DEVELOPMENT OF A GENE REGULATORY MODEL TO ENHANCE PREDICTION ACCURACY OF BIOETHANOL FERMENTATION BY *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The environmental pollution attributed to fossil fuel emissions has increased the scientific attention towards the production of biofuels. Bioethanol stands as an alternative energy source based on its carbon-neutral profile and environmentally friendly nature [1]. *Saccharomyces cerevisiae* employed in alcoholic fermentation, utilizing lignocellulosic biomass as an energy source to produce bioethanol [2]. Dynamic mathematical models comprise valuable tools for understanding, designing and evaluating processes that significantly reduce the cost required to conduct experiments and predict the time evolution of complex biosystems. Although bioprocess kinetics could be typically predicted using empirical Monod-type models, a gap still exists in connecting bioprocess performance to the molecular events that control the efficiency of bioethanol production. The development of experimentally validated models of key genetic circuits could improve the prediction of the kinetic properties of the microorganism [3]. Herein, a logic model was developed to describe transcription from important genes involved in *S. cerevisiae* during alcoholic fermentation. The logic model developed incorporated mRNA production from glucose sensing processes, activation of hexose transporters (*HXT*s), the pathway of glycolysis and bioethanol production. Thus, a Boolean model combining logic gates was constructed to describe important regulatory loops, while Hill functions were used as input functions to the most important genes of each process (*HXK2*, *PDC5* and *ADH1*). The genetic circuit model was combined with a dynamic bioprocess model that expressed bioethanol, biomass and glucose concentrations in the system, aiming to form a hybrid mathematical model that employed ordinary differential equations to predict the performance of *S. cerevisiae*. Statistical analysis was conducted to evaluate and compare the predictive capability of the hybrid and Monod type models, calculating a standardized metric value. Normalized Root Mean Square Error (NRMSE) confirmed that the Monod model yielded values exceeding unity in most cases (0.64-10.09), while the hybrid model generated substantially lower values, between 0.57-0.75, offering a substantially improved prediction of the bioprocess. Moreover, the model demonstrated high accuracy (<0.5, NRMSE) in predicting mRNA expression of each gene's activity. The substantially improved accuracy of the model in bioprocess prediction, compared to Monod model, confirmed the significance of employing descriptions of complex regulatory programmes for the realization of bioprocesses and their optimal design.

KEYWORDS: Mathematical model, Genetic circuit, Bioethanol, Glycolysis, *Saccharomyces cerevisiae*

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