

University of Pannonia
Chemical Engineering and Material Sciences Doctoral School

**Formation of *Streptomyces coelicolor* biofilm
in membrane gradostat reactor**

Ph.D. Thesis

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Veszprém

2025

Introduction

The production of microbial secondary metabolites is typically triggered by environmental stress factors like nutrient limitation, the presence of competitive species, etc. These compounds are of outstanding importance from both ecological and economic points of view. Among industrially relevant microorganisms, the members of *Streptomyces* genus are among the most significant species since they can produce antimicrobial and anti-tumor agents, immunosuppressants, enzymes, pigments and siderophores.

During industrial production, a sufficient amount of nutrients must be provided for microbial growth; on the other hand, limitation is required for product formation must be implemented. The balance between these two requirements during long-term operation is hard to maintain with conventional technologies, thus, their production mostly occurs in batch mode despite of the technical and economical benefits of continuous operation. A relatively new, less known equipment, the membrane gradostat reactor (MGR) offers a solution for this situation. Due to its special design, in the reactor, biofilm renewal and secondary metabolite production occur simultaneously; moreover, continuous product removal is possible.

From among the questions related to the MGR's operating principles and practical implementations, this study focuses on the inoculation of the membrane and the development of *Streptomyces coelicolor* biofilm.

Experimental

Investigating the filtration characteristic of the bacterium's fermentation broth, the relationship between pH, viscosity and specific cake resistance were described; moreover, two types of capillary ultrafiltration membrane modules were tested whether they can be applied as membrane gradostat reactors. The bacterium's colonization ability was observed on hydrophilic and hydrophobic PVDF flat sheet membranes. A simplified model system of MGR had been developed and the effect of several inoculation additives on biofilm-development were tested in this system. As an improvement of the model system, a scaled-up reactor with multichannel capillary membrane was developed and for the first time, the applicability of SevenBore membrane as MGR was investigated in this equipment.

I. During dead-end filtration of *Streptomyces coelicolor* fermentation broth, it was proven, that pH-dependence of specific cake resistance is influenced by the age of the fermentation broth; moreover, it was shown, that the relation described by filtration equation – i.e. specific cake resistance is inversely proportional to viscosity – is fulfilled by limitations.

- i. Performing the filtration at pH 4, 7 and 9, specific cake resistance had a minimum in case of broth fermented for 48 hours and a maximum in case of broth fermented for 96 hours at pH 7.
- ii. In case of broth fermented for 96 hours, minimum of specific cake resistance and maximum of fermentation broth's viscosity did not coincide.

II. It was established that Pentair X-Flow and Microdyn-Nadir Ultradyn membrane modules are not applicable as membrane gradostat reactor because of insufficient cell adhesion – under the applied experimental circumstances.

- i. During reverse filtration of *Streptomyces coelicolor* bacterium's fermentation broth – aged 48 hours and 168 hours – at pH 7, neither clogging of pores, nor gel layer development was considerable.

III. It was established that colonization ability of *Streptomyces coelicolor* on hydrophilic PVDF membranes with 0,22 and 0,45 μm pore size is slower than on solid medium; however, the characteristic of growth kinetics had not been altered during the first 13 days due to the membrane.

i. The multiplication of the parameters of Gompertz-model, i.e. maximum specific growth rate (k) and lag time (λ) – which data is attributed to the nature of growth kinetics – falls within an order of magnitude in case of membranes with 0.22 and 0.45 μm pore size and a solid medium (their values are 0.58; 0.63 and 0.43, respectively).

The model system developed for the investigation of the membrane gradostat reactor is suitable for observation of biofilms developed on single capillary membranes and for carrying out numerous parallel measurements. A utility model has been submitted for the model system.

IV. During experiments investigating the thickness of the *Streptomyces coelicolor* biofilm and membrane coverage in the above mentioned system, it was proven that – applying principal component analysis – there is no correlation between biofilm thickness and membrane coverage. Moreover, considering biofilm thickness and membrane coverage collectively, agar solution applied in 3 g/l proved to be the most appropriate.

i. From among the investigated inoculation additives – tween 80 (1 %), agar (1, 3 and 5 g/l) and carboxymethyl cellulose (2, 5 and 8 g/l) – only the samples of agar solution applied in 3 g/l were in the optimal region of the principal components' plane in case of all three parallels.

V. For the first time, the membrane gradostat reactor has been operated with *Streptomyces coelicolor* in the long run. Product formation was continuous during the entirety of the experiment (45 days); the rate of product formation varied between 2.2 and 6.3 $\mu\text{g} / (\text{day} \cdot \text{cm}^2 \text{ membrane surface})$.

VI. For the first time, the applicability of SevenBore membrane as the membrane gradostat reactor was investigated, and a stable, coherent biofilm of *Streptomyces coelicolor* bacterium has been developed on the membrane's surface, while the lumen remained cell-free.

i. The system can be applied as membrane bioreactor in general without the verified formation of any gradient.

ii. As a result of thermostation at 31 $^{\circ}\text{C}$, the water content of the biofilm increased by 6 %, the accumulated amount of the produced actinorhodin (calculated on dry biomass) increased by 29 % compared to the non-thermostated system.

Publications and presentations related to the dissertation

P. Lajtai-Szabó and É. Hülber-Beyer, 'The Concept and Practical Challenges of Membrane Gradostat Reactors', Hung. J. Ind. Chem., vol. 53, no. 1, pp. 61–66, Jun. 2025, doi: 10.33927/hjic-2025-07.

P. Lajtai-Szabó, É. Hülber-Beyer, V. Z. Vonyó, M. G. Szigeti, N. Nemestóthy, and K. Bélafi-Bakó, 'Novel Insights into Filtration Studies of *Streptomyces Coelicolor* Fermentation Broth for Inoculation of Membrane Gradostat Reactor', J. Membr. Sci. Res., vol. 11, no. 1, 2025, doi: 10.22079/jmsr.2024.2041305.1680.

P. Lajtai-Szabó, É. Hülber-Beyer, N. Nemestóthy, and K. Bélafi-Bakó, 'The role of physical support in secondary metabolite production by *Streptomyces* species', Biochem. Eng. J., vol. 185, p. 108495, Jul. 2022, doi: 10.1016/j.bej.2022.108495.

P. Lajtai-Szabó, 'Membrán gradosztát reaktorok', Membrántechnika És Ipari Biotechnológia, vol. XII, no. 2, pp. 21–25, 2021.

Presentation:

Szekunder metabolitok előállítás *Streptomyces coelicolor* baktériummal; 50. Műszaki Kémiai Napok; 2022. április 26-28., Veszprém

Inokulálás és biofilm-fejlődés vizsgálata membrán gradosztát rektor modellrendszerben; 53. Műszaki Kémiai Napok; 2025. április 15-16., Veszprém

Poster:

19. Aachener Membran Kolloquium (Aachen, Németország, 2024.12.03-05.); Piroska Lajtai-Szabó, Éva Hülber-Beyer, Nándor Nemestóthy: Inoculation techniques of single capillary membrane with *Streptomyces coelicolor*

MEMSEP & PERMEA 2025 (Chorzów, Lengyelország, 2025.06.24-26.); Éva Hülber-Beyer, Piroska Lajtai-Szabó, Viktória Zoltána Vonyó: Cultivation of actinorhodin producing *Streptomyces coelicolor* on microstructured PES membrane surface