**Implementation of Moving Bed Bioreactor (MBBR) Technology for Phototrophic Cultivation of Terrestrial Cyanobacteria.**

Jakob Walther1, Michael Stoffel1, Dorina Strieth1, Roland Ulber1*\**

*1 Institute for Bioprocess Engineering, Kaiserslautern, Germany*

*\*Corresponding author: ulber@mv.uni-kl.de*

**Highlights**

* Growth of cells without additives on HDPE carriers
* Increase of STY by 34%
* Basis for continuous operation.

**1. Introduction**

Terrestrial cyanobacteria grow relatively poorly in suspension culture, which is why they have not yet been considered as producers of interesting metabolites such as antibacterial substances. Previous work in our group have shown that surface-associated growth can significantly increase productivity [1]. Moving-bed bioreactor technology, which is already established in wastewater treatment [2], offers a possibility to carry out such growth on a larger scale. The bacteria grow on a solid substrate, which is continuously kept in suspension by gassing or stirring. Integrated cell retention on the substrate allows a continuous process, which can potentially significantly improve the production of secondary metabolites.

**2. Methods**

The terrestrial cyanobacterium *Trichocoleus sociatus* was investigated, which has shown promising antibacterial properties in previous experiments [1]. The substrate were 1.4x1.4 cm hollow HDPE cylinders with internal fins (Figure 1, right). The initial adhesion of the biomass to the substrate took place for one week in 0.5 L shaking flasks at 100 rpm. For optimization the volume of the culture-media and the amount of substrate was investigated. Then the substrate with the biomass was transferred to a glass cylinder of 9 cm diameter and 30 cm height filled with culture-media which was illuminated from the outside. In these the substrate was either suspended by gassing or operated as a fixed bed by using a pump. Filling degrees of 0.086-0.258 m³Substrate/m³Medium and protected surfaces of 0.087-0.259 m²Substrate/LMedium were investigated as moving beds.

**3. Results and discussion**

The intial adhesion of cyanobacteria to the substrate directly in the MBBR was not successful. Therefore, an upstream adhesion step was investigated for one week in a shaking flask. The optimal parameters were 30 substrate particles in 50 mL medium at 100 rpm. This immobilized over 90% of the biomass in the substrate, which is considered sufficient.Different cultivations have been carried out in glass cylinders to maximize biomass production.

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**Figure 1.** (left) Space-Time-Yield (STY) of different cultivations of *T. sociatus* in a glass cylinder: a.)Moving Bed Bioreactor (MBBR) with 120 substrate particles in one liter medium (corresponds to 25% filling degree); b.) fixed bed with one liter substrate and external circulation with a pump; c.) suspension culture. Cultivation time 25 days, 24°C.(right) *T. sociatus* (dark) on cylindrical growth bodies (white) within a moving bed reactor

In the control without substrate, a space-time yield (STY) of 0.025 gbio-dry-mass/(L·day) was met. In the fixed bed, a STY of 0.029 g/(L·day) was reached, which corresponds to an increase of 13%. In the MBBR with 25% filling degree even a STY of 0.034 g/(L·day) was achieved, which corresponds to an increase of 34% for control without substrate. Over 92% of the biomass was attached to the substrate. The next step in process development is the implementation of a long-term stable culture for the production of secreted valuable compounds such as antibacterial substances.

According to our knowledge, this is the first time that microalgae have been cultivated on a solid substrate, and not on cloth [3], in a moving bed or fluidized bed. Cyanobacteria have so far only been successfully immobilized in fixed beds on sponges [4] or on cloths [5].

**4. Conclusions**

A proof of concept for the cultivation of cyanobacteria on a solid substrate within a MBBR was provided. Biomass productivity is significantly higher compared to a suspension culture. Further challenges are the establishment of a long-term stable culture for continuous production and an up-scale, especially with regard to illumination.

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