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 01.07.2021. till 30.09.2021.

During the reporting period, experimental work continued actively both in the Laboratory of Yeast Nutritional Physiology and in the Fermentation Laboratory of the JSC Biotehniskais centrs. Experiments were continued in the Laboratory of Yeast Nutritional Physiology and a method to determine the presence of biosurfactants more efficiently and conveniently in lipid-rich fermentation media was developed. The method uses a combination of several methods, that is specifically adjusted to detect glycolipid biosurfactants. The developed method is described and will be used as a basis for a new scientific publication. The research team has also developed a method for biomass quantification based on the number of cells per mL during the reporting period. The growth of *Starmarella bombicola* was evaluated using glucose, glycerine and fatty acids as growth substrates. In each of these experiments, the biomass-specific growth rate was determined using the developed biomass quantification method.

The development of the pilot plant in Fermentation Laboratory of the JSC Biotehniskais centrs has continued. The installed tangential filtration module works well and can filter 100 mL/min. As a result of the procurement, several components of the reactor fermentation/extraction system, including peristaltic pumps, a surfactant storage tank, membranes, have been purchased and the assembly of the system has started. During the reporting period, several *S. bombicola* cultivation/fermentation experiments were carried out to determine the kinetic parameters of the process. Parameters will be used to develop options for softsensor algorithms. The results of the previous experiments were analysed, and it was observed that the foaming of the fermentation medium can be partially (or completely) prevented by maintaining the pH of the medium at low values (<3.5). This, in turn, makes it possible to avoid the use of gas contactors to produce surfactants.

In the modelling sections of the project, work continues with stoichiometric and kinetic models, which used simultaneously improves the reliability of the simulated results. A genome-scale model of *S. bombicola* is being developed to assess the engineering potential of the organism. Recent genome-scale models for *Yarrowia lipolytica* are also under consideration. The structures of kinetic models are extended to incorporate different substrate components.

To determine the environmental impact of the bioactive production scheme, additional aspects that may influence the model results were evaluated. The specificities of life cycle modelling of biological processes have been given increased attention. The most appropriate solution is being sought for modelling of fermentation processes. Work has also started on the modelling of the system dynamics of waste cooking oils by developing a conceptual model of the system and a causal loop diagram.

The research work has resulted in two scientific articles prepared and published:

1) Liepins, J, Balina, K, Soloha, R, Berzina, I, Lukasa, LK & Dace, E 2021, 'Glycolipid biosurfactant production from waste cooking oils by yeast: Review of substrates, producers and products', Fermentation, vol. 7, no. 3, 136. on the use of yeasts for production of biosurfactants from waste cooking oils and fats.

2) Stalidzans, E & Dace, E 2021, 'Sustainable metabolic engineering for sustainability optimisation of industrial biotechnology', Computational and Structural Biotechnology Journal, vol. 19, pp. 4770-4776. on the concept of sustainable metabolic engineering, which allows the sustainability of biotechnological production to be assessed and optimised by deriving it from the metabolic traits of the micro-organism used.

The project activities and results were presented to industry professionals at the COST Action Yeast4Bio: Non-conventional yeasts for the production of bioproducts (CA18229) meeting (29.09.2021, remotely).

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

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