**Cultivation of phototrophic biofilms in an aerosol-based photobioreactor**

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**Highlights**

* Optimization and characterization of an aerosol-based photobioreactor
* Long-time cultivation and characterization of phototrophic biofilms
* New downstream process for extraction of EPS and pigments

**1. Introduction**

Cyanobacteria offer a great potential for the production of biotechnological products. They can be divided into two groups: terrestrial and aquatic cyanobacteria. Terrestrial cyanobacteria are living embedded in self-produced extracellular polymeric substances (EPS) and show a pronounced surface-associated or air-exposed growth. They have so far been neglected as potential production organisms due to the lack of cultivation systems (Strieth 2018). And that regardless of the fact, that biofilms can render biotechnological processes simpler and more efficient. Thus, no (harmful to humans) chemicals for an artificial immobilization are necessary because phototrophic biofilms naturally adhere to surfaces. Product purification of secreted substances is facilitated by immobilized cells and higher productivities can be achieved in continuous processes (Strieth 2017) as the growth rate can be decoupled from the dilution rate (Muffler 2014). At the chair of bioprocess engineering an emerse photobioreactor (ePBR) was developed where the medium is given as an aerosol (Kuhne 2014, Strieth 2017). The system imitates natural habitats of terrestrial cyanobacteria in the desert and the rain forest. During the last decade, a few biofilm photobioreactors were developed imitating different natural habitats of terrestrial cyanobacteria. The latest developments are summarized, illustrated and compared in the reviews of Podola 2017 and Strieth 2018. Hitherto, the majority of biofilm photobioreactors are used in lab scale resulting in small amounts of biomass that can be utilized for further analysis. To facilitate the characterization of small amounts of biomass, a combined extraction of EPS and the pigments (i) chlorophyll-a, (ii) carotenoids and (iii) phycobiliproteins has been developed. This is important for a comprehensive characterization of the cells in order to be able to draw conclusions about the state of the cells. For example, EPS are produced as protection against suboptimal culture conditions. Carotenoids are produced, among other things, for cell wall stabilization. Essentially, the pigment composition depends on the available light spectrum, temperature and availability of nutrients.

**2. Methods**

The most important parameter of the optimized ePBR were characterized like residence time resolution of the aerosol (experimental by using high-speed camera and simulated using OpenFOAM), droplet size, the light and temperature distribution (experimental by using a quantum sensor and a laser thermometer and simulated using MatLab) and the surface texture (roughness using AFM and hydrophobicity). The terrestrial cyanobacterium *Trichocoleus sociatus* was cultivated on three different substrates (PMMA, borosilicate glass, silicone) in the ePBR at 24 °C and 24 hours of aerosol supply. In addition, the influence of different temperatures (24, 30, 37 °C), amount of aerosol per day (4, 8, 12, 24 h) and the influence of higher amounts of CO2 (0,03, 2, 5, 10 %) and flue gas were investigated. To characterize the biofilm a new downstream process was developed where the EPS was extracted in a first step using an optimized method and the pigments (phycobilisomes, chlorophyll and c-phycocyanin) in a second step. Optical coherence tomography was used to determine the biofilm thickness over the cultivation time and two-dimensional growth was measured over chlorophyll-a fluorescence using a PAM fluorometer.

**3. Results and discussion**

Different surfaces showed no influence on the biomass production and the pigment composition, whereas on hydrophilic surfaces more vertical growth and on the hydrophobic surface rather horizontal growth could be observed, which was attributed to the hydrophobicity of *T. sociatus*. In addition, increased EPS production was demonstrated on the hydrophilic surfaces due to the horizontal spread of the biofilm in combination with the hydrophilic properties of the EPS. The layer thickness stagnates in the ePBR at 600 μm, which is presumably caused by the low light availability. Thus, higher layer thicknesses of up to two mm were achieved at higher light intensities. The biofilm thus actively adapts to the availability of light, as shown by the degradation of the pigments but also in the production of EPS. The growth of *T. sociatus* at 24 °C was antiproportional to the amount of aerosol per day and higher EPS yields were achieved at longer dry times. An increase in temperature led to a desiccated biofilm were no growth could be achieved at 37 °C and 8 h aerosol supply. The interaction between aerosol delivery and temperature could be modeled via the black-box system and verified with a further experiment. A developed semi-continuous process led to increased EPS yields with targeted growth and dry phases. In addition, an increased production of an antibacterial substance under extreme conditions was demonstrated. The structure of the substance is currently being investigated. The developed process could be used in industry for the production of EPS or secreted substances. Cultivations with elevated CO2 concentrations unexpectedly led to growth inhibition. Interestingly, almost identical biomass productivities were achieved using atmospheric CO2 (380 ppm) and flue gas (10 % CO2). Thus, CO2 does not appear to be the reason for growth inhibition at elevated CO2 concentrations.

**4. Conclusions**

The cultivation of *T. sociatus* in the optimized ePBR was successful and the influence of temperature and aerosol per day could be modeled. The biomass and EPS productivities in the ePBR are higher than in submerse reactors and can be targeted influenced using different times of dry phases.

**References**

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