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Research review paper

Heterotrophic cultivation of microalgae for pigment production: A review

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ABSTRACT

Pigments (mainly carotenoids) are important nutraceuticals known for their potent anti-oxidant activities and have been used extensively as high end health supplements. Microalgae are the most promising sources of natural carotenoids and are devoid of the toxic effects associated with synthetic derivatives. Compared to photoautotrophic cultivation, heterotrophic cultivation of microalgae in well-controlled bioreactors for pigments production has attracted much attention for commercial applications due to overcoming the difficulties associated with the supply of CO_2 and light, as well as avoiding the contamination problems and land requirements in open autotrophic culture systems. In this review, the heterotrophic metabolic potential of microalgae and their uses in pigment production are comprehensively described. Strategies to enhance pigment production under heterotrophic conditions are critically discussed and the challenges faced in heterotrophic pigment production with possible alternative solutions are presented.

1. Introduction

Carotenoids are the most abundant and widely distributed pigments on earth, second only to chlorophyll. Chemically, they are lipophilic isoprenoid compounds composed of a C40 backbone. The structural diversity for the various carotenoids is provided by the number and position of the conjugate double bonds, cyclization at one or both ends and oxygenation of the backbone (Britton, 1995). Carotenes are the hydrocarbon carotenoids, while xanthophylls are the oxygenated version. In photosynthetic organisms like plants and algae, carotenoids are associated with the light harvesting complex of photosynthesis, functioning as accessory pigments and are also known for their photoprotective effect of photosystems from oxidative damage (Varela et al., 2015). Animals and humans are incapable of de novo carotenoid synthesis, and rely on dietary sources for acquirement of these essential nutrients. Carotenoids are important nutraceuticals because of their known beneficial effects including anti-oxidant, anti-ageing, anti-inflammatory, anti-angiogenic, cardio protective and hepato-protective properties (Zhang et al., 2014a,b). The global carotenoid market is estimated at US$ 1.24 billion in 2016 and projected to reach US$ 1.53 billion by 2021, with a compounded annual growth rate (CAGR) of 3.78% from 2016 to 2021 (http://www.marketsandmarkets.com/Market-Reports/carotenoid-market-158421566.html). Microalgae are being increasingly recognized as a potential source of carotenoids. Carotenoids are essential for the survival of microalgae, to protect the cells from the reactive oxygen species generated during photosynthesis and high light intensity and to dissipate excess light as heat by the xanthophyll cycle. It has been shown that disruption of the enzymes involved in carotenoid synthesis, phytoene synthase and phytoene desaturase can lead to autophagy in *Chlamydomonas reinhardtii*. Autophagy is triggered in these mutants when exposed to high light, as the photo protection provided by carotenoids is compromised (Pérez-Pérez et al., 2012). The major carotenoid of commercial interest from microalgae are β-carotene, lutein and astaxanthin. Lutein is known for its protective role against macular degeneration of the eye and dietary lutein is important as it cannot be synthesized by humans. Astaxanthin is a well-known anti-oxidant and has cardio protective, neuro protective, anti-cancerous and anti-diabetic properties. β-carotene, is pro-vitamin A and is also an anti-oxidant with cardio protective effects (Spolaore et al., 2006).

Microalgae, including cyanobacteria, are among the oldest photosynthetic organisms on earth, and together with the protists, they are the primary producers in aquatic ecosystems. The ability of microalgae to convert atmospheric inorganic carbon to organic biomass with the help of

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sunlight is their hallmark feature, and they are being increasingly recognized as a potential biofuel feedstock in the recent years. The unicellular nature of microalgae simplifies large scale cultivation and the microalgal biomass with its constituent components has applications in various industries ranging from pharmaceutical, nutraceutical and health care products (Mata et al., 2010). The global algae market is expected to be worth of about US$ 1.1 billion by 2024, with a CAGR of around 7% (http://www.transparencymarketresearch.com/pressrelease/algae-market.htm). The storage components or energy reserves of microalgae are primarily polysaccharides and lipids/triglycerides. Microalgae are capable of accumulating very high levels of lipids (55–70%) and carbohydrates (as high as 70%), when cultivated under appropriate conditions (Vitova et al., 2015). These components can be used in the biofuel industry for the production of biodiesel, bioethanol, biobutanol, biohydrogen, biomethane and so on. The other bioactive compounds extracted from microalgae, like the pigments, functional polysaccharides, and polyunsaturated fatty acids are the green and natural alternatives for a wide variety of chemical based components used in health care and cosmetics (Spolaore et al., 2006). The microalgal biomass itself or the residual biomass left after extraction of any valuable product can be subjected to thermochemical conversion, yielding biochar, natural gas and fuel oils. Complete utilization of the microalgal biomass is thus possible, with the advent of various technologies. Microalgae are very versatile with regard to its adapting technologies and very robust, as they can be seen in almost every environment. Microalgae has also been used for bioremediation of wastewater, as they can grow in marginal land and utilize the nutrients present in any wastewater for biomass production and hence alleviating the problems of eutrophication (Yen et al., 2013).

Even though microalgae can be a potential source of carotenoids, mass cultivation of microalgal biomass with optimal light supply is a major concern to be overcome. Based on their metabolism, microalgae can be photoautotrophic, photoheterotrophic, mixotrophic and heterotrophic. The most commonly cultivated microalgae are photoautotrophic, utilizing sunlight and atmospheric CO_2 as their energy and carbon source, respectively. Open systems are the most preferred systems for the large scale cultivation of photoautotrophic microalgae as they have many advantages: (i) can utilize sunlight as energy source and atmospheric air as CO_2 source, (ii) very low installation and operating costs, and (iii) lower energy consumption (Brennan and Owende, 2010). Most commercial establishments utilize open systems for cultivation of microalgae. But it is limited to certain robust species that grow under specific conditions like high salinity or alkaline pH, protecting the culture from contaminants. *Spirulina*, *Dunaliella* and *Chlorella* are successfully cultivated outdoors for single cell protein and pigment production (Mata et al., 2010). Closed photobioreactors (PBR) are often the cultivation method of choice for the production of high end pharmaceutical compounds that demand the maintenance of axenic cultures in a pure state. PBR requires successful design based on the organism to be cultivated and the product in question, accompanied by high installation and operating costs. In both open systems and PBR, efficient supply of optimal light intensity is still a major concern. In open systems, pond depths are limited to be around 20 cm which allows maximum penetration of light and in closed PBRs vigorous mixing is often needed to prevent a sub-population of cells being in the dark zone undergoing respiratory loss of biomass (Chang et al., 2016). Growing heterotrophic microalgae in the absence of light in conventional bioreactors seems to be a very viable option for economic cultivation of microalgae. Heterotrophic cultivation of microalgae is possible in the existing infrastructure for bacterial fermentations, and the major hurdle of light supply is overcome (Bumbak et al., 2011).

A number of microalgal species are capable of growing in the dark using organic carbon sources. Obligate heterotrophs, such as the marine thraustochytrids, are cultivated solely in heterotrophic mode for the production of polyunsaturated fatty acids. Several Chlorella species, like *C. protothecoides* (Shi et al., 1999), *C. vulgaris* (Liang et al., 2009), *C. zofingensis* (Ip and Chen, 2005a) and *C. minutissima* (Bhatnagar et al., 2010) and other microalgae like *Tetraselmis* (Azma et al., 2011) and *Neochloris* (Molares-Sanchez et al., 2011) are capable of both autotrophic and heterotrophic growth. The major advantages of cultivating microalgae under heterotrophic conditions can be summarized as follows:

a) The problem of optimal light supply for the culture is overcome. Heterotrophic microalgae can grow and metabolize in the absence of light or under dark conditions, using an organic carbon as the energy and carbon source (Chen, 1996).
b) Cell densities in the order of 100 g/L can be achieved in heterotrophic cultivation, which in turn simplifies harvesting of the biomass (Morales-Sánchez et al., 2015). In contract, under photoautotrophic conditions, the maximum cell density of microalgae that can be achieved in photobioreactors is around 40 g/L, while in outdoor open-pond or raceway-pond cultures, the cell concentration is usually lower than 10 g/L. This significantly increases the energy consumption of cell harvesting and the cost of biomass production (Scaife et al., 2015).
c) The heterotrophic cultivation can be carried out in conventional industrial scale fermenters, which offer a better control over the process parameters like pH, temperature, oxygen levels and carbon source (Perez-Garcia et al., 2011). Substrate inhibition with very high initial substrate concentration can be overcome by process strategies, such as fed-batch and continuous fermentations, and even at very high cell densities, the cell growth is not limited by self-shading of light supply that normally happens in photoautotrophic systems (Chen et al., 2011).
d) From the economic perspective, heterotrophic cultivation could be much more beneficial than photoautotrophic cultivation. The input energy to ATP conversion ratio is higher for heterotrophic cultivation (18% of energy obtained can be converted to ATP, while only 10% was converted under photoautotrophic conditions (Yang et al., 2000)). Similar observations were made by Behrens (2005), who calculated the conversion efficiency of input energy in the form of electricity to ATP and NADPH and concluded that heterotrophic cultivation is economically more advantageous than photoautotrophic cultivation; the cost per kg of dry biomass for heterotrophic cultivation was calculated as US$ 2, while for photoautotrophic cultivation it was about US$ 11 (Behrens, 2005).

Although heterotrophic cultivation possesses many advantages as mentioned above, it does not fix CO_2 during growth (unlike photoautotrophic growth). Instead, it generates CO_2 so does not positively contribute to the mitigation of global CO_2 emissions. From economic view, the major cost for conducting heterotrophic cultivation is the installation and equipment costs which account for up to 42% of total investment costs, as well as the cost of organic carbon source (e.g., glucose or acetate) (Lowrey et al., 2015). Compared to PBRs, the microbial fermenters are not specially designed for a particular microalgal species and the universal design can be mass produced and acquired at comparable costs (Behrens, 2005). It is also possible to reduce the costs of carbon source and other nutritional requirements by the use of waste biomass resources and recycling of nutrients and media in cultures (Lowrey et al., 2016). Comparison of cost evaluation and life cycle assessment (LCA) on pigments production from autotrophic and heterotrophic microalgal cultures has been lacking, but a comparative LCA for biodiesel production from microalgae by photoautotrophic (sunlight and CO_2), mixotrophic and heterotrophic cultivation (sugarcane and sugar beet as carbon source) is available (Orfield et al., 2015). The study showed that among the three cultivation modes, the net energy ratio (NER) was the highest for heterotrophic method. NER for heterotrophic cultivation was around 0.6 to 1.6, while for photoautotrophic cultivation it was 1.3 (Orfield et al., 2015). In a similar study, LCA assessment of biodiesel production from the heterotrophic cultivation of a marine Thrasutochyrid using glycerol as a carbon source revealed that the biodiesel derived from heterotrophic