LIÈGE université
Gembloux
Agro-Bio Tech

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Madame BRÜCK Hannah Luise,

Titulaire d'un master en bioingénieur : chimie et bio-industries, à finalité spécialisée,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 28 septembre 2020, à 14h30 précises,

Polytech Lille,

Avenue Paul Langevin - 59655 Villeneuve-d'Ascq

Cette dissertation originale a pour titre :

« Strain engineering and process design for continuous surfactin production in biofilm bioreactors with *Bacillus subtilis* 168 ».

<u>Le jury est composé comme suit</u> :

Présidentes : Prof. A. RICHEL, Prof. V. LECLERE (Coprésidente, Université de Lille, France),
Membres : Prof. P. JACQUES (Promoteur), Prof. F. DELVIGNE (Copromoteur), Prof. F. COUTTE (Promoteur cotutelle – Université de Lille, France), Prof. D. TOYE, Prof. R. TAKORS (Université de Stuttgart, Allemagne), Prof. I. MANDIC-MULEC (Université de Ljubljana, Slovénie).

Abstract

Biofilm bioreactors show promise for continuous microbial biosurfactant production due to the natural robustness of self-immobilized cells and the possible design of processes avoiding foam formation. The widely used bacterial strain *B. subtilis* 168 has the potential to produce surfactin, a powerful biosurfactant with exceptional biological activities and various industrial applications. However, *B. subtilis* 168 exhibits only poor biofilm formation capacities and thus entails limited cell adhesion capacities.

In order to improve the natural cell immobilization of *B. subtilis* 168 to adapt this strain better to biofilm cultivation, filamentous mutant strains with restored exopolysaccharide (EPS) production were generated. The impacts of the genetic modifications were evaluated through colonization assays and by measuring the biofilm formation capacity under low shear stress in a drip-flow reactor (DFR). Subsequently, the most performant strains were selected and cultivated in a newly designed continuous trickle-bed biofilm bioreactor containing highly structured metal packing elements for biofilm formation. Moreover, a bacterial growth model was built able to describe the growth dynamics of the planktonic cells and the biofilm in the system.

The colony development was strongly affected by filamentous cell growth and EPS production which was manifested through an enhanced surface spreading and colonization capacity. In the DFR and trickle-bed biofilm bioreactor, the EPS^+ mutants showed significantly increased performances regarding the biofilm formation and surfactin production capacities. Whereas cell filamentation had a minor impact on the processes, but contributed to a better cell cohesion in the biofilm and led to reduced cell detachment during the cultivation. Thus, EPS production and filamentous cell growth contributed considerably to an improved process performance in the system. In addition, continuous fermentation has shown to be favorable for a high surfactin productivity. The experimental data from the trickle-bed biofilm bioreactor were in good accordance with those obtained by simulations with the developed growth model. Hence, the growth model has been successfully validated and could be used for further process optimization.