

EXTRACTION OF ASTAXANTHIN “HAEMATOCOCCUS PLUVIALIS” FROM GREEN ALGAE

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Abstract Astaxanthin is a secondary carotenoid that is widely used as a dietary supplement and feed in aquaculture industries. Among other commercially significant microalgae, *Haematococcus pluvialis* is considered as the best non-genetically modified microalgae for accumulation of natural astaxanthin. Natural astaxanthin extracted from *H. pluvialis* has a significantly better antioxidant potential than artificially manufactured astaxanthin. Astaxanthin promotes the health conditions of humans by reducing oxidative stress and free radicals. Natural astaxanthin is recognized as one of the high-value-added products of the future, with noticeable advantages and a great growth in demand.

The present chapter describes the mass cultivation of *H. pluvialis* for high-end production of natural astaxanthin. Cultivation of *H. pluvialis* in photobioreactors and open raceway ponds via two-stage and one-stage methodologies are summarized in detail. Production processes including harvesting, cell rupturing, downstream processing, and biorefinery models were reviewed. Total cost and profit involved in the mass production of *H. pluvialis* were elaborated by lab-scale production, small-scale production, and large-scale production in detail. Thus, the chapter can serve as a baseline for entrepreneurship opportunities in the commercial production of astaxanthin from *Haematococcus pluvialis*.

Keywords Astaxanthin, *haematococcus pluvialis*, photobioreactors, open raceway ponds, mass production

Introduction

Haematococcus pluvialis is a single-celled freshwater microalga acknowledged for its preeminent organism of natural astaxanthin. The bright red-colored secondary

carotenoid of astaxanthin (3,30-dihydroxy-carotene- 4,40-dione) has distinctive biochemical characteristics. Due to the presence of carbonyl and hydroxyl functional groups, carotenoid exhibits remarkable antiinflammatory and antioxidant properties (Hussein et al..Dietary supplements, cosmetics, aquaculture, and pharmaceutical sectors are the most dominant applications utilizing astaxanthin. It fulfils several biological functions in aquatic animals, including prevention of oxidative stress, increasing immune response and pigmentation, and protection from ultraviolet radiation. The free radical scavenging efficiency of astaxanthin is 10 times more powerful than canthaxanthin, zeaxanthin, lutein, and β -carotene, 54 times stronger than β -carotene, 65 times more dominant than vitamin C, and 100 times more efficacious than α -tocopherol; Koller et al; Pérez-López et al,).

Astaxanthin slows down the growth and regulates immune responses against tumor cells (Nagendraprabhu & Sudhandiran. This carotenoid acts against various human health conditions like diabetes, cardiovascular diseases, liver diseases, and obesity. It also improves the brain, skin, and eye health (Nakagawa et al; Yamashita,), promotes the reduction of body fat percentage, and enhances physical performance (Aoi et al.). Nowadays, the natural astaxanthin extracted from *Haematococcus pluvialis* present in market is only around <1%, while over 95% is synthetically manufactured (Koller et al. Synthetic astaxanthin has 20-folds lesser antioxidant capacity than natural astaxanthin, and it is still not approved for human consumption (Koller et al; Lorenz & Cysewski,). Synthetic astaxanthin is produced from Wittig reaction with C10-dialdehyde and asta-C15-triarylphosphonium salt, and there is safety concern for human consumption due to its different stereochemistry. It is only allowed to be implemented in aquaculture. These factors increase the demand for natural astaxanthin in the global markets. *H. pluvialis* extracted natural astaxanthin has ANVISA (Brazilian Agency of Health Surveillance) status in Brazil, FDA (US Food and Drug Administration) granted GRAS (generally recognized as safe), and FSA (UK Food Standards Agency) granted “novel food” status (Capelli & Cysewski, Grewe &

Griehl,). This chapter provides the prior budgetary estimation for profitable production of natural astaxanthin from *Haematococcus pluvialis*.

By correlating the production expenditure of natural astaxanthin towards lab-scale, small-scale, and large-scale production scenarios, it be less expensive than synthetically produced astaxanthin. It also summarizes the mass multiplication process, cultivation methods, harvesting, and downstream processing of biomass. Current global market and biorefinery strategies are also discussed.

Morphology and Life Cycle of *Haematococcus pluvialis*

Haematococcus pluvialis is a freshwater single-celled freshwater microalga which is an efficacious producer of astaxanthin in massive amounts. It is also called as *Sphaerella lacustris* or *Haematococcus lacustris*. Typically present in freshwater bodies such as artificial pools, birdbaths, man-made ponds etc., and it is distributed worldwide (Shah et al.). The cell is oval to spherical with a diameter of 30 μm (Oslan et al.). At the start, *Haematococcus pluvialis* begins as a free-swimming, biflagellate greenish microalgae with a singular pyrenoid-carrying chloroplast, then transforms into a non-motile palmella by losing its flagella, and finally into the thicker-walled aplanospore via eliminating its flagella Niizawa et al. *H. pluvialis* does have a four-staged life cycle that is segmented into macrozooids, a flagellated cell stage which is also called as early growth phase, microzooids, palmella stage (immature cyst) followed by maturation, and aplanospore (mature cyst). It consists of two phases: a vegetative phase and a red non-motile encysted phase. The green vegetative phase involves the palmella stage and microzooids, whereas the reddish non-motile encysted phase includes aplanospores or hematocysts Shah et al.. Aplanospore develops from the vegetative cells due to morphological changes occurs during unfavorable growth condition and other stress factors Mota et al. Due to the formation of aplanospore, flagellum degenerates with increasing cell size and the astaxanthin content reaches about 81.2%.

Facilities Needed

H. pluvialis growth can take place in open tanks or closed photobioreactors. For the large-scale biomass production, open raceway ponds are the most popular as well

as feasible cultivation system. While photobioreactors provide higher biomass yield, level of contamination is comparatively lower than in raceway ponds Mota et al. Current production process is schematically represented.

Photobioreactor

Photobioreactors are generally designed as transparent tanks, in which environmental conditions like temperature, light illumination, pH, and carbon dioxide levels can be controlled. PBR increases the production of biomass by supplying the best growth condition for certain strains of microorganisms. Light and temperature are essential for biomass production, and they should be optimized as it occurs in nature Metsoviti et al. By limiting the delivery of optimum exposure of light to other cells, the exterior layer of the culture accepts most of the light source de Mooij et al. Light-emitting diodes LEDs are widely used light source due to their effective as well as simultaneously low of cost. When compared to open systems, PBR necessitates less area, minimizes contamination, and provides unique culturing growth parameters independent from environmental conditions. The operation of control modules and sensors in PBR systems facilitates the automation of biomass cultivation. In the aspects of disadvantages, the construction and operation of these systems are more expensive. However, it requires enormous supply of electricity to provide light illumination and complexity in designing as well as building the PBRs. Moreover, the formation of biofilm in the course of cultivation reduces light entry and causes difficulties with tank cleaning (Dębowski et al. In any case, the increased productivity and homogeneity of green algal biomass mitigate for its higher expenditure of these technologies Blanken et al. Due to their production of high value-added products, including biopharmaceuticals, nutraceutical, components of healthy food product, and high-grade cosmetics, these PBR systems on large scale is developing to be more and more popular Hubo et al. Closed photobioreactors are effective for the growth of *H. pluvialis*, as it is sensitive towards environmental changes. The selection of suitable bioreactors is a crucial step at an early stage. Tubular systems are distinguished by a huge illuminating space along heavy workload, as well as significantly greater maintenance

and operation expenditure Fazal et al. Use of the plastic bag PBRs is a cheaper alternative, but it faces some obstacles including lower light illumination, culture mixing, and mechanical damage Huang et al. Flat panel and column airlift PBRs are known for its high capital investment, longer lifespan, high light illumination, efficient mixing, and mass transfer. The rectangular form of airlift PBRs promotes the sedimentation of microbes causing complications while cleaning Ting et al.

Wan et al carried out a comparative study between raceway tanks and column photobioreactors and reported that during the photoinduction phase of *H. pluvialis*, the biomass and astaxanthin concentration in column photobioreactor were 0.9 g L⁻¹ and 2.8%, respectively. Raceway tanks showed 0.6 g L⁻¹ and 2.2%, respectively. The contamination and parametric control capabilities of flat-panel and closed tubular bioreactors are excellent; however, the CO₂ restriction and limited illumination efficiencies have an impact on biomass buildup Olivieri et al. It is essential to optimize the mixing effect in the bioreactors to avoid the accumulation of CO₂ and O₂, during growth phase. To overcome this issue, Yoo et al. Used 6 L indoor bag-type PBR with adequate hydrodynamic mixing of *H. pluvialis* and obtained a yield of 2.62 g/L biomass concentration and 78.37 mg/L astaxanthin content. Productive features of *H. pluvialis* cultured in various photobioreactors and culture mediums are enumerated.

Open Raceway Pond

Open raceway ponds are well known since the 1950 s Borowitzka, and it is more economical than photobioreactors for microalgal cultivation (Huntley et al. They are closed-loop recirculation channel which includes paddlewheel and baffles. The fixed depth of raceway pond is 30 cm, so that all the cells utilize ideal light irradiation. Paddlewheel promotes the proper mixing and circulation of the stream. Nutrients are supplemented near the paddlewheel for optimal mixing all over the pond. While baffle brings the uniformity of stream right through the curved bend plus limits the dead zone formation. The dead zones negatively impact on mixing, unwanted energy losses and enables solids to settle Bompoulakis et al. It is challenging to regulate the temperature, lighting, and evaporation rate in open

raceway ponds and thereby affecting the cooling process. Open ponds contribute a low yield of biomass, and it is prone to contamination Terry & Raymond. The integration of photobioreactor along with open raceway pond is known as hybrid two-stage cultivation. At first, photobioreactor controls the optimum growth condition, and after that, raceway pond exposes the cell to nutrient deprivation. As a result, the desired product's synthesis is enhanced Huntley & Redalje, Rodolfi et al. Productive features of *H. pluvialis* cultured in several open raceway ponds are enumerated.

Mass Production Processes Selection of Microalgal Strain

Haematococcus pluvialis is a widespread global species. Annually, a great percentage of strains were isolated and characterized all over the universe. Existing biotic biodiversity would allow higher functioning variants to be preferred for astaxanthin synthesis without any need for genetic modification Li et al. Variant selection is still an important phase in commercial microalgal cultivation. Cost for large-scale production can be diminished by analyzing many potent species and attributes like inflation of biomass and carotenoid productivity. Use of native microalgal species is best suited for commercial production as they can adapt effectively to the local environmental and weather condition. Kiperstok et al. studied the total astaxanthin and biomass production in 25 strains of *H. pluvialis* in twin layer photo bioreactor. Among the 25 strains, strain CCAC 0125 is selected as best strain for mass cultivation while whole biomass obtained and astaxanthin productivity of 91.2 g/m² and 1.4 g/m² and astaxanthin content of 1.5% dry weight. Zhang et al. selected *H. pluvialis* 26 and *H. pluvialis* WZ that are suitable superior variants for its biomass yield when compared to other two strains *H. pluvialis* 30 and *H. pluvialis*. *H. pluvialis* 26 showed astaxanthin and biomass productivity of 51.06 mg L⁻¹ and 1.83 g dry wt. L⁻¹ with 2.79 g 100g⁻¹ dry wt. of astaxanthin content. Therefore, the selection of optimal strain enhances the production process and cost effective.

Microalgae Cultivation

To attain high biomass and astaxanthin productivity, optimization of light, temperature, pH, growth media composition, etc. is essential. Carotenogenesis

initiation requires intense exposure to stress condition to accumulate higher levels of astaxanthin. The source of stress can be induced either via combination of multiple stress factors, or from high levels of one stress factor Shah et al. It is also interesting to notice that when cells are subjected to excessive stress, their proliferation completely stops and they die within a short period of time Su et al. When culture is exposed to nutrient deprivation, it triggers the astaxanthin accumulation inside the cells Saha et al. Most prevalently used medium for growth are BG-11 Rippka et al, OHM Fábregas et al, BBM Bischoff, KM Kobayashi et al, and their alterations. It is significant to supply nutrients during the day time with maximal sun irradiation, when photosynthesis occurs. The best source of inorganic nitrogen was found to be sodium nitrate Sarada, Bhattacharya, & Ravishankar, and urea can be used as an alternative source. Nitrogen starvation leads to the production of approximately double the astaxanthin ratio than the phosphorus deficiency. It might be caused due to lack of nitrogen resulting in higher cellular damage, which displays a remarkable degradation of chlorophyll Boussiba et al. Addition of NaCl 0.25–0.5% w/v to the growth media induces astaxanthin production.

Also, astaxanthin accumulation can be enhanced by combination of NaCl and 2.2 mM sodium acetate Sarada, Tripathi, & Ravishankar, 288 T. Fayaazuddin et al. The ideal temperature for *H. pluvialis* proliferation and astaxanthin generation is between 20–28 C Fan et al; Kang et al; Wan et al. It is preferred to gradually adjust the temperature, which enables better adjustment to the new circumstances Hata et. The optimal pH for *H. pluvialis* is within 7–7.8 pH Hata et al. Sarada, Bhattacharya, Ravishankar, Irregular shift in pH can have negative results on the biomass cultivation. Standard irradiance for culture improvement ranges within 40–50 $\mu\text{mol photons m}^2 \text{ s}^{-1}$ Hata et al Park et al. Optimal irradiance tends to be higher to reach high growth rates for about 70 Zhang et al to 177 $\mu\text{mol photons m}^2 \text{ s}^{-1}$ Dominguez-Bocanegra et al. The use of white or blue LED light at the ratio of 3:1 at 7000 lx induces carotenogenesis. Park et al recorded continuous escalation of light intensity results the cells with progressive conversion to cysts and

causes better accumulation of astaxanthin. It is due to the potential of cells to manage increasing stress levels.

Two-step Strategy

In this technique, the biomass is initially generated under ideal growth circumstances (green stage), and then, the growth is subjected to harsh ecological parameters to trigger astaxanthin accumulation. In this two-stage procedure, astaxanthin can be generated effectively Aflalo et al. Orosa et al. In commercial setups, astaxanthin accumulation is usually induced by increasing solar irradiance or temperature and combination of nutrient deprivation especially phosphate and nitrate del Río et al; del Río et al; García-Malea et al. Green and red phase of biomass production ranges from 0.1 to 0.5 and 0.1 to 4.8 g L⁻¹ day⁻¹, respectively. The astaxanthin content and its productivity ranged from 0.8 to 4.8% of DW. Simple method for efficient production of astaxanthin is by *H. pluvialis*. It incorporates the employment of nitrate starving in combination with a constant standard irradiance with in growth media del Río et al; del Río et al; García-Malea et al. Thus, the one-step technique was used to induce synchronous cellular proliferation and astaxanthin accumulating at a considerable level in a laboratory environment under continuous light illumination, yielding a median astaxanthin yield of 20.8 mg L day⁻¹ García-Malea et al.

On a pilot scale, researchers investigated the practicability of this approach in an outdoor tubular bioreactor. The productivity of biomass and astaxanthin was calculated to be 0.7 g L⁻¹ day⁻¹ and 8 mg L⁻¹ day⁻¹, respectively. Two-step Vs One-step Strategy However, the one-step procedure is less complicated than the two-step technique and astaxanthin manufacture occurs in a continuous fashion as preferred. But it drops two significant drawbacks. First, when compared to two-step method, the actual astaxanthin production is remarkably lower. Second, it requires light illumination during night as it is unsuitable for outdoor cultivation. Thus, it is too expensive Aflalo et al.

Harvesting

This is the most difficult and restricting component of *H. pluvialis* industrial biomass production. Harvesting of biomass is depended on the morphological features

of the cell, which includes size, shape, specific weight, and cells concentration (Lam & Lee,).Centrifugation is the most usual technique for harvesting and combined with other processes.The hematocysts are split up by means of a passive settlement,due to its high density and eventually robust after centrifugation (Lorenz & Cysewski, 2000;Olaizola,2000; Pérez-López et al., 2014).Around 13.5% of total suspended solid in the form of algal cake is acquired via this procedure (Li et al.,2011).

Disk-stack centrifugation and flotation efficiently recovered biomass of more than 95%, and both are alternatives for *H.pluvialis* harvesting technique.

Rupture of Cells

Cell disruption step can be carried out either before or after drying the biomass. It attempts to enhance the recovery efficiency of the intracellular compounds. Accumulation of astaxanthin induces the thickness of the cells, turning firm and rigid. The cell wall of hematocysts possesses three layers, one sporopollenin, and two cellulose with mannose (Hagen et al., 2002; Sun et al., 2016). Hence, it is mandatory to apply pretreatment methods capable of weakening this resistance. 290 T. Fayaazuddin et al.Mechanical process is involved widely in commercial scale and more particularly milling along with high pressure homogenizer (Razon & Tan, 2011; Shah et al.,2016). Bead milling technique follows by colliding minute spheres by revolving at intense speed (Onumaegbu et al., 2018). When the biomass concentration after accumulation is between 100 and 200 g/l, it is considered the most effective method (Greenwell et al., 2010). On the other hand, either one two displacement pumps compress the cells at elevated pressure to disrupt the thick cell wall in a high pressure homogenizer (Lee et al., 2012). It is simple and minimizes contamination. Nearly 75% of recovery efficiency can be reached in a single step. After cell disruption, it is suggested to keep cells from exposure to light and immediately execute the astaxanthin extraction (Khoo et al., 2019). Cheap and best alternative for cell disruption is the use of pulverizer. It is widely used in food industries, and the mechanism is similar to bead milling.

Drying

Drying preserves the quality of the pigment, longer shelf life, and it must occur immediately after harvesting to avoid spoilage. Most suitable methods are spraydrying and freeze-drying techniques (Li et al., 2011). Freeze-drying deals with freezing of algal cake. It inflicts less damage and more expensive on industrial scale when compared to spray-drying. Spray-drying is rooted on a forced course of hot air inside a drying compartment. It suddenly vaporizes the droplets when they get exposed to the air, and it is regarded as the most convenient process (Li et al., 2011; Panis & Carreon, 2016). Spray-drying delivers a recovery efficiency of about 95% of dry biomass in powder (Leach et al., 1998). Main setbacks of this method are certain risks in deterioration of pigments and high operational costs (Grima et al., 2003).

Astaxanthin Extraction

The recovery of specific product is feasible whenever the cell wall is shattered, and the biomass is perfectly dry. Astaxanthin is soluble in solvents and oils, as it is lipophilic in nature. Solvents used for extraction includes alcohols (ethanol, methanol, etc.), acetone, ether, concentrated acids or bases (potassium hydroxide, dimethyl sulfoxide, etc.), aliphatic hydrocarbons (hexane), edible oils, and supercritical carbon dioxide (CO₂-SC) (Khoo et al., 2019; Shah et al., 2016). Between these, CO₂-SC and conventional solvents are regarded as the most effective, well suited, and sustainable to be assigned in *H. pluvialis* (Shah et al., 2016). In commercial scale, CO₂-SC is widely employed due to its quicker extraction period, low toxicity, low degradation, cost effective, and high fineness of astaxanthin when compared to conventional solvents. While in the case of solvents, it is significant to assign the type of solvent used, since many possess higher toxicity leading to serious health issues (Khoo et al., 2019).

Establishment of Mass Production System

For establishment of the production setup, a sum of `555,900 was required for lab-scale production, 2,716,350 for small-scale production, and 17,159,400 for large-scale production. The main sections of production units are a bioreactor, raceway pond, centrifuge, pulverizer, spray dryer, and storage containers. Non-

recurring expenditure and laboratory equipment's essential for astaxanthin production is denoted. A standard two-step approach was practiced for the production of astaxanthin. The vegetative stage of *Haematococcus pluvialis* was cultivated in a 5 L photo bioreactor; here, we have accounted a total of 3 and 10 bioreactor in small-scale and large-scale production, respectively. These photo bioreactors are operated in a semi-continuous mode which supplies inoculum (cyst stage) for the pond. In small-scale production, a single pond is considered and a total of five ponds have been constructed for large-scale production. While in the case of lab-scale production, open raceway ponds are absent and single 5 L photo bioreactor is utilized for cultivation of both vegetative and cyst stage. The temperature of bioreactors is maintained between 10 and 25 C using cooler. Amount of CO₂ is also controlled in both pond as well as bioreactor to control the pH. The aeration is provided through the sparger with high pressure pumps, and bioreactor consists of both air and media filter which are involved in sterilization. A motor-powered paddlewheel is used to keep the suspension of cells by turbulence. The cells are harvested and stored in cell storage containers, which form a cell slurry. These cell slurries are dehydrated using a centrifuge, and resultant algae paste was powdered using a spray dryer. Then, dried cells were further powdered using pulverizer. Recurring expenditure for each batch of biomass production is represented.

Production Parameter

The production parameter was considered as per (Li et al., 2011). The temperature is maintained below 25C using coolers. The inoculum of size $5-9 \times 10^4$ cells/ml are required for initial cell concentration. The working cell standard and final concentration are $4.2-6.0 \times 10^5$ and $5-8 \times 10^5$ cells/ml respectively. Pond depth must be around 13-15 cm, and water flow rate is adjusted to 25-30 cm/s. The initial cell concentration for inoculation is $5-7 \times 10^4$ cells/ml. It takes up to 8 - 10 days for reddening of inoculum. The above-mentioned values are prescribed in. For the downstream processing of high-quality astaxanthin, the final dry weight is required to be 0.25-0.59 g/L. Cell slurry acquired after sedimentation needed to be around 1.1-1.6% of dry biomass. In case of cell paste, the dry biomass percentage

ranges in between 10% and 16.2%. By estimating all the above values, the astaxanthin content produced will be around 2–3.7%.

Cost Benefit Analysis

Considering the time taken for the inoculum to accumulate astaxanthin in the pond, we have assessed the overall expense per each batch which is demonstrated in. It includes temperature maintenance, cooling of culture, aeration consumption rate, power consumption of centrifuge, paddle wheels and pulverizer, rate of CO₂ utilized, oil consumption of spray dryer, air filters, and media composition cost. The direct production cost of biomass and astaxanthin per batch is around 1750 and `250 (lab-scale), 10,500 and `2000 (small-scale), and 42,000 and 9000 (large-scale), respectively. Annual production cost of biomass and astaxanthin is estimated to be 52,500, 315,000, and 1,260,000, respectively. The astaxanthin production per batch is expected around 5 kg per batch in large-scale production, eventually producing 650–800 kg each year. Astaxanthin yield may vary due to certain factors such as pH, sunlight, temperature, etc., While in small-scale production, astaxanthin production is around 0.5–1 kg per batch and produces 30 kg/year approximately. Although the astaxanthin production is comparatively lesser in small-scale production, it provides a sustainable profit. Astaxanthin production per year in lab-scale is estimated to be 1000 g. The current market value of astaxanthin varies between 166,534 and 501,118 per kg and around 300,000,000 per ton. The biomass will be harvested 30 times a year. In large-scale production, 750 kg of astaxanthin is expected to be harvested which provides a capital of 225,000,000 per year through the sales of astaxanthin. In small production, as mentioned earlier, 30 kg will be produced per year generating a total of 9,000,000 per year. Laboratory scale generates a capital of 300,000 per year. The cost may vary based on the nature and purity of the pigment accumulated. With deducting expenses, including production cost, labor cost, and some additional expenses. The net profit is estimated to be 197,500 (lab-scale production), 6,885,000 (small-scale production), and 216,740,000 (large-scale production).

Current Global Market for Astaxanthin

With the current trend of using natural components in cosmetics, food, and medical applications, there is a considerable concern about synthetic ingredients entering the human food chain. Increasing knowledge of the health benefits of natural astaxanthin, the global demand for astaxanthin extracted from *H. pluvialis* has been rapidly increasing. In 2021, Astaxanthin's global market is predicted to be worth USD 647 million. During the forecast period, it is predicted to reach around USD 1206.52 million by 2026 and recording a CAGR of 7.7% (Market Research Future, 2021). Depending upon the purity levels, the market value of astaxanthin tends to vary between USD 2000–15,000/kg (Koller et al., 2014). In the aquaculture, yeast-derived astaxanthin and synthetic astaxanthin are predominantly consumed. While for human consumption, the *H. pluvialis*-derived astaxanthin is recommended for cosmetics, dietary supplements, and food (Nguyen, 2013). When compared to synthetic astaxanthin, in medicinal and nutraceutical uses, natural astaxanthin is 3–4 times more effective and valuable than artificial astaxanthin (Han et al., 2013). Because there is an increasing interest in natural astaxanthin, mass growth of *H. pluvialis* on a commercially has a lot of promise and could be a lucrative company with huge opportunities in trading enterprises. Synthetic astaxanthin can be toxic and carcinogenic, due to its presence of stereoisomers (Khoo et al., 2019). Commercial Astaxanthin Production from Green Alga *Haematococcus pluvialis* 297 Astaxanthin business has been gaining interest due to the following reasons:

1. Bioactive profile which involves antioxidant and free radical scavenging activity.
2. Growing consumer needs on personal care, cosmetic, and nutraceutical products.
3. Huge potential for industrial applications.
4. Gaining popularity in several health spa service providers and cosmetic producers.
5. Rise in health consciousness and demand for natural food additives.
6. Natural astaxanthin promotes better pigmentation in some species of fishes.

7. U.S. FDA approved astaxanthin as safe (GRAS) for dietary supplements and authorization from several European food organizations.

As a commodity of extraordinary commercial value, astaxanthin alone explains the high price of *H. pluvialis* cultivation and its processing setups, making it an economically practical job (Shah et al., 2016). Application of biorefinery models for the astaxanthin production from *H. pluvialis* turns into more sustainable and feasible. In this method, incorporation of bioprocesses leads to production of desired products and co-products of more superior value with slightest waste. Biorefinery of microalgae enables the processing of various biomass products, which includes carbohydrates, proteins, lipids, and bioenergy utilizing a single raw material. As follows, revenues are maximized (Chew et al., 2017). Simultaneous production of astaxanthin, triglycerides, and polyhydroxybutyrate (PHB) can be obtained and becomes a possible source of biodiesel and biopolymers, respectively. The residual microalgal biomass can be utilized in biogas plants for its production processes (Prieto et al., 2017). The residual biomass performs vital role in producing methane to generate thermal energy for industrial facilities, protein rich feed for animal consumption, and carbon source for fermentation industries (Chew et al 2017; Oliveira et al., 2020).

Conclusion

Haematococcus pluvialis holds the highest capacity in accumulation of astaxanthin, and it is the source for obtaining the carotenoid with tremendous market value. Even it comes with high production costs, these can be reduced with the addition of effective methods of cultivation and processing of biomass. Biorefinery modelling makes it feasible to attain high-value added products and co-products. Organic astaxanthin has a higher demand than artificial astaxanthin, leading to increased rate of production. Also, the animal feed industry has exhibited prominent growth and demand for astaxanthin. As a result, this bioproduct has the potential to reach the top of the market. Commercial synthesis of astaxanthin using *Haematococcus pluvialis* is indeed a potential using current modern technologies. The total manufacturing expenditure of astaxanthin per batch in large-scale production is estimated to be 51,000. While in lab-

scale and small-scale production, the production cost is evaluated to be 2000 and 12,500, respectively. When compared to current industrial processes, the production cost is considerably inexpensive. Lab-scale production of astaxanthin can be preferred for optimization of production processes, due to its low yield. The large-scale production is more efficient due to better quality and quantity of photobioreactors, raceway ponds, and other instrumentation used in the cultivation process. Small-scale production is budget friendly and best option in the case of low investment scenarios.

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