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Designing Bioprocesses *In Silico*: Data-Driven Bioprocess Optimization of Molasses and Corn Steep Liquor for Yeast Protein Production

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Background: The transition toward sustainable and circular food systems requires resource-efficient strategies for the valorization of industrial by-products into high-value outputs. Molasses and corn steep liquor (CSL) represent abundant yet underutilized streams with significant potential for microbial protein production. In practice, their efficient use is constrained by complex nutrient interactions that are difficult to resolve through time consuming conventional experimental optimization.

Aims: This study aimed to develop a computationally guided framework for digital bioprocess design and optimization of molasses-CSL-based media for protein production by *Saccharomyces cerevisiae*, with a focus on identifying key process parameters governing metabolic trade-offs.

Methods: A genome-scale metabolic model based on yeast-GEM (Yeast9) was systematically analyzed using flux balance analysis (FBA) under physiologically relevant constraints, including non-growth associated maintenance. Molasses was represented as a mixed carbon source, while CSL was approximated as a complex nitrogen input. Sensitivity analysis and multi-objective optimization were applied to quantify metabolic trade-offs. A design-of-experiments (DOE) approach was implemented *in silico* to map the process design space across varying carbon-to-nitrogen (C/N) ratios and oxygen availability, generating a response surface for protein production.

Results: The model demonstrated robust and biologically predictive behavior corroborating expected trends in biomass formation under varying substrate conditions. Simulations indicated the presence of a transition region associated with changes in nutrient limitation, as well as a strong influence of oxygen availability on metabolic efficiency. The response surface analysis suggested the existence of an optimal operational window for protein production within intermediate carbon-to-nitrogen ratios and sufficiently aerated conditions. Overall, the results highlight consistent relationships between nutrient balance and protein synthesis, supporting the applicability of the model for process optimization.

Conclusions: This study highlights the potential of integrating computational modeling with biotechnological process design to improve the efficiency of protein production from complex substrates. By supporting systematic exploration of complex process spaces while reducing experimental burden, the proposed framework contributes to the development of digitally enabled, sustainable fermentation processes aligned with future food production and green transition priorities.