Summary of dissertation entitled: Enhancement of fumaric acid production by fungi of the genus *Rhizopus*

Production of fumaric acid by microbiological synthesis using fungi belonging to the genus *Rhizopus* is an alternative to methods based on chemical transformations. The research conducted within the framework of my PhD thesis was aimed at improving the method of cultivation of the above mentioned fungi both in terms of improving productivity and efficiency, as well as lowering the costs of the biosynthesis process by using cheap substrates with the character of by-products of other technologies, such as food and petrochemical industry.The aim of the study was to develop optimal conditions for the culture of a selected strain of fungus of the genus *Rhizopus* with the use of alternative carbon sources, including glycerol and nitrogen sources for intensification of fumaric acid biosynthesis.

The aim was achieved by the following specific objectives:

1. Taxonomic identification of obtained fungal isolates and determination of their chemotype.
2. Determination of the ability of selected strains - producers of fumaric acid to use glycerol in the crude form as a source of carbon.
3. Selection of wild strains with natural predisposition to produce an increased amount of fumaric acid in media containing, among others, crude glycerol of various origins.
4. Use of alternative sources of carbon and nitrogen in media for culture of fungi of the genus *Rhizopus* sp.
5. Development of a simplified procedure for culture in a bioreactor without the separate stage of pre-culturing.
6. Conducting bioreactor cultures on an increased scale using a selected strain of *R. oryzae* fungus characterized by the highest productivity in terms of fumaric acid.

The dissertation deals with known solutions in microbiological production of fumaric acid combined with a new approach based on the strategy of co-utilization of glycerol in the crude form with carbohydrates, the application of which would allow to increase efficiency and reduce process costs and thus production costs of fumaric acid. Moreover, an important element of the

conducted research was shortening the time of culturing, obtaining an appropriate form of fungal biomass and limiting the number of operations posing a risk of culture infections, which may be of key importance for the application of the developed fumaric acid technology by microbiological fermentation with the use of fungi of the genus Rhizopus.

The basis for the strategy was to divide the process into two typical phases: biomass growth and production phase, without the need to conduct typical preculture. An innovative element of the proposed culture method was the use of waste glycerol as a carbon source to obtain the fungal biomass of a selected strain of *Rhizopus oryzae* R-45. As a result of this procedure the growth of the hyphae was slowed down, which allowed to receive an appropriate form of biomass. Through the supplementation of the growing medium with ammonium sulphate, high productivity was achieved, while a supplementation of potassium nitrate (0.2 g/l) during the saccharide addition process maintained the biomass viability to 400 h (fed batch). The highest concentration of fumaric acid, 93.31 g/l, was obtained by applying the culture strategy proposed in this work. The process was characterized by moderate final productivity (0.39 g/lxh), but the partial productivity after the sequential addition of glucose and fructose syrup as a co-substrate reached 0.77 g/lxh. Other experiments carried out within the framework of this study allowed, among others, to observe a positive effect of temperature increase (from optimal 30°C to 35°C) on the glycerol utilization in the group of environmental isolates belonging to the *Rhizopus stolonifer* species, which were also able to produce fumaric acid, but in smaller amounts in comparison to the *R. oryzae* species.

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