



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 01.07.2022. till 30.09.2022.

With the finishing of previous project reporting period, the work on the life cycle assessment has been completed and the results are summarised in an LCA report. In the meantime, analysis on sophorolipid pathway in various organisms is still ongoing, with plasmid constructs for heterologous expression of the synthesis pathway being generated. Studies to characterise the growth phenotype of *S. bombicola* are also ongoing. Experiments with model medium (sophorolipid solutions) on the established filtration system are being carried out in the laboratories of the collaborating partners JSC "Biotehniskais centrs". A plan for the filtration experiments has been established and is being implemented. The data obtained will be further used to model the performance of the filtration process. During the reporting period, the optimal composition of the culture medium in which to efficiently stimulate the synthesis of sophorolipids was also established. Experiments with model media (sophorolipid solutions) are being carried out on the developed filtration system. Based on the above, experimental data (for both fermentation and filtration processes) are being actively accumulated, which will further allow the adaptation/development of an efficient control system.

Additional literature data are being collected to develop kinetic models for biosurfactant production and to optimise it in order to define more accurate model parameters. Possible differences between the pathway parameters of *Y. lipolytica* (after engineering) and *S. bombicola* are also sought. The types of reaction kinetics equations are examined. Evolutionary algorithms are being trained to design strains for simultaneous searches for deletions and insertions to improve speed and convergence properties. The implementation of growth-coupled production methods in optimisation continues. Ten strain designs based on the synergy of stoichiometric and kinetic models have been developed.

Final refinements have been made to the life cycle assessment model. The inaccuracies that had arisen during the previous project period by assigning two different functions to the model have been eliminated. The results of the life cycle assessment have been prepared for presentation at an international scientific conference. The report on the results of the life cycle analysis has been finalised. Work on the development of the system dynamics model also continued during the reporting period. The model is divided into two main structures: the waste generation system and the biosurfactant market. The interlinks between the two models are being explored. Socio-economic indicators affecting the system have been discussed during the reporting period. A literature analysis is carried out to identify the most appropriate indicators to describe the system.

Work on the determination of growth parameters of *S. bombicola* has been completed in the Yeast Physiology Laboratory. A review of the physiology of the standard strain of *S. bombicola* has been prepared. A set of *Yarrowia lipolytica* *goldengate* plasmids was purchased and deposited. Gene sequences of *S. bombicola* sophorolipid fusion proteins corresponding to the GoldenGate cloning system for *Y. lipolytica* were generated. Biosurfactants produced by *Y. lipolytica* have been characterised. Tests with *Pseudozyma antarctica* have been initiated to test the ability of this micro-organism to convert waste cooking oils.

Cultivation/fermentation experiments are continuing in the laboratories of the project partner JSC "Biotehniskais centrs". Based on the results of these experiments, a softsensor algorithm is being developed. Screening experiments to identify yeast extract components that stimulate sophorolipid biosynthesis in *S. bombicola* cells were completed during the reporting period. Further experiments will be carried out with an appropriate composition of the fermentation medium in a 5L bioreactor for proof of concept.

Empirical models are being developed to predict the main performance parameters of the sophorolipid extraction system and theoretical scaling is being realised. The work on the report is ongoing. The optimum composition of the medium in which to efficiently stimulate sophorolipid synthesis was established. Based on the results of the cultivation/fermentation experiments carried out, the existing version of the algorithm was further developed. The efficiency and overall functionality of the algorithm is tested with existing experimental data. The development of the optimisation algorithm is under active development. The first versions are being tested on the experimental data.

During the reporting period, several communication activities were carried out, three presentations on the project results were given at three international scientific conferences.

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

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27.10.2022.