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MicrOLED-photobioreactor: Design and characterization of a milliliter-scale Flat-Panel-Airlift-photobioreactor with optical process monitoring

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ABSTRACT

Small-scale cultivation systems with real-time-monitoring of suspension parameters are important for high-throughput bioprocess development. This manuscript describes the design and characterization of a new photobioreactor (PBR) approach using 3D-printing and organic light emitting diodes (OLEDs) in the design step. The structurally complex miniaturized PBR periphery was manufactured from polyamide using the selective laser sintering technology. The MicrOLED-PBR - the first Flat-Panel-Airlift photobioreactor (FPA-PBR) with a working volume below 20 mL - was equipped and validated with non-invasive optical sensors for cell- (microalgal dry weight concentration and chlorophyll fluorescence) and suspension parameters (pH, dO_2 and dCO_2) allowing multiparametric high-resolution physiological studies of microalgae growth at low photon flux densities. The OLED modules used in the MicrOLED-PBR were characterized with respect to their spectral photosynthetically active radiation efficiency (35.31%), maximum photon flux density (83 μmol m^{-2} s^{-1}) and resulting photon flux density profiles across the layer thickness of the FPA-cultivation chamber (10 mm) according to Lambert-Beers law (150 μmol m^{-2} s^{-1} for dual-plane external illumination). The hydrodynamic properties of the FPA-cultivation chamber, i.e. its volumetric oxygen transfer coefficient k_La (1.5–57 h^{-1}), superficial gas velocity (0.8–42 m h^{-1}), mixing time (1.5–34.5 s) and gas hold-up (0.016–0.2) were comparable to those for lab- and production-scale FPA-PBRs at volumetric aeration rates of 0.5–5.0 L h^{-1}. The application of the MicrOLED-PBR was demonstrated for optimizing the CO_2 conditions during batch-mode growth of *Chlamydomonas reinhardtii* 11-32b. By analyzing the suspension dynamics in real-time limitations of dissolved carbon dioxide were identified at a CO_2 amount of 0.1 vol% whereas 2.0 vol% CO_2 was identified as optimum conditions for growing *C. reinhardtii* 11-32b in the MicrOLED-PBR.

1. Introduction

1.1. Photobioreactors (PBRs)

Phototrophic microorganisms (PMs) like microalgae and cyanobacteria are promising production systems for a great variety of valuable products such as polyunsaturated fatty acids, lipids, carbohydrates, and pigments [1]. In recent research PMs have been also used as production hosts for recombinant proteins [2].

PM cultivation is challenged by an adequate supply of photosynthetically active radiation (PAR) and dissolved carbon dioxide [3,4]. A wide range of fully automated and sensor-equipped lab-scale (V > 0.5 L) PBRs including stirred tank reactors [5,6], plate-based reactors [7], cylindrical bubble columns [8], and flow tube PBRs [9] have been developed so far.

Abbreviations: HS, High-Salt; FPA, Flat-Panel-Airlift; OLED, Organic light emitting diode; PAR, Photosynthetically active radiation; PFD, Photon flux density [μmol m^{-2} s^{-1}].

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However, the establishment of milliliter-scale cultivation systems for PMs which offer high-throughput operation (e.g. strain screening and optimization of process conditions), the reduction of experimental effort and a real-time monitoring of process parameters is still under research and development [10]. Recently, the mPBR, an orbitally shaken 24-well microplate PBR platform with LED-illumination was described by Ojo et al. [11]. The application of the mPBR was demonstrated for optimizing the culture condition of the microalgae *Chlorella sorokiniana* in batch-mode operation. A partially automated miniaturized bubble column PBR made of polymethylmethacrylate (EOSS-PBR) was introduced by Taleb et al. [12]. The EOSS-PBR (V = 30 mL) allows a semi-continuous process operation by automated medium feeding and cell harvesting. Kandilian et al. [13] presented a parallelized flat-plate PBR (V = 70 mL) with online measurement of microalgal dry mass to achieve an optimum incident illumination of *Nannochloropsis oculatat* during batch-mode growth. As demonstrated, there are promising approaches to realize bioprocess development for PMs in small-scale PBRs. However, to our knowledge there has been no milliliter-scale Flat-Panel-Airlift-photobioreactor allowing the real-time monitoring of suspension dynamics during microalgae cultivation described so far.

1.2. Sensing in micro-scale (photo-) bioreactors

High-throughput operation systems for rapid strain screening and automated process optimization are gaining more and more importance. During the last decades a stepwise reduction of cultivation volumes to the micro- and milliliter-scale was realized which is associated with the need for non-invasive sensor systems allowing the real-time monitoring of bioprocesses in this scale [14]. Optical sensors whose operation-principle is based on fluorescence intensity and lifetime measurements are the most promising technologies to meet these space-limiting requirements. They are widely used in parallelized cultivation systems for heterotrophs, e.g. stirred tank reactors [15,16], shake flasks [17,18,19], microtiter plate cultivation devices [20] or single-use bioreactors [21]. The photo-sensitivity of these optical sensors has impeded their usage for phototrophic bioprocesses and thus a further reduction of the cultivation volume of PBRs so far. Commonly, tailor-made sensor solutions are applied in small-scale PBRs. Nedbal et al. used a built-in densitometer and fluorimeter in their 350 mL FPA-PBR to realize an online cell density and phycobilin fluorescence detection [22]. The ePBR of Lucker et al. was equipped with custom-made microelectrodes for the process parameter pH and temperature whereas the cell density was also measured online by using a 940 nm NIR-LED and a photodiode [10]. A similar approach for online cell density measurement (680 nm and 720 nm LEDs) was realized in the commercially available MC1000-OD multi-cultivator of Photon Systems Instruments.

1.3. OLED lighting — an upcoming PBR illumination technology

In small-scale PBRs, especially the type of artificial light source, their controlling and the regulation of heat transfer (thermal management) are critical parameters for their design [23]. Up until now, primarily fluorescent lamps and light emitting diodes (LEDs) were used as artificial light sources in miniaturized PBRs [24]. An upcoming and alternative way of PAR-generation could be organic light emitting diodes (OLEDs) that are widely used in display applications (digital cameras, computer displays, flexible displays, mobile phones, and television), emergency lighting, automotive lighting, and specialized applications such as luminous clothing or decoration [25].

OLEDs can be manufactured as single layer devices that emit monochromatic light or multilayer devices containing red, green and blue organic emitters (small molecules or polymers) whose combined emissions generate a white light emission [26]. The typical used OLEDs can be divided into two types based on the way they release light: bottom-emitting and top-emitting (Fig. 1) [27]. The OLED's emission spectrum, light extraction efficiency and intensity of emitted light depend on the applied voltage and the composition of its organic layers. Conventional OLEDs with a planar-organic layer structure can achieve a light extraction efficiency of approximately 20%. However, various patents and papers published in recent years have described methods that can be used to increase the light extraction efficiency to as much as 80% [28]. The lifetime of OLEDs recently achieve ca. a fifth (10,000–15,000 h) of the widely-used LED light sources (up to 50,000 h).

OLED devices have a number of other beneficial properties that could be advantageous for PBR design: they can be very thin (approximately 1 mm thick), offer completely freedom of geometric design, and exhibit low self-heating, which could be advantageous for space-limited small-scale PBRs. In addition, OLEDs can be deposited on a variety of substrates including metals, glass, or silicon by ink-jet printing processes.

1.4. Additive manufacturing — design of structural complex PBR components

Additive technologies have revolutionized production and manufacturing in many industries [29,30]. Even very complex geometrical structures can be created directly from synthetic plastics, polymers, metals or ceramics in a layer-by-layer fashion by using computer-aided design in conjunction with a 3D-printer [30]. Due to the design flexibility, the short production time and material efficiency this manufacturing technology could have a substantial impact in the field of bioprocess engineering. First applications of additive technologies in biotechnological contexts were presented by Lode et al. (2015) [31]. The authors showed the fabrication of microalgae-laden 3D-immobilization structures by a 3D-bioprinting approach which could be applied to medical and biotechnological issues. In a following study the authors reported an increased cell viability at increasing temperature and time of light exposure conditions within the 3D-hydrogel-environment compared to suspension cultures of *Chlamydomonas reinhardtii* 11-32b and *C. sorokiniana* UTEX1230 [32]. Further applications of additive manufacturing in biotechnology are the design of artificial tissues [33] and single-use lab equipment [34]. However, additive technologies have not yet been used for the fabrication of PBRs.

This work describes the combination of OLED-lighting and additive manufacturing, to design a small-scale Flat-Panel-Airlift-(FPA) PBR approach (MicrOLED-bioreactor) for studying microalgal physiology during varying growth modes. Real-time non-invasive information about cellular parameters (microalgal dry weight concentration and chlorophyll fluorescence) and suspension parameters (pH and concentrations of dissolved oxygen and carbon dioxide), respectively, were obtained by optical sensor systems. The OLED modules were characterized with respect to their PAR-efficiency and photon flux density. The FPA-PBR's hydrodynamic properties (i.e. mixing time, volumetric oxygen transfer rate, gas hold-up and evaporation rate) were analyzed and compared to lab- and production-FPA-PBRs. To demonstrate the applicability of the multi-parametric MicrOLED-PBR culture conditions and suspensions dynamics were analyzed during batch-mode growth of the microalgae *C. reinhardtii* 11-32b.

2. Material and methods

2.1. Microorganism, media and preculture

C. reinhardtii 11-32b was purchased from the Culture Collection of Algae at Goettingen University (Göttingen, Germany). *C. reinhardtii* 11-32b shake flask cultures were inoculated from Tris-Acetate-Phosphate (TAP) [35] agar plates into 50 mL liquid High-Salt (HS) medium (300 mL Schott Duran without baffles, Wertheim, Germany) and subcultivated every 48 h. Shake flask precultures were incubated in an illuminated incubator (WB750, mytron Bio- und Solartechnik GmbH,