**Surfactin production optimization in biofilm bioreactors using genetically modified *Bacillus subtilis 168* strains with improved adhesion capacities**

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**Highlights**

* The presence of exopolysaccharide improves significantly the support colonization
* The surfactin productivity of immobilized cells is higher in a continuous process mode
* A high dilution rate enhances biofilm formation on the reactor support

**1. Introduction**

Biofilm bioreactors have shown to be efficient cultivation systems for the production of bacterial biosurfactants [1]-[3]. They provide improved productivity and process stability through cell immobilization while avoiding foam formation. The widely known Gram-positive bacterium *B. subtilis* 168 is a potential producer of a very powerful biosurfactant, surfactin, with many applications in different industrial sectors [4]. The genome of *B. subtilis* 168 is completely sequenced and the strain can easily be genetically modified [5]. However, as a result of its domestication process, this laboratory strain is not able to produce surfactin anymore due to genetic mutations in the *sfp* gene coding for a co-factor required for the synthesis [6]. Furthermore, *B. subtilis* 168 possesses only poor biofilm formation capacities. This is mostly due to a deficiency in exopolysaccharide production [7]. In this work, different surfactin producing mutants of *B. subtilis 168* with increased adhesion and biofilm formation capacities have been investigated for the cultivation in a trickle‑bed biofilm reactor [2].

**2. Methods**

The used *B. subtilis* 168 mutants contained all a functional *sfp* gene necessary for surfactin production. Firstly, a mutant with restored exopolysaccharide production (*epsC+*) has been selected to optimize the natural immobilization of the bacterial cells on the bioreactor support. Secondly, cell filamentation has been additionally provoked through the deletion of the *sepF* gene which is involved in the cell division process [8]. The idea was to promote further the initial cell adhesion step as well as the support colonization through this change of cell shape. The surfactin productivity and biofilm formation capacity of these mutant strains have been studied under batch and continuous process conditions in a trickle-bed biofilm reactor containing a structured metal packing with a high specific surface area for the cell colonization [1], [2]. Moreover, the effect of the dilution rate on biofilm formation has been examined.

**3. Results and discussion**

As expected, the *epsC+* mutants showed significant improved attachment capacities on the biofilm bioreactor support resulting in an increased surfactin productivity compared to the control strain. The restoration of the exopolysaccharides permitted the cells to produce a biofilm matrix which helps to stick the cells together for their immobilization on the reactor support. The surfactin productivity could be further increased through the transition from a batch to a continuous production mode. An increased dilution rate (D=0.5 h-1) permitted to enhance the biofilm formation and thus higher cell densities on the reactor support could be achieved. By choosing a dilution rate higher than the maximum specific growth rate, the number of suspended cells could be reduced by washing out the cells. Microscope images of biofilm samples of the *epsC+* *ΔsepF* mutant revealed strongly filamentous cells. The deletion of *sepF* did not affect the cell metabolism and had a minor impact on the support colonization in comparison to the presence of exopolysaccharides.

**4. Conclusions**

A continuous production mode is more favorable for surfactin production than the production in a batch reactor. A dilution rate that is higher than the maximum specific growth rate permits to reduce suspended cells and increase the biofilm formation in the trickle-bed biofilm reactor and thus improve the surfactin productivity. Exopolysaccharide production is important for an increased support colonizationby *B. subtilis 168* whereas induced morphological changes seem to have a lower impact on the final biofilm formation but may facilitates initial adhesion on the reactor support.

**References**

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