NATIONAL DEVELOPMENT PLAN 2020

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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 01.10.2022. till 31.12.2022.

During the reporting period, the Computational Systems Biology Group continued to refine and extend their models to provide the most complete representation of the mechanism of sophorolipid synthesis. Meanwhile, in the Sustainability Modelling Group, work continued to improve the system dynamics model and describe the dynamics with mathematical equations. A publication based on the results of the life cycle analysis is under preparation. Planned experiments on a sophorolipid filtration system have been completed in the laboratories of the partner *Bioreactors.net*. The data obtained were partially compiled and analysed. Empirical mathematical relationships between filtration rate, effective filtration area, membrane pore size and retentate circulation rate were constructed. These empirical relationships (models) are being validated. In the yeast Physiology Laboratory, constructs for the expression of the *Starmarella bombicola* sophorolipid synthesis pathway in *Yarrowia lipolytica* have been developed. Also, experiments with *Pseudozyma antarctica*, started in the previous reporting period, were continued to test the ability of this micro-organism to convert waste cooking oils. Experiments were also continued with the optimal composition of the fermentation medium found previously. The experimental data are collected and analysed to adapt the process control system. During the reporting period, more attention was paid to mass balances as well as to the identification of process markers able to describe the current state of the bioprocess (synthesis of sophorolipids/biomass growth/formation of other metabolites).

Three kinetic models have been developed to model and optimise the scale kinetic model of the sophorolipid production pathway: two models describing sophorolipid production by *S. bombicola* on different substrates and one model describing the production of a surfactant precursor in *Y. lipolytica*. The models have been subjected to TOP optimisation, resulting in the identification of metabolic engineering targets. The development of new strain designs is continued with introduction of environmental and social sustainability criteria, using the OptGene approach as the basis for the search algorithm. The developed projects are tested for their ability to incorporate the product production flows described in the above three kinetic models.

To assess the sustainability of sophorolipid production using waste cooking oil, additional literature data is being collected to improve the existing structures of the system dynamics model. In collaboration with colleagues of the Yeast Physiology Laboratory, an approach is under development to model the conversion of waste oils to biological surfactants, taking into account the relative proportions of substrates. The effect of the glucose-waste oil ratio on the yield of sophorolipids is planned to be used as a co-relation. Based on the literature analysis different scenarios for the multicriteria analysis are being developed. Socio-economic indicators have been identified, from which the most relevant ones will be selected to compare the scenarios developed.

A soft-sensor algorithm for sophorolipids has been developed in the project partner's *Bioreactors.net* laboratory. Relevant information (algorithm and description of methodology) was submitted for evaluation. An effective strategy was found to reduce foaming without impairing oxygen mass transfer. The evaluation of the techniques investigated, and the description of the final effective strategy have been submitted for evaluation. Based on the obtained results, a prototype fermentation/extraction system is being developed. The resulting filtration process models are used to evaluate the predicted efficiency and relevant system performance parameters during the prototype development. During the reporting period, the MPC control algorithm was significantly enhanced (integrating efficient mass balance accounting algorithms and testing

several process efficiency markers (RQ, OUR, CER) and their combinations). Work on the improvement of the MPC system is ongoing. The latest version of the MPC control optimisation algorithm demonstrated good results and proved its efficiency in bioprocesses to produce sophorolipids. Additional experimental data are being collected to further improve the performance of the algorithm.

Several communication activities were carried out during the reporting period. A poster presentation and an abstract on the project results were presented at the International Scientific Conference "LCA Foods 2022". A general presentation on the project and its results was also given to colleagues from other departments of the University of Latvia.

The project team visited the production site of Happy Fish Ltd, a company producing biosurfactants from oil. During the visit, cooperation opportunities were discussed, and the participants exchanged experience on aspects of the production of biosurfactants.

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

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