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 01.04.2022. till 30.06.2022.

During the reporting period of the project, synergies between kinetic and stoichiometric models have been developed through optimisation experiments. Preliminary results of the environmental impact assessment have been presented at two conferences and the system dynamics model of the waste cooking oil is becoming more concrete. Experimental work in the laboratory continued with experiments on *Starmarella bombicola* and the development of a mutant of the yeast *Yarrowia lipolitica*. Work is also continuing on the characterisation of the operating parameters of the extraction/downstreaming process. Empirical mathematical relationships have been established which will allow future modelling and/or designing of similar systems for different scales of sophorolipid production.

Laboratory tests were carried out in the Yeast Physiology Laboratory to determine growth parameters (glucose consumption, growth rate, mannitol accumulation, biomass components) of *S. bombicola* in batch fermentation tests with glucose. The experiment to eliminate the ura3 gene with 5-FOA was completed. By eliminating the ura3 gene, uracil needs be added, but this facilitates manipulation of the organism functioning. The ura3 mutant of *Yarrowia lipolytica* that was created has been deposited in the microorganism collection at the Institute of Microbiology and Biotechnology. Preparations (first tests) have started to use this strain as a basic chassis for genetic engineering.

In the project sections devoted to kinetic and stoichiometric metabolic modelling, metabolic pathways of the genome-wide models are built. They may operate in parallel to the pathways included in the kinetic models. The analysis of the variability of these pathway fluxes allows to determine their potential impact on the process under study at specific values of biomass growth. Different types of constraints are applied in the kinetic models, which improve the likelihood of applicability of the developed solutions in biological experiments. Genome-wide stoichiometric models are optimised using different combinations of deletions and insertions, maximising the total carbon flux to the products, which minimises the release of carbon into by-product compounds. The convergence rate during optimisation is evaluated. Work is in progress on the first strain designs.

The environmental impact assessment of the baseline model has been completed during the reporting period and environmental hotspots have been identified. Several scenarios to address the hotspots have been developed and presented as suggestions at an international conference. In consultation with an external expert, the points requiring further attention in LCA model have been identified: 1) Functional unit, 2) Use scenario, 3) Waste scenario. The literature analysis and the expert suggestions have been used to address the existing uncertainties and gaps. In the system dynamics modelling activity, causal loops have been identified and characterised. Data analysis is carried out to describe the loops by mathematical formulae. The construction of a yeast metabolite database to describe the environmental impact of each metabolite entering and leaving the yeast cell has started.

Project partners JSC "Biotehniskais centrs" worked on the characterisation of the operating parameters of the extraction/downstreaming stage of sophorolipid production process. Empirical mathematical relationships were established which will allow future modelling and/or design of similar systems for different scales of sophorolipid production processes. Cultivation/fermentation experiments also continued during the reporting period. These experiments included the accumulation of relevant data and the development of softsensor algorithms for testing. Since the start of the project, due to the constraints imposed by Covid-19, the

experiments foreseen under the activity have been delayed and the activity is expected to be completed by 31 December 2022.

Screening experiments using different fermentation media compositions (varying concentrations of both amino acids and other organic/inorganic components) were continued to identify yeast extract components that stimulate sophorolipid biosynthesis in *S. bombicola* cells. Experimental and theoretical information is combined to look for correlations.

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

12.07.2022.