Evaluation of diversified bioprocessing schemes for biosurfactants production from *Lactobacillus* strains using cheese whey

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Abstract

Biosurfactants or microbial surfactants constitute a group of amphiphilic molecules, comprising of both a hydrophilic (e.g. long-chain fatty acid, hydroxyl fatty acid) and a hydrophobic moiety (e.g. a carbohydrate, an amino acid, peptides, phosphate, alcohol). Those surface-active molecules can be produced by a variety of microorganisms and gained importance lately in several fields (e.g. bioremediation, pharmaceuticals, food applications), owing to their unique properties such as biodegradability, biocompatibility, and lower toxicity.Regardless the broad spectrum of end-applications, large scale production of biosurfactantsis is still hindered because of the high cost of manufacture (low yields, high raw material, recovery and purification costs). Additionally, the majority of biosurfactants derive from potentially pathogenic strains, including *Pseudomonas*, *Aspergillus*, *Candida* and *Bacillus* species, demonstrating limitations for novel food applications.

The utilisation of renewable resources confers several benefits since the reduction of production costs could be achieved through proposing environmentally benign approaches within the concepts of circular economy. As a matter of fact, used cooking oils, molasses, glycerol, soap stocks but also lignocellulosic biomass and cheese whey have been implemented as low-cost fermentation resources to reduce the cost of manufacture for the synthesis of biosurfactants. Likewise, non-pathogenic bacteria, like *Lactobacillus* strains that are Generally Regarded as Safe (GRAS) are studied to assess the biosurfactant production ability. Previously published studies reported on the exploitation of lignocellulosic biomass and cheese whey as onset material for the fermentative production of *Lactobacillus* derived biosurfactants.

The current study targets the evaluation of several bioprocessing strategies on the production of biosurfactants during the incubation of selected *Lactobacillus* strains using cheese whey as fermentation substrate. Shake flask batch cultures were initially employed, followed by fermentations in a 2L bioreactor under controlled pH and temperature conditions. Initially, the effect of pH value and incubation temperature was separately evaluated using an one-factor-at-a-time approach. Aiming to optimise the fermentation substrate, the effect of different nitrogen sources (e.g. beef extract, peptone and yeast extract) and mineral medium was also investigated. The latter experiments were performed using a microplate reader and results were assessed in terms of specific growth rate (μ, h^{-1}). Bioreactor cultures were carried out by combining optimum results obtained by the initial experimental design. Surface tension, emulsification activity (E_{24}, %), total dry weight (TDW, g/L) and lactose consumption were measured, during several fermentation time points, to estimate bacterial growth and biosurfactants production. Surface tension reduction was assessed both for cell-bound biosurfactants after extraction with PBS solution as well as for extracellularly produced biosurfactants in the supernatant. On top of that, methods employing organic solvent extraction and precipitation were also implemented to isolate biosurfactants from the supernatant fraction. Biosurfactants characterization was also conducted considering that *Lactobacillus* strains produce mainly glycolipids or glycoproteins. Results indicated the significance of the composition of the fermentation substrate to direct *Lactobacillus* cell metabolism towards biosurfactants production. Future studies will focus on fed-batch fermentation strategies to further enhance production and maximize productivity.

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