NATIONAL DEVELOPMENT PLAN 2020

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INVESTING IN YOUR FUTURE

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 01.01.2022. till 31.03.2022.

During the reporting period, experiments have continued to determine the optimal fermentation environmental conditions to produce sophorolipids by fermentation. The process of production of biological surfactants is becoming more and more concrete. During the reporting period, activities for the development and optimisation of kinetic and stoichiometric models continued. Work on modelling the environmental impact of the process is also continuing and the model is being adapted to match experimental results. An environmental impact assessment report for the baseline scenario has also been prepared.

The Yeast Physiology Laboratory carried out laboratory tests on a variety of yeasts, using both raw cooking oil and waste cooking oil. The tests showed that *Starmarella bombicola* does not lose its viability, while *Yarrowia lipolitica* cells die after the fifth day of the experiment. Vitamin auxotrophy of *S. bombicola* was also determined. The tests showed that the presence of biotin is critical for the growth of *S. bombicola*. A literature analysis of the sophorolipid production pathway in *S. bombicola* metabolism is being carried out to allow genetic engineering in the strain. Deletion of the Ura3 gene results in *S. bombicola* no longer producing uracil. Carbon source consumption was also analysed. It is concluded that glucose, fructose, and glycerol are the most consumed. This is important information in the search for a gene to knock out in the engineering process. An experiment has been launched to knock out the ura3 gene with 5-FOA. By knocking out the ura3 gene, uracil will have to be artificially added, but this will allow to control the process.

In the Fermentation laboratory of the project partner JSC "Biotehniskais centrs", the second prototype fermentation/extraction system for biosurfactants has been built and successfully tested in a fed-batch fermentation process. The optimum operating parameters (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 the synthesis of biosurfactants. Based on these parameters, a mathematical model of the process was developed and a softsensor algorithm is under development. Screening experiments are being carried out to identify the yeast extract components that stimulate soforolipid biosynthesis in *S. bombicola* cells. The metabolic diversion of amino acids in yeast extract towards the biosynthetic pathway of biosurfactants was experimentally determined. Experiments are being carried out to determine which amino acids stimulate biosynthesis and at which concentrations. Fermentation of *S. bombicola* using different synthetic/complex media compositions was carried out. Syntheses of bioactive substances were initiated, and the kinetic parameters of the processes were evaluated. Based on the kinetic parameters, a process control algorithm was developed and refined.

In the modelling tasks of the project, an initial version of the *S. bombicola* model has been established and will be refined in the light of experimental results. The genome-wide adaptation of the two *Y. lipolytica* surfactant-producing strains to the project is ongoing. Models of other surfactant-producing organisms are also being analysed with a view to their inclusion in the optimisation workflow. The metabolic pathways involved in surfactant production in *Y. lipolytica* are analysed in detail. The resulting steady-states of metabolism are transferred to genome-scale stoichiometric models to determine the feasibility of pathways taking into account biomass production. The framework of the complex optimization algorithm in the COBRA toolbox has been developed, which allows determining the steady-state fluxes of the organism while it is being optimized. The resulting steady-state fluxes are tested in kinetic models.

During the reporting period, a study on the main flows of edible oils was carried out and an initial model was developed. Optimisation of the structure of the environmental impact assessment model was performed. The model identifies the phases with the highest environmental impacts and seeks solutions/scenarios for mitigation in cooperation with project partners. Environmental impact indicators have been selected. It is considered to use them to obtain single indicator using normalisation and weighting techniques.

During the reporting period, several communication activities were carried out. ". The results of the project were presented at the 80th International Scientific Conference of the University of Latvia. Two additional abstracts for participation in international scientific conferences were submitted. The project was featured on the Latvian Radio programme "Zināmais Nezināmajā" (Known in the Unknown). In addition, participation in a national event for professionals and the general public was carried out by giving a presentation at the Vidzeme Innovation Week 2022 event. During the reporting period, an information webinar for professionals and the general public on the project results and future activities was also organised, as well as an international training workshop Biomodelling Spring 2022.

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

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