Warsaw, 1st December 2025

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ABSTRACT OF THE DISSERTATION
"*Intensification of mass transfer in a wave-mixed bioreactor*"

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Single-use bioreactors with wave mixing stand out from classic bioreactors and other single-use devices because of the mechanism used to agitate the liquid phase, which is induced by the oscillatory movements of a disposable polymer bag-like container mounted to a dedicated platform. The polymer containers of wave-mixed single-use bioreactors are made of welded sheets of multi-layer film and are partially filled with the liquid phase to ensure continuous or periodic flow of the gas phase over the flowing liquid. Despite the relatively short period of availability of commercially offered equipment on the market, and thus the short period of routine use, wave-mixed bioreactors are widely used in modern areas of the biopharmaceutical industry. The mixing and mass transfer efficiency which characterise the liquid agitation mechanism used in this type of equipment are many times lower than in classic bioreactors. For this reason, the applicability of wave-mixed bioreactors is currently limited to bioprocesses using biomass with moderate proliferation rates and moderate oxygen assimilation rates.

The aim of the research was the development of a prototype and bioprocess validation of modifications for intensification of mass transfer in polymer Cellbag™ containers of the *Ready-ToProcess* WAVE™ 25 bioreactor. The scope of the research included: characterisation of mixing efficiency in the liquid phase subjected to oscillations as a function of the operating parameters of the bioreactor, investigating the impact of changes of selected properties of the liquid phase on mass transfer efficiency, the development of an original adaptation of the colorimetric method for measuring mixing time in the system and analysing the structure of the oscillating gas-liquid interface, and the bioprocess validation of prototype modifications to polymer tanks during a series of batch cultures of *Nicotiana tabacum* BY-2 cells.

The influence of the liquid phase composition and the addition of an anti-foaming agent, as well as the viscosity of the liquid phase, on the mass transfer efficiency through the waving gas-liquid interface was investigated. It was demonstrated that an increase in the viscosity of the liquid phase in the tested bag-like container caused a decrease in mass transfer efficiency, quantified by measurements of the $k_L a$ coefficient with an adaptation of the gassing-out method. Based on the results of the experiments, it was concluded that the presence of an anti-foaming agent and the change in the composition of the liquid phase together affected the change in the ratio of the k_L coefficient and the specific surface area a, causing a decrease in mass transfer efficiency in the tested system.

Mixing times were determined for the full range of operating parameters of the wave-type mixing bioreactor. Using the DoE experimental design methodology, a statistically significant influence of the amplitude α and frequency ω of the bioreactor platform deflections (the strongest influence) and the tank filling level fl on the mixing time was identified, and no significant influence of the acc parameter was found. Correlation equations were proposed to estimate the mixing time depending on the values of the bioreactor operating parameters in Cellbag™ containers with two different nominal volumes, enabling the estimation of the operating parameters at which the mixing times in the compared tanks were similar. To efficiently and accurately measure the mixing time in a wave-type mixing bioreactor system, an adaptation of the colorimetric method was developed based on a system designed for the purposes of the study to record the video material and an original algorithm for image processing and analysis.

Prototypes of Cellbag™ polymer tanks were developed, with structural modifications to increase mixing efficiency and mass transfer through placement of hemispherical protrusions on the bottom surface inside the tank. The selection of the modification geometry was verified based on the results of CFD simulations of liquid flow inside the tank for three modification variants. The selected variant was then quantitatively characterised in terms of its impact on mixing time and $k_L a$ coefficient values, identifying statistically significant changes in parameter values indicating the desired increase in mixing intensity and mass penetration in the modified polymer tank.

At the final stage of experimental research, bioprocess validation of the applicability of the selected modification variant was carried out by comparing the values of parameters characterising the performance of *Nicotiana tabacum* BY-2 cell cultures carried out in unmodified and modified tanks. In the case of cultures in modified tanks, measurable changes in the culture performance were observed: an approximately one-day beneficial extension of the period of dissolved oxygen availability in the culture medium, with a simultaneous slowdown in the rate of oxygen concentration decline compared to the control culture, a shift in the maximum metabolic activity of the cultured cells by about one day earlier compared to the control culture, an increase in the final yield of the culture, and changes in the distribution of sugar concentration in the culture medium during cultivation, indicating an increase in the rate of metabolic changes occurring in cells cultivated in a tank where flow turbulence was increased through the use of protrusions.

Keywords: wave-mixed bioreactor, mixing time, volumetric mass transfer coefficient, colorimetric method, *Nicotiana tabacum* BY-2 cells, mass transfer intensification, ReadyToProcess WAVE™ 25.