

An interdisciplinary -omics study to boost continuous bolaform sophorolipid production.

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Why are we still using petroleum and palm oil derived surfactants for personal care and cleaning applications when more sustainable biobased surfactants are available? Because of the latter's high production costs. Hitherto, the production efficiencies of fermentation processes for biosurfactants are often insufficient to be economically viable, which is mainly due to the lack of knowledge about the complex interactions between multiple parameters in (industrial) bioreactors and biosynthetic pathways. In this study, we aim at reducing the production costs for innovative bolaform sophorolipids (BLSL), produced with the yeast *Starmerella bombicola* $\Delta sble\Delta at$. An interdisciplinary polar metabolomics and lipidomics driven strategy was set up to gain insight in bolaform sophorolipid biosynthesis and to substantiate an industrial biosurfactant production process with high volumetric productivity.

A novel continuous retentostat bioprocess set-up was investigated. It resulted in a volumetric productivity of $0.63 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$, a 186.36% increase in comparison to previous reports. Unfortunately, this high productivity could not be maintained for more than 543h and the reason for the drop in productivity was investigated by performing a multi-omics study. Whole genome sequencing and subsequent variant analysis discovered no evidence for genomic variations for up to 1306h of retentostat cultivation. To reduce confounding factors, a second independent set-up was performed. Hereby, The stirring rate was increased excessively in a fed-batch process and resulted in a permanent loss of BLSL production compared to a control fed-batch process.

Untargeted polar metabolomics and lipidomics analyses were subsequently performed on bioreactor broth samples of abovementioned experimental set-ups, in order to define metabolites that are associated with either high or low BLSL productivity. Untargeted fingerprints upon polar metabolomics and lipidomics analyses rendered 22,897 and 21,962 components, respectively. Subsequently, principal component analysis (PCA) and supervised orthogonal partial least squares discriminant analysis (OPLS-DA) were performed. These analyses revealed 139 and 44 discriminating components in the polar metabolomics and lipidomics dataset, respectively.

These components are linked to BLSL productivity and therefore subsequent identification and pathway mapping will allow the development of rational strategies for metabolic- and/or process engineering and, in the end, to achieve an economically viable (continuous) production process.

Please tick at least one box in the list below (you may tick more boxes if applicable)

- Microbial Biosurfactants
- Plant based Biosurfactants
- Biocatalytic production of Biosurfactants
- Chemical production of Biosurfactants
- Biosurfactant production from waste- and/or side streams
- Functional characterisation of Biosurfactants
- Process development for Biosurfactants
- Molecular methods to improve Biosurfactant Biosynthesis
- LCA and TEA studies on Biosurfactants
- Industrial Perspectives of Biosurfactants
- Applied research with Biosurfactants:
 - mining
 - food/feed
 - agro
 - detergents
 - personal care, cosmetics
 - other(s): specify

- Other: