



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 01.07.2023. till 30.09.2023.

During the final period of the project, the main focus was placed on obtaining and summarizing results to conclude the project activities. Final steps of sustainability assessment were conducted using multi-criteria decision analysis (MCDA). Additionally, recombinant strains of yeast *Yarrowia lipolytica* were developed and described. Final steps of model predictive control (MPC) system optimisation were carried out.

The project team finalised the sustainability assessment for the years 2020, 2030, and 2050 and analysed the results. Future scenarios were calculated by assuming changes in electricity mix in future scenarios and by calculating future prices of oil substrate. Two reports on the sustainability assessment results have been prepared. One report includes step-by-step selection on sustainability criteria while other report describes MCDA methodological choices and results.

The project team has completed the construction of the GoldenGate construct in the *Yarrowia lipolytica* yeast strain, and recombinant strains were obtained. A report has been prepared regarding the results of the activity. The *Y. lipolytica* wild-type strain does not produce glycolipid bio-surfactant substances. The ability of the *Y. lipolytica* wild-type strain to transform with a GoldenGate construct containing GFP was also demonstrated, and GFP was successfully produced. The level of sophorolipids in the recombinant strains did not reach the detection threshold. It is possible that part of the synthesis pathway is inhibited, or *Y. lipolytica* clones with suppressed sophorolipid synthesis have been selected. A report on the activity results has been prepared. In addition, the project team member participated in special international professional training on *Y. lipolytica*.

At the partner institution, JSC "Biotehniskais centrs", fermentation was optimized using the Model Predictive Control (MPC) system. The optimal composition of the fermentation feedstock was determined, and a prototype MPC algorithm was developed and tested. The obtained result will provide a foundation for further development and implementation of MPC systems in the production of bio-surfactant substances. The assessment of the algorithm's operation was conducted under conditions of input data disturbances (stability analysis of the algorithm). The main objective of the work package was achieved, and a report on the relevant results was provided.

During the reporting period, various dissemination and communication activities were carried out for the project. Results from the environmental impact assessment were published in a scientific article: Balina K., Soloha R, Suleiko A, Dubencovs K, Liepins J, & Dace E, 2023, "Prospective Life Cycle Assessment of Microbial Sophorolipid Fermentation". This paper was published in the journal "Fermentation," [DOI:10.3390/fermentation9090839].

A presentation on the project results was delivered to industry professionals at the COST Action WIRE (Waste biorefinery technologies for accelerating sustainable energy processes - CA20127) scientific workshop held in Cottbus, Germany.

Overall, this project has made significant progress in the development of a scalable and sustainable biosurfactant production technology. The project team has made important contributions to the scientific knowledge base on *S. bombicola* and *Y.lipolytica* metabolism and sophorolipid production. The developed genome-scale metabolic model and life cycle assessment study will be valuable resources for future research

and development in the field, while the developed technology prototype is prepared for scale-up and application in biosurfactant production industry.

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

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