PAT platform for scale up/down of *Streptococcus thermophilus* processes

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Abstract

Oscillatory conditions, which usually appear in large scale production due to extended mixing times, lead to cell stress and increased occurrence of population heterogeneity. Such heterogeneities are regularly observed in industrial production; however, the underlying mechanisms are seldom perceived or considered in bioprocess development. Since inefficient subpopulations may have a significant impact on the productivity of industrial cultures, cellular heterogeneity shall be quantified with appropriate process analytical tools. For lactic acid bacteria batch processes in particular, pH-gradients are the main concern when facing scale-up, since they lead to growth retardation and a loss of productivity. In order to study the consequences of these heterogeneous conditions on the cell population, two- and three-compartment scale-down bioreactors (Two-CR and Three-CR, respectively) were applied to mimic pH-gradients of the large scale in the lab scale in *S. thermophilus* fermentations [1].

The aim of this research was to compare physiologic and morphologic population heterogeneity under optimal and oscillating environments, based on multi-parameter flow cytometry and automated microscopic detection of the length of cocci chains, respectively. The application of these tools, together with *atline* electrooptical measurements of the average cell anisotropy of polarizability (AP) and size [2, 3], confirm differences between a homogeneous and heterogeneous growth environment. The cocci chain length reached minimum values during high growth rates and under optimum conditions, whilst chains remained rather long under pH-gradients as induced in Two- and Three-CR scale-down fermentations. Furthermore, a lower cell viability was observed under oscillating conditions. A similar profile was

measured in the production scale, in which the *atline* polarizability as well as the cell size showed a similar time course than in the scale-down cultivations (Figure 1).

This methodology coupled to a population balance model will enable the prediction of the growth performance based on the chain length distribution. Furthermore, this distribution could be applied as morphologic scale-down criterion to properly mimic conditions of the large scale without the exact knowledge of gradients and conditions in the liquid phase.

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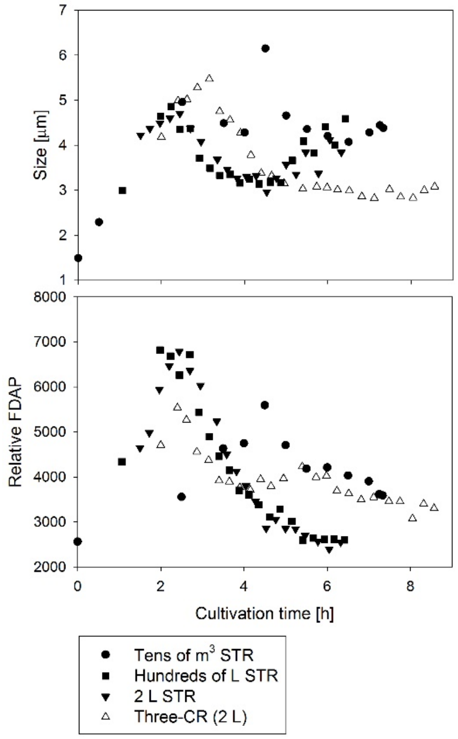
References

1. Lemoine, A. et al., Response of *Corynebacterium glutamicum* Exposed to Oscillating Cultivation Conditions in a Two- and a Novel Three-Compartment Scale-Down Bioreactor. Biotech Bioeng 2015, 9999(xxx):1-12.

2. Junne, S. et al., Electrooptical measurements for monitoring metabolite fluxes in acetone-butanol-ethanol fermentations. Biotechnol Bioeng, 2008. 99(4): p. 862-869.

3. Junne, S. et al., Electrooptical monitoring of cell polarizability and cell size in aerobic *Escherichia coli* batch cultivations. J Ind Microbiol Biotechnol, 2010. 37: p. 935-942.



Size [µm]
Relative FDAP
Cultivation time [h]
Tens of m³ STR
Hundreds of L STR
2 L STR
Three-CR (2 L)