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Algal growth in photo-bioreactors: Impact of illumination strategy and nutrient availability

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ABSTRACT

The objectives of this study were to first identify illumination strategies which support long term sustainable algal growth in photo-bioreactors, and second to address contradictions in literature regarding the algal nutrient uptake behavior under a sustainable growth regime. Experiments were conducted at different light intensities with continuous illumination and intermittent illumination of 12-h light and dark periods. Sustainable algal growth was characterized with algal specific chlorophyll-a content of more than $\sim 20\,m\,g\,g^{-1}$. Experiments were also conducted to address contradictions in literature regarding Droop versus Monod formulations for nutrient uptake, and representation of growth limiting factors using Liebig's law of the minimum versus the multiplicative rule. Intermittent illumination of 12-h light and dark periods at a light intensity of $246\,\mu mol\,m^{-2}\,s^{-1}$ was found to be the most optimal strategy for sustainable algal-growth. It was further concluded that the impact of several growth limiting factors on algal growth could be best described by the Liebig's law of the minimum with Droop's formulation rather than multiplicative rule and Monod formulation.

1. Introduction

Numerous applications of algal photo-bioreactors for wastewater treatment, hazardous contaminant removal, for hydrogen production, algal biomass production for bio-fuel recovery, carbon sequestration, etc., have been reported in the literature (Ai et al., 2008; Di Termini et al., 2011; Griffiths, 2009; Muñoz et al., 2009; Perales-Vela et al., 2006; Tamburic et al., 2011). The advantages of such photo-bioreactors over the traditional open algal ponds are numerous and extensively reviewed elsewhere (Rawat et al., 2013; Singh and Sharma, 2012).

There are some instances of algal photo-bioreactors being operated under continuous illumination at low light intensities (Kim et al., 2013; Ryu et al., 2014; Wang et al., 2010). However in most cases, algal photo-bioreactors are operated with alternate light and dark periods, with duration of light and dark periods ranging from milliseconds to hours (Cabanelas et al., 2013; Ji et al., 2014; Wang et al., 2010; Yoshimoto et al., 2005). Irrespective of the illumination strategy adopted, the main focus during the operation of such reactors is on maximizing the algal growth. Light intensity and illumination strategy have been accepted as important factors which govern the algal growth dynamics (Soletto et al., 2008). At low light intensities, algal growth is limited by light availability. However, algal growth under high light intensity conditions is also potentially problematic and phenomena such as algae photo inhibition and photo toxicity have been reported (Béchet et al., 2013). Literature reports also suggest that chlorophyll-a content of the algal cell declines at higher light intensities (Bonente et al., 2012). This is because the Photo System II (PS-II) in algal cells is stressed at high light intensities and may under certain circumstances be irreversibly damaged (Mulo et al., 2012). Depletion of algal chlorophyll content beyond a certain threshold may thus jeopardize the long-term viability of algal culture maintained in photo-bioreactors (Shriwastav et al., 2014).

The review of literature reveals that most studies investigating the effect of illumination regime on algal growth dynamics focus solely on algal biomass growth. However, there is enough evidence in the literature to indicate that prolonged exposure to light or exposure to light of high intensity may result in irreversible damage to the long-term viability of the algal cell as indicated by depleted algal chlorophyll content. Often the evidence of such damage may not be apparent in short-duration experiments which only monitor algal biomass growth. Hence it is important to identify illumination strategies which maximize the algal growth while maintaining the culture in sustainable state to ensure long term viability of algal systems.

Additionally, once the illumination regimes suitable for sustainable algal growth are identified, further studies on the

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algal growth dynamics and nutrients uptake under these conditions are necessary for the comprehensive understanding of all factors required to achieve a sustained and high rate algal growth in photo-bioreactors. Uptake of nutrients (N and P) and consequent algal growth has been studied by several researchers (Termini et al., 2011; Wang and Lan, 2011). The fact that algae often take up nutrients in excess of their immediate growth requirements and store excess nutrients internally has also been known for some time (Droop, 1974; Nambiar, 1979; Rhee, 1973). Despite such insights, many experimental and modeling studies still utilize the Monod formulations, which do not account for internal storage of nutrients (He et al., 2012; James and Boriah, 2010; Malve et al., 2007). Further, the effect of multiple growth limitation factors on algal growth is still debatable, as studies supporting the multiplicative effect of several growth limiting factors (James and Boriah, 2010; Malve et al., 2007) as well as those advocating Liebig's law of the minimum (Chapra et al., 2007; Klausmeier et al., 2008) are both widely used. Such contradictions need to be addressed in a systematic manner in order to gain a comprehensive understanding of sustained and high rate algal growth.

Specifically the objectives of the present study were the following:

- To use algal chlorophyll content as an indicator for the long-term sustainability of algal cultures and thus identify illumination regimes suitable for sustained algal growth in photo-bioreactors.
- To investigate the nutrient uptake and algal growth dynamics in photo-bioreactors under illumination regimes suitable for sustained algal growth and thus gain insight into the limiting factors impacting algal growth.

This study links algal chlorophyll content with light induced stresses and thus suggests that algal chlorophyll content can be used as proxy for culture viability in algal systems where autotrophic growth is predominant. In addition, a systematic analysis was carried out to address contradictions in literature regarding the mechanism for algal nutrient uptake. Achieving a comprehensive understanding of all factors associated with algal growth in photo-bioreactors will lead to the development of a realistic model for describing algal growth dynamics.

2. Materials and methods

2.1. Light chamber

Standard BOD bottles of 300 mL capacity were used as photo-bioreactors. These reactors were placed inside a wooden light chamber (dimensions: 0.85 m × 0.6 m × 0.45 m). Mixing of the photo-bioreactor contents is important for efficient light utilization by algae cells during photosynthesis (Mitsuhashi et al., 1995), however high levels of mixing result in cell damage (Gudin and Chaumont, 1991). Hence, for mixing, the base of the light chamber on which the reactors were placed was mounted on bearings. This base was attached to a crankshaft operating at a crank speed of 30 rpm. The mixing thus imparted was sufficient to keep the photo-bioreactor contents completely mixed, without creating violently agitated conditions. Compact fluorescent lamps (CFL) were fitted inside the light chamber to provide the required illumination. Two types of lamps were used, 14 W CFL lamps (make: Philips, India), 100 W CFL lamps (make: Ajanta, India). The lamps could be turned on and off as required using a timer fitted to the electrical circuit.

2.2. Characterization of the light sources

To determine the light emission spectra from a typical light source of each type, i.e., 14 W and 100 W CFL lamps, the optical probe extension of a spectro-fluorometer (Fluorolog-3, JOBIN VYON HORIBA, USA) was placed inside a typical reactor. The reactor was then placed in the vicinity of the light source in a dark room. Typical spectra obtained from the CFL lamps are presented in Fig. 1a. Based on the emission spectra as shown in Fig. 1a, cumulative fractional photon distribution curve, i.e., plot of the fraction of photons below a particular wavelength versus the wavelength, was generated for both sources as shown in Fig. 1b.

2.3. Measurement of light intensity inside a photo-bioreactor

The average value of the rate of photon incidence inside the reactors was determined using potassium ferrioxalate actinometry (Hatchard and Parker, 1956; Parker, 1953). Actinometry experiments were conducted in a room illuminated with red light. Seven reactors were each filled with 270 mL of 0.006 M potassium ferrioxalate solution in 0.1 N H_2SO_4. One bottle was immediately kept in dark and used as reference. Remaining six bottles were kept in the light chamber under mixed conditions and exposed to light of a particular intensity for different exposure times. After exposure for the required duration, 10 mL aliquots were removed from all bottles (in duplicate) and mixed with 5 mL of acidic acetate buffer and 2 mL of 0.1% w/w 1,10-phenanthroline solution and kept in complete darkness. Fe^{+2}-1,10-phenanthroline complex formation was complete in 1 h, after which the absorbance of the solutions was measured at 510 nm using a UV–visible spectrophotometer (Helios Epsilon, Thermo Scientific, USA) and a 1 cm path length quartz cuvette. The number rate of incident photons (in quanta s^{-1}) in the 253–430 nm range was calculated using the equation below (Rabek, 1982).

$$n_a = \frac{D \times V_1 \times V_3 \times 10^{-3} \times N_L}{t \times \phi \times L \times \varepsilon \times V_2} \quad (1)$$

where,

n_a = number of photons absorbed by the actinometer per second, quanta s^{-1}.

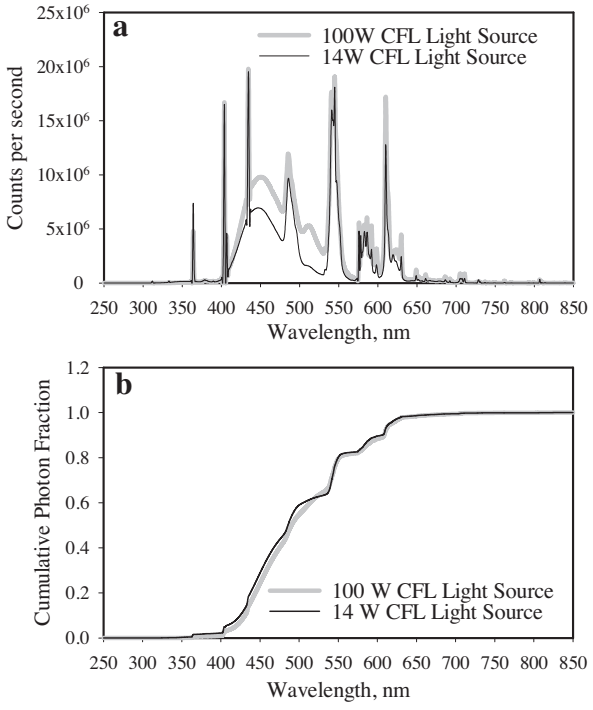

Fig. 1. Characterization of light sources: (a) typical emission spectra; (b) cumulative fractional photon distribution diagram.