**Mechanical stress during scale up to membrane aerated stirred bioreactors for rebeccamycin production in filamentous *Lentzea aerocolonigenes***

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**Highlights**

* Filamentous *L. aerocolonigenes* produces the antitumor antibiotic rebeccamycin.
* Particle-induced mechanical stress on *L. aerocolonigenes* increases rebeccamycin titers*.*
* Simulations can be used to characterize the optimal mechanical stress.
* Membrane aerated bioreactor cultivations result in comparable rebeccamycin titers.

**1. Introduction**

Filamentous microorganisms represent the majority of natural producers of antibiotics and other active pharmaceutical ingredients (APIs). *Lentzea aerocolonigenes* is a filamentous actinomycete producing the antitumor antibiotic rebeccamycin. The microorganism exhibits a complex morphology ranging from freely dispersed mycelia to dense pellets [1]. The morphology is linked to the product formation and can be controlled by e.g., inoculum concentration and viability, pH, medium composition, hydromechanical stress, addition of inorganic salts or particles [2]. Rebeccamycin formation in *L. aerocolonigenes* was increased by the adjustment of mechanical stress induced by glass particles in shake flask scale [3]. Regarding a scale up from shake flasks to a laboratory scale stirred bioreactor the induced mechanical stress is an important factor.

**2. Methods**

Cultivations of *L. aerocolonigenes* were conducted in 250 mL shake flasks with 4 baffles and 50 mL filling volume. Glass particles were added for adjustment of the induced mechanical stress. The flasks were incubated on an orbital shaker at 120 min-1 (50 mm amplitude) at 28 °C for 10 days.

A scale up to a stirred bioreactor (Applikon, The Netherlands; 6 blade Rushton turbine) with 1.2 L filling volume was performed. Aeration in this bioreactor was conducted via an oxygen permeable silicone membrane (0.2 mm thickness; Reichelt Chemietechnik, Germany). Stirrer speed was set to 400 min-1 and pure oxygen was used for aeration (aeration rate 0.2 L min-1).

**3. Results and discussion**

The influence of mechanical stress on cultivations of *L. aerocolonigenes* in shake flasks was investigated using glass beads in different sizes and concentrations. The highest rebeccamycin concentration of 70 mg L-1 after 10 days of cultivation was achieved with 100 g L-1 glass beads of 969 µm diameter compared to an unsupplemented control (rebeccamycin concentration after 10 days of cultivation with 5 mg L-1) (**Fig. 1**).

A stirred bioreactor usually generates higher mechanical stress than a shake flask. Therefore this parameter is important to consider during scale up, especially since product formation is influenced. As conventional bubble aeration and agitation induce mechanical stress, a bubble free membrane aeration was chosen to reduce the influencing variables. A first membrane aerated cultivation without glass beads resulted in a rebeccamycin titer of 12 mg L-1 after 6 days of cultivation which is comparable to a regular unsupplemented shake flask cultivation (**Fig. 2**).

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| **C:\Users\katpomme\Documents\Vorträge, Poster, Abstracts\Eigene\Grafiken\KontrolleoptimalesGlas.wmf**  **Figure 1.** Cell dry weight concentration and rebeccamycin concentration of shake flasks cultivations without and with 100 g L-1 glass beads after 10 days of cultivation. | C:\Users\katpomme\Documents\Vorträge, Poster, Abstracts\Eigene\Grafiken\Modellreaktor_1Lauf_englisch.wmf  **Figure 2.** Rebeccamycin concentration of a silicone membrane aerated bioreactor cultivation without glass particles at an agitation rate of 400 min-1. |

Coupled CFD-DEM simulations have already been conducted to characterize the induced mechanical stress in shake flasks with glass particles. A similar simulation of the membrane aerated stirred bioreactor will allow a comparison of the induced mechanical stress and thereby an adjustment to the optimal stress found in shake flasks.

**4. Conclusions**

Rebeccamycin production in *L. aerocolonigenes* is increased by setting an appropriate mechanical stress by glass bead addition in shake flask scale. This knowledge can be used for scale up. A first membrane aerated stirred bioreactor cultivation led to a comparable rebeccamycin titer as in unsupplemented shake flask cultivations. Further adaption of mechanical stress by stirrer speed and geometry as well as the addition of glass beads could lead to even higher rebeccamycin titers.

**References**

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