Project title: Sustainable Microbial Valorisation of Waste Lipids into Biosurfactants (Waste2Surf)

Project No. 1.1.1.1/19/A/047

Project leading partner: University of Latvia

Project partners: JSC "Biotehniskais Centrs"

Project report on the tasks completed in the period from 01.10.2021. till 31.12.2021.

During the reporting period, work on both mathematical modelling of metabolism and modelling of environmental impacts has been ongoing. A system dynamics model is also beginning to take shape, but further work is needed to refine the model and develop potential scenarios. During the reporting period, experimental work continued actively both in the Laboratory of Yeast Nutritional Physiology and in the fermentation laboratory of the project partner JSC "Biotehniskais centrs".

A prototype of fermentation/extraction system for surfactants was successfully validated in the yeast physiology laboratory with model environments. The specificity of the anthrone and DNA methods (lipids, other surfactants) was tested. A protocol for the application of the methods for the determination of biosurfactant in lipid-rich fermentation media was prepared. Work on the recombinant strains' method has started: literature analysis is being carried out to select the optimal *Starmerella bombicola* transformation and recombinant selection methods.

Work on optimisation of the fermentation and extraction system for biosurfactants is ongoing in the fermentation laboratory of the project partner JSC "Biotehniskais centrs". Optimal operating parameters (pressure, flow rate) for the removal of sophorolipids/biomass from the fermentation medium were determined. During the reporting period, several *S. bombicola* cultivation/fermentation experiments were carried out to determine the dynamics of biosynthesis. Based on these parameters, both – a mathematical model of the process and a softsensor algorithm are being developed. Screening experiments are being carried out with the aim to identify yeast extract constituents that stimulate biosynthesis of sophorolipids in *S. bombicola* cells. The experimental determination of the metabolic diversion of amino acids in yeast extract to the biosynthetic pathway of biosurfactants was carried out. Experiments are being carried out to determine which amino acids stimulate biosynthesis and at which concentrations.

The modelling teams of the project continue the process of building a genome-scale model of *S. bombicola*. In collaboration with the yeast physiology laboratory, the selection and combination of the most appropriate models for Yarrowia lipolytica continues. Parameters of the kinetic models are being determined by parameter estimation methods in the COPASI environment, based on published and experimental data generated within the project, to determine the pathway of biosurfactant production. The design of strains based on the synergy of stoichiometric and kinetic models has been initiated by optimising the available models according to the developed prototype solution ranking criteria.

During the reporting period, the basis of a life cycle model of sophorolipids production was developed. The model simulates the environmental impact of the fermentation process and identifies the production steps where environmental impacts occur. The model defines parameters that can be changed to be adapted to the fermentation process. Work has started on the preparation of the life cycle assessment report. During the reporting period, work has started on the development of a system dynamics model and data are being collected to identify the patterns that determine the dynamics of the model.

Two abstracts were submitted to the international scientific conference "Conference for Young Scientists on Biorefinery Technologies and Products", which will take place on 27-29 April: https://www.btechpro.lv/

Information about the project at the partner's website: https://www.bioreactors.net/wastetosurf

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