

# MIXING EFFICIENCY ON PLANT CELL GROWTH AND PROTEINASE PRODUCTION IN A STIRRED TANK REACTOR

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Mixing efficiency is one of the most significant factors in bioprocess productivity. The major role of agitation is to improve broth homogenization, mass and heat transfer inside the bioreactors. The increase of agitation and aeration and a good geometry of the reactor provide better mixing and mass transfer; however, excesses might result in high hydrodynamic stress with negative effects, such as loss of cell viability, rupture of cells, autolysis, changes in membrane integrity, release of intracellular compounds, morphological alterations, variations in intracellular respiration and a decrease in enzymatic production affecting the culture growth [1, 2]. The biomass production and metabolites accumulation in a STR depends on the resistance of the cells to shear stress, balanced with efficient mass transfer and mixing. Impeller design plays a major role in the mixing of fluids and suspensions. Its design influences fluid movement, and in turns the type of liquid involved influences the impeller design. Three-phase mixing, of which cell suspensions are an example, is extremely complex, with heat and gas distribution and solid suspension demanding certain requirements which are heavily influenced by the rheology of the mixture and the levels of shear stress tolerated by the cells. Multiple-impeller systems in the reactor are being applied where shear sensitivity of microorganisms is an important criterion for the process design, due to their efficient gas distribution and lower power consumption per impeller when compared to single-impeller systems [3-5].

Plant cell cultures, due non-Newtonian characteristics and the formation of heterogeneous aggregates, are an important biological model to study cell hydrodynamic stress correlated with oxygen mass transfer rate capacity in stirred tank. Cultures of *Centaurea calcitrapa* cells, presenting aspartic

proteinases with milk-clotting activity, were established in a 7 L stirred tank reactor (STR), at initial mass transfer conditions constant ( $4 \text{ h}^{-1}$ ). The aim of this work is the understanding of the mixing efficiency on cell growth and proteinase production. In this study were tested two types of impeller, a Rushton impeller and a marine propeller, where the cultures were grown in STR equipped with single or double impellers.

*C. calcitrapa* cultivated in a STR equipped with two impellers, presented high proteinase activity (235 U/mg DW) and growth, similar to the proteolytic value obtained with single-marine propeller (214 U/mg DW). This system has a very low dissipation energy rate ( $\bar{\epsilon}$ ),  $10 \times 10^{-4} \text{ W/kg}$ , what may correspond to the higher homogeneity and optimal mixing reached by two-Rushton turbines or by single marine-impeller systems. These results suggest that the mixing efficiency is a key factor for the minimizing shear stress driving the success of a bioprocess in terms of the biomass or the product formation. The choice of the impeller system and mixing conditions were crucial for proteinase and biomass production of *C. calcitrapa* cell suspension.

#### References

- [1] Busto, V.D., Rodríguez-Talou, J., Giulietti, A.M., Merchuk, J., Effect of Shear Stress on Anthraquinones Production by *Rubia tinctorum* Suspension Cultures. *Biotechnol. Prog.*, 2008, 24, 175-181.
- [2] Ducros, E., Ferrari, M., Pellegrino, M., Raspanti, C. & Bogni, C. Effect of aeration and agitation on the protease production by *Staphylococcus aureus* mutant RC128 in a stirred tank bioreactor. *Bioprocess Biosyst. Eng.*, 2009, 32, 143-148.
- [3] Gogate, P.R., Beenackers, A.A. & Pandit AB., Multiple-impeller systems with a special emphasis on bioreactors: a critical review. *Biochem. Eng. J.*, 2000, 6, 109-144.
- [4] Puthli, M.S., Rathod, V.K. & Pandit, A.B., Gas-liquid mass transfer studies with triple impeller system on a laboratory scale bioreactor. *Biochem. Eng. J.*, 2005, 23, 25-30.
- [5] Xia, J., Wang, Y.-H., Zhang, S.-L., Chen, N., Yin, P., Zhuang, Y.-P. & Chu, J., Fluid dynamics investigation of variant impeller combinations by simulation and fermentation experiment. *Biochem. Eng. J.*, 2009, 43, 252-260.