ZENTRUM ANGEWANDTE CHEMIE

Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Partner in der Forschung

Leibniz Universität Hannover

Evaluation of a novel scaleable and disposable membrane bioreactor for enhanced secondary metabolite and recombinant protein production in filamentous microorganisms

C. Endres[1], S. Beutel[1], S.J. Fraser[2], W. Edwards[2], A. Kocourek[3] and T. Scheper[1]

[1]Institut für Technische Chemie, Universität Hannover, Callinstr. 5, 30167 Hannover, Germany, [2]Synexa Life Sciences, Cape Town, South Africa, [3]Sartorius Stedim Biotech GmbH, Göttingen, Germany

Introduction

Compared to traditional fermenters an important advantage of membrane-based bioreactors is the provision of an artificial environment for an increased biomass density and enhanced productivity of shear stress-sensitive organisms.
The novel Quorus GLS bioreactor investigated in this study consists of capillary ceramic membranes and therefore provides an ideal platform for the immobilization of aerobic shear stress-sensitive microorganisms. Due to its design it is suited for a steady-state cultivation and continuous production of valuable secondary metabolites or recombinant products.

Quorus GLS bioreactor

- Disposable and scalable ceramic membrane bioreactor
- Stable and continuous process
- Low shear stress environment
- Suited for cultivations of aerobic filamentous organisms
- Exploitation of steady-state biofilms for the production of secreted compounds such as recombinant proteins or secondary metabolites
- Cultivation controlled through nutrient flux

Fig. 1: Quorus GLS bioreactor

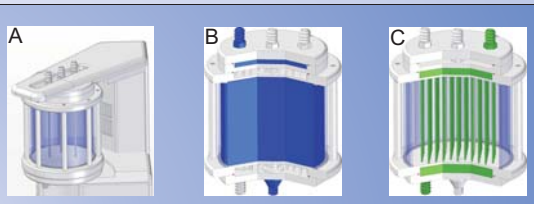

Fig. 2 A: Quorus GLS disposable bioreactor unit. B: Aerated extracapillary space (ECS) for biofilm formation and product recovery. C: Intracapillary space (ICS) for nutrient supply.

Quorus GLS mode of operation

- Biomass is grown in the extracapillary space (ECS) with nutrient medium flowing unidirectional from the lumen of the fibres towards the ECS
- With a sufficient thickness of the biofilm a radial nutrient gradient is established across the biofilm
- Nutrient gradient is controlled to support primary growth of the microorganism and to induce secondary metabolite production
- Secreted products are collected with medium stream passing the biofilm

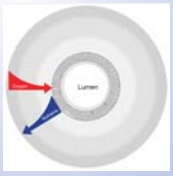

Fig. 3: Oxygen and nutrient gradients during biofilm formation around the ceramic membranes

Fig. 4: Schematic illustration of *Streptomyces coelicolor* biofilm growth and differentiation over time, cultured using the Quorus GLS Biroeactor.

Evaluation of Quorus GLS productivity

- Cultivation of the fungi *A.niger* in a Quorus 2 L 50 membrane bioreactor and monitoring of the production of a secreted recombinant protein
- Cultivation of the filamentous bacteria *S.coelicolor* in a Quorus 2 L 25 membrane bioreactor and monitoring of the production of a secondary metabolite
- Benchmarking in traditional fermentation vessels like shaking flasks, 2 L Biostat A plus, and 10 L Biostat C
- Comparison of the productivities in all used systems

A.niger
Recombinant protein production

- Stable protein production in the Quorus GLS bioreactor for more than 40 days
- Quorus space-time-yield significantly higher than in all other cultivation vessels

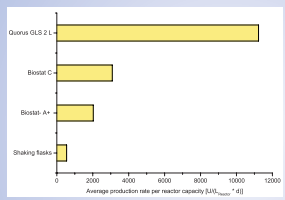

Fig. 5: Average production rate of a recombinant protein per reactor capacity in various cultivation vessels while cultivation of *A.niger*.

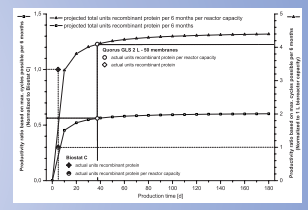

Fig. 6: Modelling of a Quorus GLS cultivation of *A.niger* to produce a recombinant protein. Effect of increasing Quorus GLS production time up to 180 days onto the total product output and space-time-yield to estimate the break-even with a Biostat C. All values are normalized to the accumulative output of the maximum number of Biostat C batches in 180 days.

S.coelicolor
Secondary metabolite production

- Stable protein production in the Quorus GLS bioreactor for 25 days
- Quorus space-time-yield higher than in the Biostat C fermenter and the shaking flasks

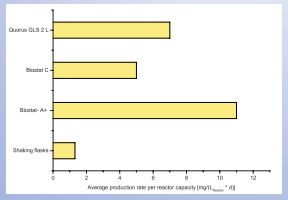

Fig. 7: Average production rate of a secondary metabolite per reactor capacity in various cultivation vessels while cultivation of *S.coelicolor*.

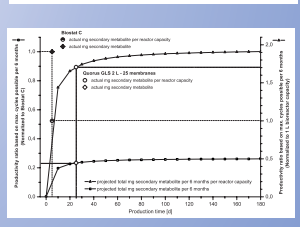

Fig. 8: Modelling of a Quorus GLS cultivation of *S.coelicolor* to produce a secondary metabolite. Effect of increasing Quorus GLS production time up to 180 days onto the total product output and space-time-yield to estimate the break-even with a Biostat C. All values are normalized to the accumulative output of the maximum number of Biostat C batches in 180 days.

Summary

The productivity of the Quorus GLS bioreactor was extensively studied by comparing production rates in long term cultivations of two filamentous organisms to a series of batch cultivations in traditional STR´s and shaking flasks. Especially during the cultivation of *A.niger* the Quorus GLS showed a significantly higher space-time-yield than all other cultivation vessels. Even though medium throughput in this continuously operated system is higher than in batch reactors, expensive downtime and the risk of contamination of single STR batches is kept to a minimum.

Acknoledgements

This project was supported by Synexa Life Sciences and Sartorius Stedim Biotech GmbH

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