**A 5-Regime Kinetic Model for the Study of Heterogeneities in *Bacillus Licheniformis* Aerobic Fed-Batch Fermentation Processes.**

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**Highlights**

* A 5-regime kinetic model for *Bacillus licheniformis* has been developed and validated
* Glucose and oxygen variations are considered for a differential cell performance
* The model can be used to assess the impact of gradients on aerobic fed-batch fermentation processes

**1. Introduction**

The combination of computational fluid dynamics (CFD) and microbial kinetic models is of great interest to investigate the occurrence and magnitude of gradients in process variables [1-3] in industrial-scale reactors, since detailed information of the fermentation environment can be generated. Nevertheless, coupling both models can become rather challenging due to the increased computational effort these types of simulations require. Therefore, it is important that simple, but accurate, kinetic models are developed for the study of heterogeneities.

In this work, a mechanistic model for the industrial workhorse *Bacillus licheniformis* [4] has been developed experimentally and validated. Its large-scale process consists of a high cell density aerobic fed-batch fermentation. Hence, the presence of sugar and oxygen gradients is likely to occur [5]. These gradients lead to the potential occurrence of five different metabolic regimes (acetate limited, fully oxidative, microaerobic and two overflow levels), depending on the concentrations of glucose and dissolved oxygen. With this kinetic model, the already well-established [1-3] CFD models can be extended and glucose and oxygen gradients in *B. licheniformis* fermentation processes can be investigated.

**2. Methods**

The model considers the different performance of *B. licheniformis* SJ4628in five metabolic regimes depending on the concentration of glucose and oxygen in the fermentation broth (Figure 1). The switch between regimes is assumed to be instantaneous and based on critical concentrations of oxygen and glucose and on critical specific growth rates. Therefore, fermentations with varied concentrations of oxygen and glucose were performed to investigate the behaviour of *B. licheniformis* under different conditions.



**Figure 1.** Scheme of the metabolic regimes of the kinetic model and the conditions at which the switch occurs.

The model describes growth with classical Monod kinetics and uses stoichiometric yield and maintenance coefficients to account for glucose, oxygen and acetate uptake and acetate production. More details regarding the equations setup will be given in this contribution.

**3. Results and discussion**

This work consists of the development and validation of a 5-regime kinetic model for *B. licheniformis* fermentation processes. The model takes into account the glucose and oxygen conditions at which the microorganism is grown, and uses different parameters and equations accordingly. Therefore, it resembles glucose and oxygen uptake in fully oxidative, overflow and microaerobic regimes, acetate re-assimilation under glucose-limiting conditions and acetate production under overflow and microaerobic conditions. Although all these different metabolic behaviors have already been described in literature [6], it is the first time that a 5-regime model with immediate metabolic switches has been experimentally developed and validated. Besides, it is also the first time that the growth, substrate uptake and acetate production kinetics of *B. licheniformis* have been investigated.

In this contribution, besides assessing the model uncertainty, the applications of this model to study glucose and oxygen gradients in fermentation processes will be discussed and exemplified. The implications of such gradients in industrial *B. licheniformis* fermentation processes will also be tackled.

**4. Conclusions**

With the execution of this work, it has been proven that the behaviour of *B. licheniformis* SJ4628 can be described with an unstructured mechanistic model including five different metabolic regimes. It has also been shown that the model is suitable for the study of glucose and oxygen gradients in industrial fermentation processes.

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