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## Oxygen gradients enhance heterogeneity of *Trichoderma reesei* in bioreactors

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In large scale bioreactors, microorganisms are submitted to spatial heterogeneities that affect their performance during bioproduction. This phenomenon is more pronounced when considering the filamentous fungus *Trichoderma reesei*, which is the microorganism of choice to produce cellulases, in the context of production of second-generation (2G) bioethanol from lignocellulosic materials (Lopes Ferreira et al. 2014). However, *T. reesei* develops hyphae during growth, thus generating high viscosity medium with shear thinning behavior (Hardy et al. 2015) that enhances these heterogeneities. As a consequence, oxygen and substrate gradients as well as pH variations appear in the bioreactors that may affect the microorganism. The objective of this work was to study the effects of heterogeneities in dissolved oxygen on *T. reesei* morphology, its enzyme production capacity and its transcriptome.

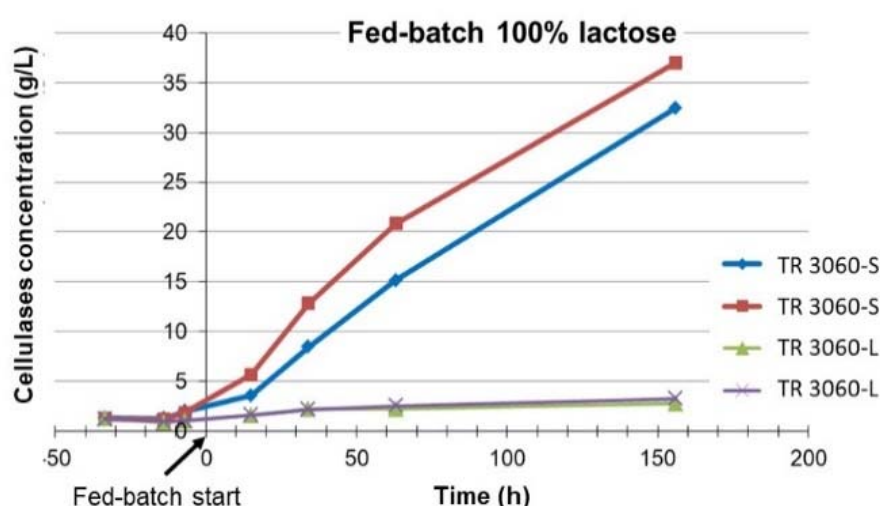
To answer these questions, we developed a scale-down approach using two lab-scale bioreactors connected together in series ("bizone" configuration), one being aerated, the other one not. By collecting samples at the beginning (19 h) and the end (212 h) of a fed-batch fermentation, we observed an increase of the proportion of large colonies (TR 3060-L, 24 %) that differed from the small colonies of the original strain (TR 3060-S), when spread onto agar plates (Fig. 1).

Strain TR 3060-S	Strain TR 3060-L
	
Cellulases final concentration = $2.0 \pm 0.4 \text{ g.L}^{-1}$	Cellulases final concentration = $0,7 \pm 0.3 \text{ g.L}^{-1}$

*Fig. 1: Difference in morphology and cellulase production by the original strain of T. reesei and its variant obtained from dissolved oxygen heterogeneities*

The ability of these colonies to produce cellulases was quantified on 7 isolates after cultivation in microplates. From Fig. 1, the cellulases concentration showed a significant difference ( $p < 0.05\%$ ) between the two variants.

Isolates from these two variants were further cultivated in fed-batch cultures under controlled conditions, with a lactose feeding. Specific growth rates were not different by considering the two variants, but final fungus concentrations were significantly lower with the variant T 3060L. Kinetics of cellulases production were strongly different, as shown on Fig. 2. The final cellulases concentration was lowered by 90 %, from  $34.2 \pm 2.8 \text{ g.L}^{-1}$  for the variant TR 3060S to  $3.5 \pm 0.2 \text{ g.L}^{-1}$  for the variant TR 3060L and the cellulase production rate was reduced by 92 %, from  $0.203 \pm 0.013 \text{ g.L}^{-1}.\text{h}^{-1}$  to  $0.016 \pm 0.002 \text{ g.L}^{-1}.\text{h}^{-1}$ , respectively.



*Fig. 2. Kinetics of cellulases production by the two variants of T. reesei T 3060*

These results indicated that oxygen heterogeneities, as generated in “bizone” bioreactor, enhanced heterogeneities at the level of the microorganisms, thus modifying the performance of the bioprocess. In the future, identification of the genes showing differential expression between the two variants will help understanding this behavior.

Hardy et al. 2017. Chem. Eng. Sci. 172, 158–168.

Lopes Ferreira et al. 2014. Biotechnology and Biology of *Trichoderma*. Elsevier.