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 01.01.2023. till 31.03.2023.

During the recent phase of the Waste2Surf project, considerable strides were made by our Genome and Sustainability Modelling groups. The Genome Modelling group augmented their focus on microorganism strain modelling by fusing kinetic and stoichiometric models. Simultaneously, the Sustainability Modelling group honed the system dynamics model, seeking optimal ways to integrate its diverse structures. Meanwhile, the Yeast Physiology Laboratory demonstrated their proficiency by successfully cloning heterologous genes into the *Yarrowia lipolytica* genome following the Golden Gate (GG) protocol. Concurrently, the team at JSC "Biotehniskais Centrs" pressed forward with experiments using an optimally determined growth medium.

We are thrilled to announce that the prototype of the biosurfactant extraction system has been seamlessly integrated into the bioprocess operating system. This allows us to collect vital experimental data from fermentation and separation processes, thereby refining our control of the continuous process. Furthermore, the prototype completion signifies a notable accomplishment in our project activities.

The Metabolic Modelling group, during this reporting period, fortified their application of kinetic and stoichiometric model synergy, leading to higher potential for positive outcomes. Increased success rates were achieved by implementing limitations initially omitted in the model, while details of the stoichiometric model continue to be enhanced.

Our Sustainability Modelling group has now pivoted its focus towards developing a waste cooking oil valorisation module within the system dynamics model. The module focuses on three crucial areas: identifying various waste oil usage pathways like biosurfactants, biodiesel, and biogas; devising an optimal biosurfactant production pathway, considering factors such as glucose to waste oil ratio and maximum biosurfactant yield; and initiating work on the biosurfactant consumption and economic modules.

During the last reporting period, we made considerable strides in research on Multi-Criteria Decision Analysis (MCDA) methods. Our objective was to identify the most apt and relevant MCDA methods in line with our project goals. A series of in-depth discussions and exchanges of ideas took place among working groups to ensure a logical analysis of scenarios. The subsequent plan is to implement the identified MCDA methods to scrutinize data and draw conclusions.

In the Yeast Physiology laboratory, the first successful cloning of heterologous genes in the *Y. lipolytica* genome has been accomplished per the GG protocol, with active gene expression demonstrated. Our team will now proceed with the cloning of the remaining sophorolipid pathway genes and the preparation of vectors in accordance with the GG protocol. Notably, surfactant by-product potential was explored as an additional additive for *Starmerella bombicola* biomass production.

Finally, this period marked the completion of the biosurfactant extraction system prototype. The prototype employs a two-step continuous filtration of the fermentation broth, initially separating biosurfactants, and then enhancing their concentration. The creation and validation of the prototype entailed conducting experiments with both simulated and real fermentation broths.

During this period, we accumulated additional experimental data to augment the MPC control algorithm. Concurrently, we tested the algorithm's stability using artificial data.

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

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