

Initial validation of the xT smart_DoE research management system (RMS) and AI-based design of experiments (DoE) for improved expression of the viral structural proteins in bioreactors.

In order to test the application of the xT smart_DoE software developed by Exponential Technologies Ltd. for protein production, the recombinant *E. coli* strain, pet-CMV-ntt 830, producing virus-like particles (VLPs), was cultivated in 6 parallel bioreactor system. The work was carried out in the Institute of Microbiology and Biotechnology of the University of Latvia.

VLPs are non-infectious supramolecular protein assemblies that can be synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. *E. coli* cultures were grown in TY medium containing kanamycin (25 mg/l) on a lab scale bioreactors Sartorius Q+. As variable parameters in order to maximize biomass production and highest expression levels of individual structural proteins according to earlier reports (Zeltins et al., 2017, Balke et al., 2018) have been selected: pH, temperature, isopropyl- β -D-thiogalactopyranoside (IPTG) concentration, agitation, air flow, as well as added glucose concentration. To evaluate the optimal cultivation parameters the following optimization criterion was used:

$$K = X * Y_p * 100;$$

where X is cell biomass (grams dry wt), Y_p is the amount of target protein produced (expressed as a percentage of the total protein).

By successively analyzing the obtained results and optimizing the cultivation conditions with xT smart_DoE software, within three fermentations we were able to increase the value of the optimization criterion almost two times - from 10,9 to 19,1. Corresponding parameters were: glucose concentration = 1.5%; IPTG = 250 μ M; agitation = 400 rpm; $t = 32$ °C; air flow = 0.7 l/min; pH = 7,7. Moreover, the xT smart_DoE has succeeded not only in improving the best overall value of the optimization criteria, but also within each subsequent iteration during the batch cultivation experiments. Compared to conventional methods, where value of only one parameter is varied during the cultivation, xT smart_DoE allows for the variability and subsequent analysis of all (in this case 6) variable parameters thus significantly reducing the experimental time. It was found that such an approach justified itself as it succeeded in significantly improving the value of the optimization criterion in at least one reactor at each time of cultivation.

Also, xT smart_DoE should be further validated/refined with more extensive studies of a similar type, taken together obtained results indicate that xT smart_DoE potentially might serve as useful tool in different sectors of biotechnology to reach optimal cultivation conditions within shorter periods of time.



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