Bioresource Technology 221 (2016) 607–615

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Utilising light-emitting diodes of specific narrow wavelengths for the optimization and co-production of multiple high-value compounds in *Porphyridium purpureum*

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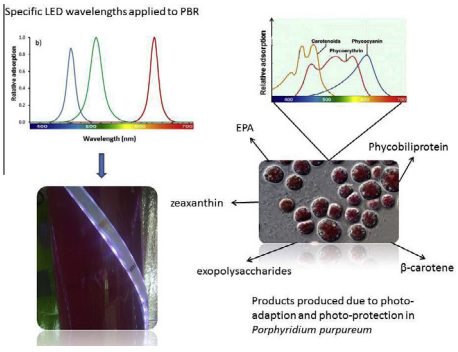
HIGHLIGHTS

- Green light plays a significant role in the growth of *Porphyridium purpureum*.
- Multi-chromatic LED wavelengths accumulated the highest yields of valuable products.
- Photo-adaption and photo-protection are suspected to boost product yield.
- Specific wavelengths can increase product ratios increasing biomass value.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 21 September 2016
Accepted 22 September 2016
Available online 24 September 2016

Keywords:
Porphyridium
Phycobiliproteins
Fatty acids
Pigments
FTIR
Exopolysaccharides

ABSTRACT

The effect of specific narrow light-emitting diode (LED) wavelengths (red, green, blue) and a combination of LED wavelengths (red, green and blue – RGB) on biomass composition produced by *Porphyridium purpureum* is studied. Phycobiliprotein, fatty acids, exopolysaccharides, pigment content, and the main macromolecules composition were analysed to determine the effect of wavelength on multiple compounds of commercial interest. The results demonstrate that green light plays a significant role in the growth of rhodophyta, due to phycobiliproteins being able to harvest green wavelengths where chlorophyll pigments absorb poorly. However, under multi-chromatic LED wavelengths, *P. purpureum* biomass accumulated the highest yield of valuable products such as eicosapentaenoic acid (~2.9% DW), zeaxanthin (~586 μg g^{-1} DW), β-carotene (397 μg g^{-1} DW), exopolysaccharides (2.05 g/L^{-1}), and phycobiliproteins (~4.8% DW). This increased accumulation is likely to be the combination of both photo-adaption and photo-protection, under the combined specific wavelengths employed.

1. Introduction

There is significant and increasing pressure within the cosmetic, pharmaceutical, biopharmaceutical and nutraceutical sectors to

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http://dx.doi.org/10.1016/j.biortech.2016.09.093
0960-8524/

deliver so called "green pharmaceuticals", i.e. those derived from a more environmentally friendly and sustainable production process (Ottman, 2011). This demand is the result of an increased consumer awareness and demand for personal care products, cosmetics, and pharmaceuticals to be derived from natural products rather than synthetic origin (Gernaey et al., 2012).

Porphyridium purpureum has the potential to fill a number of niche areas within these industrial sectors by possessing the ability to produce a broad range of commercially valuable chemicals such as phycobiliproteins (PBP) (Guihéneuf and Stengel, 2015), sulphated exopolysaccharides (EPS) (Fuentes-Grünewald et al., 2015), and polyunsaturated fatty acids (PUFAs) (Durmaz et al., 2007). Phycobiliproteins are a group of coloured water-soluble, αβ heterodimeric proteins that constitute the major complex light-harvesting pigments of cyanobacteria, red algae, glaucocystophytes, and cryptophytes (Bermejo Román et al., 2002; Roy et al., 2011). PBPs can be applied as a colourant in the food and drink industry, as a pharmaceutical agent, and as a fluorescent agent (Bermejo Román et al., 2002; Spolaore et al., 2006). Due to the wide range of applications of PBPs, the total market value is estimated to be >US$ 60 million (Borowitzka, 2013). EPS are complex sulphated polysaccharides composed of different sugar monomers such as xylose, glucose, and galactose (Fuentes-Grünewald et al., 2015; Patel et al., 2013; Sun et al., 2008). *Porphyridium* spp. has been shown to synthesize and secrete EPS into the culture medium, which can be easily extracted from the culture medium (Fuentes-Grünewald et al., 2015; Sun et al., 2012; Velea et al., 2011). A number of studies have demonstrated antiviral, anti-radiation and antioxidant activities, antitumor and immunomodulatory activities properties of this compound (Patel et al., 2013; Raposo et al., 2013; Sun et al., 2012). Polyunsaturated fatty acids (PUFA) have been shown to be the predominant fatty acids detected in *Porphyridium spp*., reaching 43.7% of total fatty acids (Durmaz et al., 2007), PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to have a variety of health benefits such as hypotriglyceridemic and anti-inflammatory properties (Bergé et al., 2002; Ryan et al., 2009; Spolaore et al., 2006).

P. purpureum possess several mass production advantages such as ease of culturing, the ability to grow in a range of latitudes, and a broad range of useful products enabling the potential for a biorefinery approach to be adopted; which focuses on the co-production of multiple value added product streams, improving process economics, and therefore market viability (Guihéneuf and Stengel, 2015; Vanthoor-Koopmans et al., 2013). However, in order to realise the value of such molecules, algal production facilities must adopt control systems which guarantee quality, purity, potency, consistency, efficacy, efficiency, and safety. Photobioreactors (PBRs) allow for the common biotic (e.g. pathogen contamination and competition with other microorganisms) and abiotic (e.g. temperature, gases, pH, and nutrients) bottlenecks in microalgal growth to be greatly reduced or even removed in a managed environment. Therefore, in autotrophic cultivation, light becomes the most critical processing parameter (Carvalho et al., 2011). Although sunlight offers a zero-cost source of illumination, the diurnal fluctuations of light can significantly decrease the total biomass concentration and consistency of product formation (Pérez-López et al., 2014). Algal cells are only able to utilise light energy in the photosynthetically active radiation (PAR) range (400–700 nm), which equates to ~48% of total solar energy (Park et al., 2011). Furthermore, calculations have shown that only 10–14.4% of solar energy in the PAR range can in theory be converted into algal biomass (Benemann, 2008; Park et al., 2011; Williams and Laurens, 2010). However, typical yields gained by outdoor cultures have a photosynthetic conversion efficiency of only 1.3–2.4% of total solar radiation (Benemann, 2008; Park et al., 2011). Functionally, photons are harvested by the phycoerythrin-chlorophyll complex in rhodophytes, or peridinin-chlorophyll complex in dinoflagellates, which increase the portion of the spectrum that can be used for photosynthesis (Williams and Laurens, 2010). The pigment composition of algae defines the PAR utilisation range and differs according to the pigments acquired or lost during the organism's evolutionary history (Schulze et al., 2014). Light-emitting diodes (LEDs) are a relatively cheap, highly versatile, rapidly advancing modern lighting technology that can provide narrow spectral output (Glemser et al., 2016) and colour blending capability using red, green, blue (RGB) lights. A number of studies have demonstrated that single wavelength LEDs at various light intensities can be used in the culturing process to adjust the biochemical composition of the biomass produced by microalgae (Atta et al., 2013; Das et al., 2011; Wang et al., 2007). Therefore, due to the unique spectral properties of LEDs when compared to other light sources, the specific light responses of microalgae can be analysed (Glemser et al., 2016). The cyanobacteria *Nostoc* sp. has been shown to exhibit significant changes in the levels of PBP when grown under specific LED wavelengths. Red wavelengths resulted in an overexpression of phycocyanin, whereas green wavelengths resulted in an overexpression of phycoerythrin (Johnson et al., 2014). The chromatic adaptation of *P. purpureum*, especially under green and RGB wavelengths, positively influences PBP production due to its specific role in adaptive photosynthetic activity (Chen et al., 2010).

The aim of this study was to determine the effect of specific LED wavelengths on the formation of multiple high-value products derived from the microalgae *P. purpureum*, and to assess if particular wavelengths trigger specific metabolite induction. All wavelengths tested were assessed for biomass yields and value-added product formation which would allow for bio-refinery processing to be adopted. To the best of the authors knowledge this is the first study to investigate the production of multiple high-value products by *P. purpureum* in response to specific LED wavelengths.

2. Materials and methods

2.1. Strain, medium and pre-cultivation conditions

The Rhodophyta microalgae *P. purpureum* (CCAP 1380/3) was conditioned in indoor cultures at the Centre for Sustainable Aquatic Research (CSAR), Swansea University, UK. Non-axenic cultures of this species were scaled up using natural autoclaved seawater (20 min at 121 °C) from 250 mL to 1 L flasks using standard F/2 commercial media (Cell-hi F2P, Varicon). The flask cultures were grown in triplicate for seven days, illuminated with approximately 60 μmol photons m^{-2} s^{-1} using cool white fluorescent tubes perpendicular to the culture, with a light/dark cycle of 18/6 h. The flask cultures where then pre-adapted using the relevant LED wavelength for a minimum of 3 days prior to inoculation into the 10 L^{-1} bubble columns.

2.2. Culture conditions

The flask cultures were used to inoculate 3, 10 L bubble columns (acrylic plastic, 0.1 m diameter/light path, 1.2 m height) to an initial cell density of 1.30×10^5 cells mL^{-1} using a standard F/2 commercial media (Cell-hi F2P, Varicon). The columns were maintained in a controlled temperature room at 19–21 °C, aerated with filtered (0.2 μm) ambient air (0.039% CO_2) at a rate of 0.1 min^{-1} (v/v) into the base of the tube through a 1 mm plastic capillary tube as described by Mayers et al. (2013). Each column was illuminated by a 1 m length flexible cable of 60 tri-coloured LED chips (red, green, blue LED) (Fig. 1), coiled externally around the column. The LED light strips were purchased from Taotronics,