Abstract

Surfactants or "SURFace ACTive AgeNTs", are a class of chemicals that intervene in almost every aspect of our daily lives. With USD 20 billion, surfactants are one of the largest markets of bulk chemicals in their totality (S. L. K. W. Roelants & Soetaert, 2022). As for the carbon contained within the total surfactant market, only 4% is fully biobased, from which <0.5% are produced biologically (S. L. K. W. Roelants & Soetaert, 2022). Moreover, low biodegradability, bioaccumulation, ecotoxicity and reported dermatological issues are other challenges associated to synthetic surfactants (Wongsirichot et al., 2021). To address these challenges, investigations for finding natural biobased alternatives to petrochemical surfactants initiated in the 1960s leading to the development of "wholly bio-based surfactants" or "biosurfactants" as a promising alternative (Vieira et al., 2021). An example are sophorolipids (SLs), a glycolipid biosurfactant produced in titers >200 g/L and volumetric productivities up to 3.7 g/L.h by the yeast *Starmerella bombicola* (*S. bombicola*). Due to the efficient SL production machinery and non-pathogenicity, *S. bombicola* has attracted both industrial and academic interests. However, for successful commercialization, the goal is to create greater variability between products and more uniformity within each product in the framework of an efficient low-cost process. The problem of batch-to-batch variations and the production of different types of biosurfactants can be solved by strain engineering. Also, utilization of industrial waste/side streams as feedstock can significantly contribute to the economic feasibility of the process and valorize those streams in the framework of a sustainable circular bioeconomy.

To address these challenges, this master dissertation focused on the fermentative production of novel biosurfactants from a modified microorganism on organic biowastes (organic biowaste1 (OBW1), OBW2 and OBW3) and a hydrophobic feedstock. OBW1 was the growth medium, OBW2 or OBW3 were the fed-batch hydrophilic carbon substrate and the hydrophobic feedstock was the hydrophobic carbon substrate. The biowastes were subjected to pretreatment before fermentation, consisting of two general sections: enzymatic hydrolysis and filtration. Enzymatic hydrolysis was performed using hydrolytic enzyme 1 and hydrolytic enzyme 2 for OBW2 or OBW3, and hydrolytic enzyme 2 and hydrolytic enzyme 3 for OBW1. Hydrolysis optimization tests were also conducted to reduce enzymatic loads and treatment times as much as possible. These tests resulted in a 50000 folds decrease in hydrolytic enzyme 3 load, and a 280 folds decrease for hydrolytic enzyme 2 load for OBW1 hydrolysis. Also, an 11 folds reduction in hydrolytic enzyme 2 load, and 2 folds reduction in hydrolytic enzyme 1 load for OBW 2/3 hydrolysis was achieved. Moreover, a 17h reduction of hydrolytic enzyme 1 treatment time (from 18h to 1h) was attained for OBW2 and OBW3 hydrolysis. It was proven that novel biosurfactants can be produced on organic biowaste and hydrophobic feedstock. Further optimization led to the production of novel biosurfactants in a titer, volumetric productivity, and product to total substrate yield of 119 g/L, 0.59 g/L.h, and 0.44 g/g, respectively. Interestingly, these results were achieved with a hydrophilic carbon substrate starvation strategy throughout the fermentation. For comparison, the reference condition (filtered and hydrolysed OBW1 with sufficient carbon source throughout the fermentation) resulted in a titer, volumetric productivity and yield of 66 g/L, 0.33 g/L.h, and 0.28 g/g. However, in terms of growth, the condition in which non-filtered OBW1 was used as growth medium yielded the highest cell dry weight (CDW) of 26.68 g/L in comparison to 23.40 g/L of the reference condition. Based on these results, the prospective shall be to conduct a fermentation with not filtered, not hydrolysed OBW1 and with a hydrophilic carbon starvation strategy, in order to check if such a strategy leads to maximum growth and production simultaneously. Moreover, further enzymatic hydrolysis optimization tests shall be planned.

Keywords: Novel biosurfactants, Sophorolipids, *Starmerella bombicola*, Second-generation feedstock, Enzymatic hydrolysis, Optimization