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Steps toward a Digital Twin: Biomass and growth rate modelling in batch and fed-batch bioprocess of Lactobacillus rhamnosus

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Lactobacillus rhamnosus (*L. rhamnosus*) represent a valuable potential for applications in the continuously growing multi-billion euro functional-food industry. This commensal microorganism is known for its multiple health benefits including immunomodulatory and gut-stimulating properties. Optimizing the production of *L. rhamnosus* biomass can enhance the efficacy of its use. In this study, three artificial neural networks (ANNs) were developed to predict biomass and growth rate in batch and fed-batch bioprocesses using a dairy-based substrate. Additionally, the immunomodulatory effect of *L. rhamnosus* was examined, revealing anti-inflammatory properties.

This research aims to maximise the production of *L. rhamnosus* biomass through optimized and robust control of bioprocesses. *L. rhamnosus* was cultivated in bench-scale studies on industrially suitable dairy-based feedstocks, namely skim milk powder, 90% demineralised whey and whey permeate.

Based on on-line and at-line measurements of bioprocess data, three ANNs were developed to predict biomass and growth rate in batch and fed-batch bioprocesses. This can be used to estimate biomass and growth rate digitally in real-time, creating a digital twin of the process. This estimator can be used to regulate the addition of feed to the bioprocess, leading to more precise control and increased productivity. These findings demonstrate the potential of ANN modelling and digital twin development for bioprocess optimisation of *L. rhamnosus* for application as a functional food ingredient. The immunomodulatory effect of *L. rhamnosus* was furthermore investigated and in-activated cell samples were found to have anti-inflammatory properties.

Overall, this research provides a comprehensive approach to the optimization of L. rhamnosus production through ANNs, digital twin development, and process control. The findings are significant for the functional food industry, demonstrating the potential for using *L. rhamnosus* as a functional food ingredient.

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