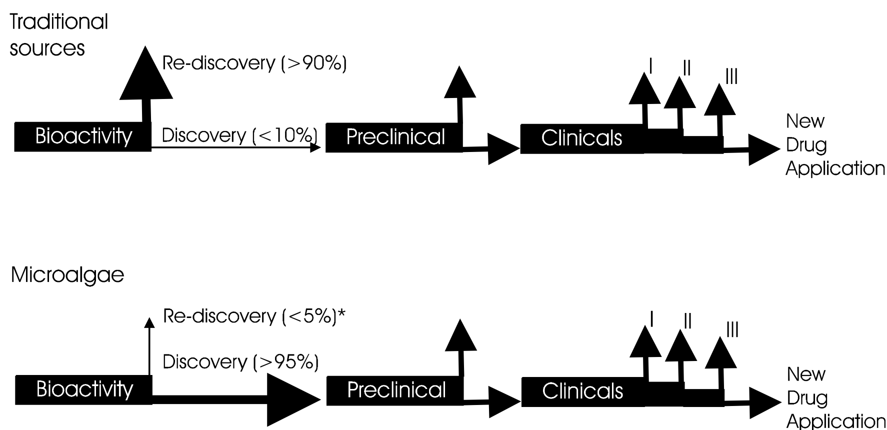

Fig. 1. A big advantage of using little studied organisms such as microalgae is an expected much lower rediscovery rate of compounds. This translates into higher probability of developing new drugs. *Based on 178 bioactive compounds from our contracted collection at University of Hawaii (G. Patterson, personal communication).

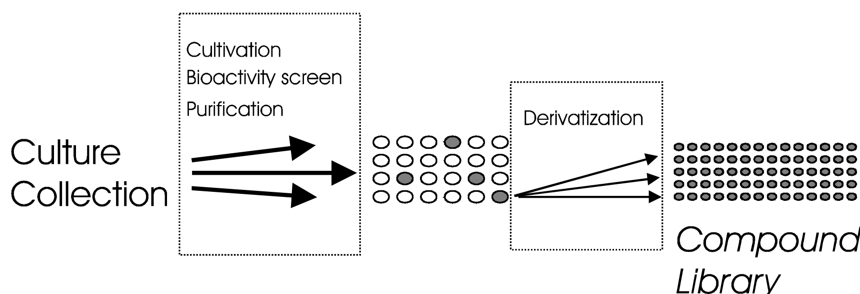

Fig. 2. Techniques such as enzymatic biocatalysis can be used to multiply the number of bioactive leads in drug discovery. Here, each bioactive compound (gray circles) is transformed into a compound library, multiplying the probability of producing chemicals with desired characteristics.

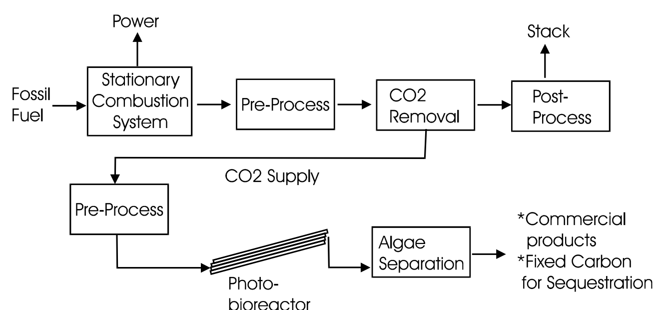

Fig. 3. Diagram showing our concept for a microalgal-based carbon capture and sequestration scheme. High value products obtained from the microalgal biomass would offset the cost of CO_2 removal.

higher than $10 per ton of biomass, this cost can be offset entirely by using microalgae that produce high value compounds. We have embarked in a search and discovery effort consisting in screening a substantial number of microalgae to determine their suitability for this purpose. Specifically, we are searching for species that

- can withstand warm growth temperatures (up to 35 °C, since one could expect that flue gases would raise the algal medium's temperature),
- show broad pH optima (since one could expect changes in rate of CO_2 supply from a combustion source as, for example, power demand changes),
- can withstand the mixture of gases that would accompany the CO_2 in typical combustion systems (e.g. natural gas-, fuel oil-, and coal-fired) such as SO_x and NO_x gases,
- accumulate high value metabolites under stressing and non-stressing growth conditions, and
- are scalable to industrial-sized PBRs.

Our efforts so far have resulted in the identification of several microalgal strains that not only can withstand warm temperatures, changes in pH and thrive in combustion gas mixtures but that also accumulate high value carotenoids at a culture scale of 3.3 l chemostats (Table 1). By combining our high value compound discovery efforts with the carbon sequestration efforts, we are able to lower the effective cost of sequestration and generate valuable byproducts in the process.

3. Production

3.1. Scale-up

One of the major problems with the development of drugs from natural products is the fact they are in limited supply (by definition this is precisely the case with non-common or low abundance organisms such as microalgae). While such organisms offer advantages for the discovery phase, the availability of material needed for further testing may be very limited [27].

Over the last decade, the consensus among microalgal biotechnologists is that commercial photoautotrophic production of high value metabolites from microalgae requires outdoor enclosed PBRs [2,28–31]. Tredici [2] has reviewed the development of PBRs over the last few decades. While many experimental PBRs have been designed, constructed and deemed successful, very few have actually succeeded at commercial scale. The commercial application of PBR technology remains limited mainly to the production of two Chlorophyte algae: *Chlorella* and *Haematococcus* [31,32].

Scale up of research PBRs to commercial scale is not trivial (see Tredici [2] for examples of two commercial failures due to improper scale up). PBR scale up needs to take into consideration changes in illumination, gas transfer and temperature (all three affected by the turbulence in the reactor) and their control. Indeed, scale up is an engineering problem, not a biological one. Much work has been done to describe the light field inside PBRs and general recommendations as to possible maximum scales have been made [33].

Our own scale up procedure involves establishing culture conditions in computer-controlled experimental outdoor pilot PBRs of up to 2000 l capacity. The design is essentially the same as for our commercial scale PBR (the 25 000 l capacity Mera Growth Module, MGM) except for the diameter of the reactor itself (0.18 vs. 0.41

Table 1
Highest percent carotenoid per dried biomass obtained in growth experiments and predicted pigment production rates at a biomass production rate of 13 g dry biomass m^{-2} per day, a typical rate for microalgae grown in Mera Growth Modules (25 000 l PBRs)

Strain ID	Treatment which gave highest % pigment	Compound	Content as %DW	Predicted production rate
AQ0011 (chlorophyte)	5 h strong sunlight	Lutein	0.28	0.037 g m^{-2} per day
AQ0011 (chlorophyte)	5 h strong sunlight	Zeaxanthin	0.12	0.016 g m^{-2} per day
AQ0012 (cyanobacterium)	Standard conditions	Zeaxanthin	0.15	0.020 g m^{-2} per day
AQ0033 (rhodophyte)	Standard conditions	Zeaxanthin	0.21	0.027 g m^{-2} per day
AQ0036 (rhodophyte)	Standard conditions	Zeaxanthin	0.13	0.017 g m^{-2} per day
AQ0052 (chlorophyte)	Standard conditions	Lutein	0.21	0.027 g m^{-2} per day
AQ0052 (chlorophyte)	8 h strong sunlight	Zeaxanthin	0.05	0.006 g m^{-2} per day
AQ0053 (chlorophyte)	5 h strong sunlight	Lutein	0.35	0.049 g m^{-2} per day

m). We are using these scale up reactors in our DOE program on carbon sequestration and high value compound discovery efforts. The scale up reactors allow us to produce enough biomass for thorough testing of bioactivity and to establish the general parameters for production of the desirable strains.

There are two significant differences between laboratory chemostats and the MGM that could affect culture productivity at this scale. These differences concern (1) light field and (2) the mixing, dissolution and distribution of gases. The purpose of the pilot MGM experiments is to examine scale-related effects precisely. The design of the MGM permits us to change the flow characteristics at will (Reynold's number between 2×10^3 and 2×10^5) to study these effects. So far, we have successfully scaled up two Cyanobacteria (*Lyngbya sp*. and another unidentified filamentous strain), two Chlorophytes (*Haematococcus pluvialis* and an unidentified small-5 μm coccoid strain) and a Rhodophyte (*Porphyridium sp*.).

3.2. Commercial photobioreactors

From a commercial (i.e. business) point of view, a PBR must have as many of the following characteristics as possible:

- high area productivity (g m^{-2} per day), since many costs scale with plant size;
- high volumetric productivity (g l^{-1} per day), since some costs scale with the amount of water needed for culture;
- large volume (l PBR^{-1}), since some costs scale with the number of reactors needed;
- inexpensive to build and maintain (\$ PBR^{-1});
- easy to control culture parameters (temperature, pH, O_2, turbulence); and
- reliability.

PBRs of different designs attempt to achieve these characteristics differently [2]. Obviously, from a commercial point of view, the optimum PBR design will be the one that reliably produces the high value compound sought at the best possible quality for the least amount of money. Examples of commercial applications of PBR technology today are those used for *Chlorella* and *Haematococcus* production [31,32].

Our own PBRs have capacities of up to 25 000 l and occupy an area of just 100 m^2. They are of the serpentine type (Fig. 4) and made of clear polyethylene tubing (41 cm diameter) and PVC parts (bends and control unit). Temperature and pH are computer-controlled, which provides for very tight tolerances independent of variability in ambient conditions (Fig. 5), which is necessary to produce a consistent product.

3.3. Harvest

Harvesting entails concentrating the biomass produced from a concentration of <1 g DW l^{-1} in the PBR to as much as 250 g DW l^{-1}. The harvesting technique to be used is dependent on characteristics of the microalgae, such as size and density. Reviews of the different techniques available (including flocculation, filtration, centrifugation and air flotation) have concluded that centrifugation is possibly the most reliable technique and only slightly more expensive than other techniques [1,34].

Fig. 4. The Mera Growth Module, a 25 000 l PBR.

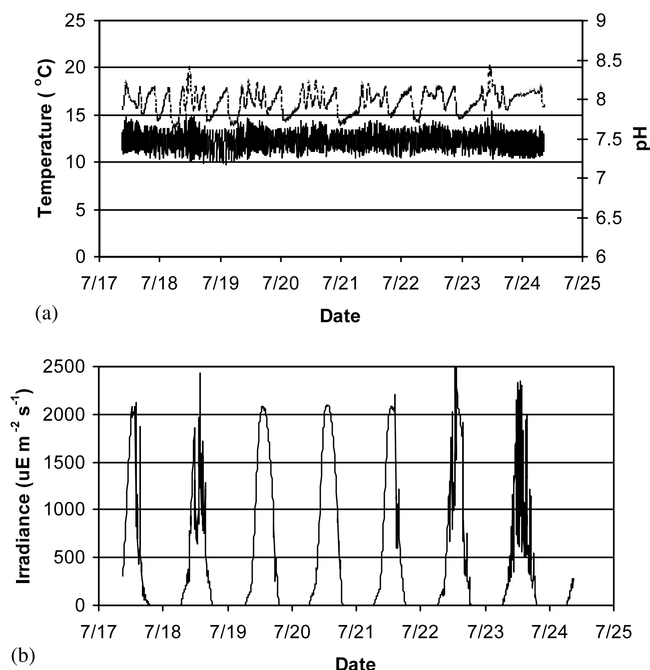

Fig. 5. (A) Temperature (broken line) and pH (solid line) traces for a 25 000 l MGM culture showing the tight tolerances maintained over a 7-day period (e.g. over 97% of the pH values are between 7.3 and 7.8). (B) Trace of solar irradiance measured near the surface of the MGM for the same period.

In the case of *Haematococcus* biomass for the production of astaxanthin (Mera's first high value product), we take advantage of the fact that *Haematococcus* cells become large and heavy during the carotenogenesis and encystment phase. The cysts coalesce into larger flocks that settle out of the growth medium quickly; we have observed settling velocities of >1 cm min^{-1} for single cells and even faster for flocks (unpublished observation, see also [35]). This provides for an efficient concentrating step ($15\times$) in the cultivation units. The slurry thus produced is further concentrated with centrifuges (concentration factor $22\times$).

3.4. Separation and recovery

Here we have assumed that the goal of microalgal biotechnology efforts is to recover a high value product from the microalgal biomass. Thus, the high value product needs to be separated from the biomass. Depending on the process, the microalgal cells may need to be physically disrupted. Both ball mills and high pressure homogenizers have been used successfully to disrupt microalgal cells [36,37] to enhance recovery of astaxanthin from *Haematococcus* at commercial scale, but other methods may be possible [38]. Use of solvents and enzymes might help with cellular disruption and product recovery but care must be taken regarding what aids are used if the product is intended for human consumption.

Depending on the product to be recovered, the next step in the process might entail reducing the water content of the microalgal biomass. Absence of water in the biomass enhances the recovery of lipid soluble components such as astaxanthin and β-carotene. Microalgal biomass can be dehydrated in spray dryers, drum dryers, freeze dryers and sun dryers. In the case of heat sensitive compounds such as astaxanthin, commercial producers have developed technologies that limit exposure of astaxanthin to conditions known to cause degradation (specially high temperatures and oxygen [32]). Following dehydration, astaxanthin is recovered from the biomass using supercritical CO_2 extraction or oil extraction techniques. At the present time commercial producers of astaxanthin do not purify astaxanthin from the extract thus obtained.

In some cases the biomass may not need to be dehydrated, and extraction and fractionation can be carried out on the wet biomass (e.g. fatty acids [39], biliproteins [40], carotenoid pigments [41]). Further downstream processing may be needed to isolate the active compound depending on the intended final product [34,42].

3.5. End products and formulation

There are very few commercial microalgal high value products in the market today (e.g. fatty acids (FAs), and carotenoids). We assume that future drugs developed from microalgal products would be prepared and packaged as other pharmacological compounds are today.

In the case of extracted FAs and carotenoids, these products can be offered in bulk at different purities, incorporated into other products or encapsulated. For example, Martek (http://www.martekbio.com) is a successful producer of docosahexaenoic acid (DHA) from *Crypthecodinium cohnii*. They market the bulk, blended product, to infant formula manufacturers but also offer it in capsules.

To illustrate the learning curve that sometimes needs to occur when putting together a new product we will look at nutraceutical astaxanthin. Dried, astaxanthin-rich, *Haematococcus* algal meal can be pressed into tablets. However, the astaxanthin in these tablets is degraded easily by oxidation. Producers of astaxanthin have attempted to suspend *Haematocccus* biomass in edible oils instead, expecting that the oil would create a barrier between atmospheric oxygen and the astaxanthin-rich biomass. Cyanotech (http://www.cyanotech.com) tried suspensions in rosemary oil but found that astaxanthin was very unstable in this formulation [43]. Our own observations are that dried particles suspended in oil can cause leaks in gelatin capsules resulting in a product unacceptable to the consumer. The solution to these issues has been the development of

extraction methodologies using non-petrochemical solvents. For human applications, use of petrochemical solvents could create health and/or acceptability concerns because of possible residues in the final product. Mera Pharmaceuticals has developed a proprietary oil extraction method in which edible oils are used as the extraction solvent. Alternatively, super-critical CO_2 extraction can be used to produce an astaxanthin-rich oleoresin that is then diluted with edible oils to the appropriate concentration for encapsulation [43].

4. Marketability and profitability

In the end, the objective of microalgal biotechnology is to make money by selling a product for a higher price than it costs to produce. To sell a product there must be a market, a group of consumers that are willing to purchase the product. The preferred approach is to first find a market and, then provide the product desired. “The goal is to develop a product that fills a need; one should avoid developing a product in search of a use” [44]. Indeed, the marketers should be the ones guiding the efforts of the researchers.

There are very few commercial high-value products from microalgae available today. Perhaps the three best known are β-carotene (*Dunaliella*), DHA (*Crypthecodinium*), and astaxanthin (*Haematococcus*). We will use astaxanthin as a case study to illustrate some of the pitfalls that may be encountered when bringing a microalgal biotechnology product to market.

4.1. Astaxanthin case study

The largest consumer of astaxanthin today is the salmon feed industry. In the 1980s and 1990s, *Haematococcus* was identified as an organism that could be cultivated as a rich source of astaxanthin with a readily identified market, the salmon feed industry. Over the last 15 years several companies have attempted to establish commercial operations to supply natural astaxanthin to the feed market. In practice, reliable production of *Haematococcus* astaxanthin at industrial scale was not accomplished until the late 1990s [36,37].

However, the largest producers of astaxanthin today do not produce astaxanthin from *Haematococcus* but via chemical synthesis (BASF and Hoffman-La Roche). Synthetic astaxanthin has a different ratio of stereoisomers (mainly $3R,3'S$) than natural astaxanthin ($3S,3'S$ in, e.g. *Haematococcus*). The $3S,3'S$ is the main stereoisomer found in wild Pacific and Atlantic salmon species. Since salmon are unable to modify the chemical configuration of the astaxanthin molecule, one can detect whether an individual fish was fed natural or synthetic astaxanthin [45].

Why does the salmon feed industry use synthetic astaxanthin? First, microalgal producers had assumed that *Haematococcus* astaxanthin would be cheaper to produce. The synthetic producers have established the world market price for astaxanthin at about US$2000 kg^{-1}. We suspect that the actual production cost for synthetic astaxanthin may be < US$1000 kg^{-1}. To beat this cost, and assuming a 3% astaxanthin content, *Haematococcus* biomass would need to be produced at significantly less than $30 kg^{-1}. Considering the added costs of producing astaxanthin (e.g. cell breaking), we feel that this low cost cannot be achieved by commercial producers at this time. It is possible that as the production technology is optimized (e.g. find a strain that accumulates 10% astaxanthin) and production is transferred to low cost locales (e.g. China) *Haematococcus* astaxanthin will become cost competitive as a feed supplement.

Second, producers of *Haematococcus* astaxanthin had also assumed that natural astaxanthin would have better acceptability than the synthetic counterpart would. However, the public, at large, does not appear to demand and is not willing to pay a higher price for naturally pigmented salmon. We feel this is due to a lack of awareness by the consumer. Most consumers probably do not realize that most of the salmon consumed today is farmed, that pigment is added to their diets and that the pigment added is a synthetic product. As long as the consumer is uninformed there will be very little demand for natural astaxanthin-fed salmon, and astaxanthin producers will have to compete on price alone. Once the consumer is educated, or regulations favor the use of natural products, we would expect to see a premium price for natural versus synthetic astaxanthin as has occurred in the vitamin E and β-carotene markets [46,47].

There are a few applications where natural astaxanthin is preferred over the synthetic product (koi, chicken, red seabream diets) because of enhanced deposition of pigment in the tissues or regulatory requirements. However, these markets, at the present time, appear too small to sustain an enterprise engaged in the commercial production of astaxanthin.

4.2. Astaxanthin's second chance

In the 1990s the antioxidant characteristics of the astaxanthin molecule became well established. Several in vitro and animal model studies demonstrated a number of possible roles for astaxanthin in disease treatment and prevention [48]. This has opened the possibility of a new market for *Haematococcus* astaxanthin: human nutraceuticals. Retail price of nutraceutical grade astaxanthin is > $100 000 kg^{-1} [32] which more than justifies the increased cost of producing natural astaxanthin from *Haematococcus*.

The size of the astaxanthin nutraceutical market today is probably less than a few million US$. However, we estimate the current production capacity for nutraceutical *Haematococcus* astaxanthin to be at least 40–50 million US$ (retail) in Hawaii alone (Cyanotech, Mera Pharmaceuticals and MicroGaia). Thus, the industry is poised for rapid growth. However, most consumers remain unaware of astaxanthin, much less understand why it is good for them! We expect that as the producers undertake consumer awareness campaigns (advertising and public relations), demand will rise and will outpace present production capacity.

4.3. Future of Haematococcus astaxanthin

We believe that, through consumer education and by lowering production costs, the future of *Haematococcus* astaxanthin is bright. As mentioned above, while astaxanthin is a valuable product with important benefits for human health [48], most consumers have never heard of it. Thus, the market is still very small, and it is up to the producers to create awareness for the product. Doing so will require significant capital.

As price is always a factor in consumer acceptance, we are pursuing two strategies to lower our production costs. First, we are continuously improving our production technology to produce *Haematococcus* biomass with a higher final astaxanthin content. Any improvement in astaxanthin content of the final product translates directly into lower costs, since the costs are proportional to the amount of biomass produced and processed. Second, we plan to lower our production costs by expanding our production capacity into locales with lower land, labor and energy costs such as China.

4.4. Lessons learned from Haematococcus astaxanthin

Haematococcus astaxanthin has not yet realized its potential because of mistakes made by the producers. These can be summarized as:

- overly optimistic cost projections,
- lack of market research (consumers do not care...but would they if they knew?),
- an “if we make it consumers will buy it” attitude,
- lack of marketing resources to build consumer awareness, including distribution of product safety and efficacy studies results.

5. Future of microalgal biotechnology

Microalgal biotechnology has not yet attracted the attention of large (i.e. have money to fund research) pharmaceutical companies. This may be because of the lack of success stories so far. While we, microalgal biotechnologists, are convinced of the potential of microalgal biotechnology, we have little success to justify our optimism.

Moving forward, microalgal biotechnology may fulfill the following roles.

5.1. Drug and high value chemical discovery

This is perhaps the most promising aspect of microalgal biotechnology. As stated earlier, the diversity of the microalgae leads us to believe that this is a very fertile ground for search and discovery with low rediscovery rates. Generation of compound libraries based on bioactive microalgal metabolites could attract the attention of biopharmaceutical companies with the necessary resources to fund clinical trials; bringing a new drug to market costs hundreds of millions US$ [49], which your average microalgal biotech does not have. Furthermore, PBR technology has advanced to the point where it is relatively easy to scale up cultures to produce enough material for research efforts beyond initial discovery.

5.2. Drug and high value chemical manufacture

While microalgae can be fast growers (high primary productivity) many desirable chemicals are the product of secondary metabolism triggered under conditions not conducive to fast growth. In addition, once a chemical is discovered and characterized it might be produced synthetically. Furthermore, the biochemical pathway that results in the desired chemical may be transferred to an easily cultivable organism (e.g. [50]). It would seem that the future of microalgae in manufacturing might be limited to chemicals complex enough that they cannot be chemically synthesized or the pathways of which cannot be transferred to other organisms. For those chemicals that will be produced by microalgae we will need to develop new strains (faster growth, higher chemical concentration), whether by classical selection or genetic manipulation, and improve PBRs to the point where 40–60 g m^2 per day of microalgal biomass are produced consistently.

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